Universidad de Jaén

Escuela de Doctorado

TESIS DOCTORAL·

THERAPEUTIC MODEL OF VASCULAR REGENERATION: DEVELOPMENT OF A NOVEL BIOREACTOR FOR RECELLULARIZATION OF HUMAN ARTERIES

PRESENTADA POR:
SHIVAN BARUNGI

DIRIGIDA POR:
Dra. MACARENA PERÁN QUESADA
Dra. ELENA LÓPEZ RUIZ

JAÉN, 2023

ISBN

INDEX

ABSTRACT 10

INTRODUCTION 16

1.1. Cardiovascular diseases – an Overview 16

1.2. Coronary angioplasty and stent implantation 19

1.3. Tissue-engineered vascular grafts (TEVGs) 27

1.4. Inflammation leads to atheroma formation and tumour cell mass initiation 32

HYPOTHESIS 42

OBJECTIVES 46

MATERIAL AND METHODS 51

2.1. Bioreactor design 51

2.1.1. USB serial communication 51

***2.1.2.* Installation and configuration of the serial monitor** 52

2.1.3. Serial interface 54

2.2. Biological samples obtention 59

2.2.1. Peripheral blood collection 59

2.2.2. Arteries of human origin obtention 59

2.3. Cell isolation and cell culture 60

2.3.1. Isolation and culture of endothelial progenitor cells (EPCs) 60

2.3.2. Culture of human umbilical vein cells (HUVECs) 60

2.4. Cell characterization 60

2.4.1. Phenotypic and functional characterization 61

2.4.2. Colony morphology, count and size 61

2.4.3. Cell proliferation assay with Sericin 61

2.4.4. Immunofluorescent staining 62

2.5. Nodal induction 62

2.6. Artery decellularization 63

2.7. Human Artery recellularization 65

2.8. Statistical analysis 66

RESULTS 69

3.1. Bioreactor design and construction 69

3.1.1. Bioreactor description 69

3.1.2. Cell injection system 72

3.1.3. Continuous flow of cell/cell medium mixture 72
3.1.4. Unilateral cell/cell medium mixture flow 73
3.2. Key advantages of the novel bioreactor 74
3.2.1. Further applications of the novel bioreactor 75
3.1. EPCs isolation and culture 77
3.2. EPCs characterization 79
3.3. Nodal induction in EPCs culture 83
3.4. Nodal enhances migration of EPCs 84
3.3. Biological validation of the novel Bioreactor 90
3.3.1. Characterization of decellularized human arteries 90
3.3.2. Collagen coated vessels support endothelial cell growth 92
3.3.2. Recellularization of human vessels grown into the bioreactor 93
DISCUSSION 98
CONCLUSIONS 113
BIBLIOGRAPHY 116

ABSTRACT

ABSTRACT

Cardiovascular diseases (CVD) are the leading causes of death and disability in the world, with coronary artery disease (CAD) being the most common. Currently there is no effective alternative to treat vascular diseases. The final treatment of these involves a vascular replacement. The development of techniques such as electrospinning and 3D printing have facilitated the fabrication of scaffolds that mimic natural vessels' structures. Nevertheless, synthetic grafts that are available on the market usually exhibit poor mechanical properties and the occurrence of infections, induction of intimal hyperplasia or thrombosis. As an alternative, the replacement of the damaged vasculature by the development of tissue-engineered vascular grafts (TEVGs) has been proposed, for instance, the use of decellularized vessels. The decellularization strategy relies on obtaining an acellular biological scaffold by removing donor tissue-resident cellular populations in order to minimise adverse host immune rejection, while simultaneously preserving the extracellular matrix and tri-dimensional structure. However, in order to have a practical clinical application as a biological scaffold, the decellularized vessels must be previously re-cellularized, preferably, with the patient's own cells. The vascular graft replacements should have mechanical properties similar to native arteries; ensure the correct formation of the endothelium layer to avoid thrombosis and should be easily insertable in the injured area.

Considering the limitations of conventional therapies, the main aim of the present study was the creation of a therapeutic model of vascular regeneration using human decellularized arteries.

To do so, we first optimised endothelial progenitor cells (EPC) isolation and culture. The use of Sericin and Nodal supplementation was proven to improve EPC growth and the maintenance of the endothelial phenotype. Human artery decellularization processes were improved and the absence of cells was demonstrated via histological and immunological analysis.

The main achievement of this doctoral thesis has been the design of a novel bioreactor with the goal of re-cellularizing human arteries. The bioreactor has improved characteristics that promote better performance with respect to previous bioreactors. These are i) a reduced size, adequate for being fitted into the laboratory hood and the culture incubator; ii) it can be can be completely sterilised by autoclaving its components; iii) it has an innovative system that allows the control of the intravascular flux, allowing it to reproduce the physiological blood flow; iv) the culture chamber can rotate which facilitate the complete dispersion of the cells on the internal wall of the vessel; v) the culture chamber has got a culture medium supply that allows the refreshing of the medium, independent of the medium influx.

We have proved the viability of this novel bioreactor by covering human decellularized arteries with biocompatible molecules and later with human endothelial cells that grew attached to the internal walls of the vessels. The correct formation of the endothelium layer was evaluated by histological and immunocytochemical assays.

In short, we present a compact and flexible bioreactor that simulates the physiological conditions and favours the cell coupling necessary to obtain vascular tissue with characteristics that reproduce those of the native vessels.

INTRODUCTION

INTRODUCTION

1.1. Cardiovascular diseases – an Overview

Cardiovascular diseases (CVD) are the leading causes of death and disability in the world, with coronary artery disease (CAD) being the most common (1). Current high-income countries' lifestyles, like low physical activity and diets with high fat and glucose content (2,3), contribute to the formation of an atherosclerotic plaque in low diameter vessels such as coronary arteries that supply blood to the myocardium (4). Atherosclerosis progressively occludes blood vessels and, in certain situations, the atheroma could break after the complete occlusion and form a clot that collapses the vessel (5). In both cases, the patient will suffer a myocardial infarction (MI) that can cause death as a result of the defective irrigation of cardiac muscle by the affected vessels if it is not quickly and properly treated (6).

Current treatments for atherosclerosis consist of repairing the vascular lumen of the affected vessels to allow a normal blood flow to the compromised muscle (7). Complementary pharmacological treatments may contribute to a decrease in atherosclerosis progression, for example by controlling the cholesterol and glucose blood levels as well as trying to prevent thrombosis (8). If the pathology is sustained during a long period without treatment or if the patient suffers MI, the cardiac cell death and adverse cardiac remodelling can lead to terminal heart failure (THF) (9,10). The recovery of normal blood flow can be performed percutaneously, by angioplasty, or surgically, by coronary artery bypass (CAB) (11) Figure 1. In the first case, the procedure is minimally invasive, a coronary catheter with a balloon is introduced until the stenotic area, the balloon is inflated and the artery recovers its normal lumen after which the balloon is deflated and removed (12). In most cases, a stent is implanted when the balloon is inflated to keep the vessel open. In the second case, the coronary bypass implies a surgery that uses other healthy autologous vessels, whose replacement is innocuous

(usually mammary and saphenous veins or the radial artery(13), to connect the aorta to a point of the coronary artery distal to the stenotic area. Therefore, this surgical intervention may allow the ‘’by-passing’’ of the atheroma and the recovery of normal irrigation in the compromised myocardium (14).

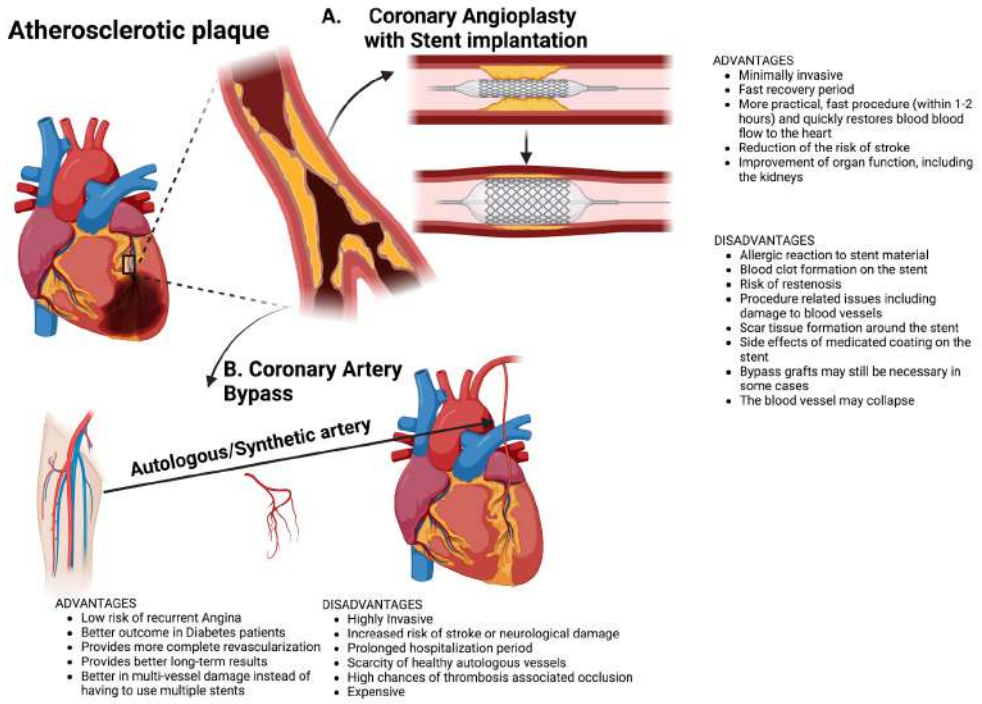


Figure 1. Common clinical procedures to treat coronary occlusion by atherosclerosis. A) Coronary angioplasty with stent implantation and B) Coronary artery bypass, either with an autologous artery or synthetic artery. Main advantages and disadvantages of both techniques are listed.

While MI requires a quick recovery of the blood flow to reduce necrosis at maximum, angina represents a non-critical situation which can be solved with slower procedures implying surgical interventions. Thus, angioplasty can be used for both angina and MI whereas CAB is usually reserved for angina (14). Even so, these procedures still have important drawbacks to consider. On one hand, stent implantation stimulates vascular wall cells proliferation which promotes

re-occlusion of the vessel through a process known as restenosis (15). To overcome restenosis and to provide a long-lasting solution, new stent designs have been developed which include drug-eluting stents or bioresorbable stents. On the other hand, although CAB surgery has been demonstrated to be more efficient than angioplasty (16), it is still an expensive and invasive procedure and in 5-40% of cases, the graft can collapse from thrombosis-associated occlusion (17). Moreover, healthy autologous vessels are not always available and there are additional difficulties such as the absence of commercially available small-diameter (<6 mm) vascular conduits needed for coronary artery replacement (18).
Comparison of clinical outcomes between percutaneous coronary revascularization vs coronary artery bypass grafting has revealed that both procedures resulted in similar rates of mortality, myocardial infarction, or stroke (19).

Because of the current limitations in CVD treatments, revascularization therapies have been focused on biological approaches with the aim of restoring, improving and maintaining tissue function over prolonged periods of time (20). Some of these novel strategies include the use of nanotherapy to prevent in-stent restenosis, or advances in vascular tissue engineering to develop tissue-engineered vascular grafts (TEVGs). A variety of approaches such as electrospinning and 3D printing have been employed to fabricate TEVGs while other strategies are based on the use of allogenic or xenogenic decellularized scaffolds (21). Parallelly, developing new therapeutic drugs with the potential to promote the atherosclerotic plaque reabsorption or, at least, mitigate its progression to gain time before obtaining an appropriate TEVG may represent a complementary approach. In this line, it is of key importance to understand the biological nature of atherosclerotic plaque formation (22).

Current evidence supports the role of inflammation in the initiation and evolution of plaque, with promotion of cell migration and proliferation that lead to lesion progression (23). In this respect, atheroma or plaque formation has been described as a kind of neoplasm of vascular smooth muscle origin, establishing similarities between atherosclerosis and cancer (24). Thus, stent implantation

represents an external agent that could cause restenosis, but other intrinsic agents such as patients' genetic alterations could increase the probabilities of developing the atheroma (25,26). The parallelism between alterations of molecular pathways between restenosis and tumour progression could help to determine the best therapeutic option (23).

Here we review common CVD treatments and novel therapeutic approaches focusing on the use of nanotherapy and tissue-engineering strategies. Furthermore, atherosclerosis progression and molecular/physiological events that support the correlation between inflammation and atherosclerosis are highlighted in order to open new avenues and opportunities to develop efficient drugs which may prevent and treat atherosclerosis.

1.2. Coronary angioplasty and stent implantation

Coronary angioplasty, developed by Dr.Gruntzig in 1977, was the first method described to perfuse stenotic vessels by recovering the vascular lumen through the inflation of a catheter-guided balloon to the atheroma (27). The procedure is minimally invasive, relatively non-expensive, effective, and the patient, in most cases, can leave the hospital the same day (28). Nevertheless, it has been shown that after a while, angioplasty vessels progressively return to their occluded status in between 30-40% of the cases (29) thus, to avoid the abrupt vessel collapse after angioplasty, a little expandable coil or stent is implanted (30).

It was in 1986 when the first human coronary artery implant was carried out using WALLSTENT® (Schneider AG). This was a structure made of a stainless-steel wire-mesh that was self-expanding (31). Nonetheless, it was taken off the market a few years later due to existing limitations in the stent delivery system which limited its clinical utility. Over the years, many more stents were developed like Multi-link® (Advanced Cardiovascular Systems), Micro® (Applied Vascular Engineering) and Wiktor® (Medtronic) to mention but a few. This was a major advance in the field of coronary angioplasty but it did not come without a number of drawbacks as most of the early stents were bulky and hard to manage

technically due to their high metallic density which ultimately led to a high rate of sub-acute thrombosis (31). Moreover, the mechanical stimuli over the cell wall provoked cytokine release that stimulates cell proliferation and migration to the injury. This phenomenon is named in-stent restenosis and may provoke coronary artery re-occlusion through an inflammatory and proliferative response against the foreign body (32,33). Hence, despite there being reduced restenosis in comparison to the plain old balloon angioplasty, the occurrence of in-stent restenosis was still high because of the migration and proliferation of cells within the stents (31). In fact, in-stent restenosis was associated to a high mortality and morbidity rate (34). According to these data, restenosis and the immune response against the stent are two of the main hurdles in this therapeutic approach. Remarkably, both events are characterized by a pro-inflammatory response (35), so it seems reasonable to assume that the immune reaction against the stent may be deeply involved in the promotion of in-stent restenosis. With the aim to solve in-stent restenosis associated complications, a new generation of stents including drug-eluting stents (DES) and bioresorbable stents (BS) have been developed (33). Figure 2 schematically shows the implantation of the stent and the subsequent appearance of restenosis together with different types of stents.

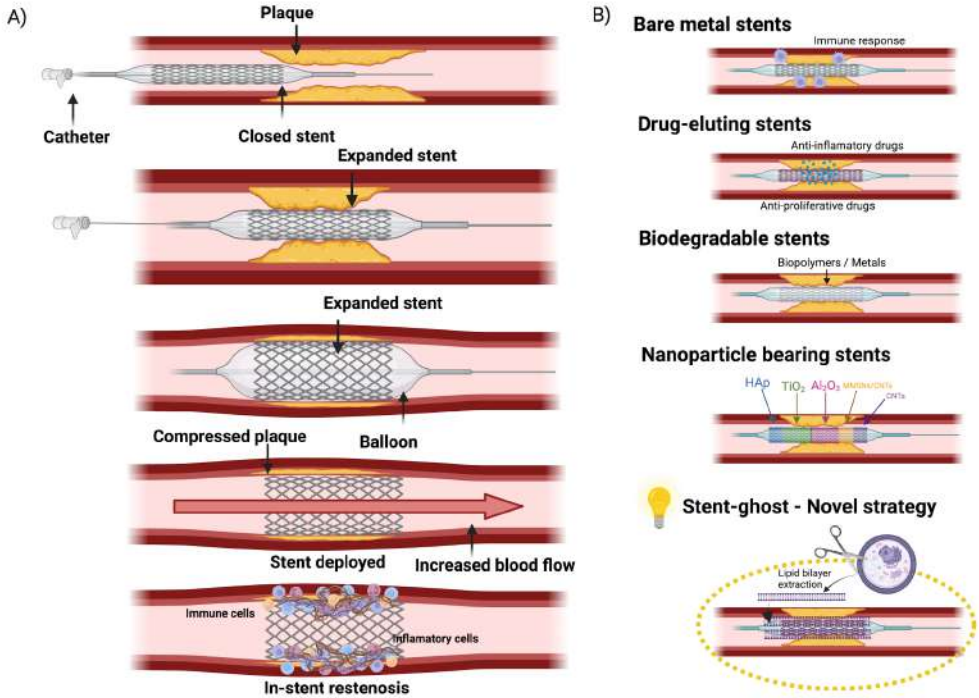


Figure 2. Stent strategy A) Representative stent implantation procedure, including implantation, expansion and lastly, in stent-restenosis formation. B) Representation of different Stents used in clinic: Bare metal stents; Drug-eluting stents; Biodegradable stents; nanoparticle bearing stents. The use of a "Stent ghost" which is a stent coated with cell membranes could represent an innovative therapy to escape the immune surveillance.

Regarding DES, different compounds have been used to cover bare metal stents (BMS) to target the proliferation of vascular smooth muscle cells (SMC), platelet activation, inflammation and thrombosis. Examples of DES are heparin-coated BMS, used to prevent thrombosis, and BMS loaded with phosphorylcholine which imitates the cell membrane, but the benefits have been limited (31). DES can also be coated with bioactive compounds that avoid vascular SMC activation and proliferation**,** reducing restenosis development and also modifying the healing process after implantation (36,37). Everolimus, sirolimus

and paclitaxel have been shown as antineoplastic agents with a high potential to reduce neointimal overgrowth, maintaining a bigger vascular lumen. Their mechanism of action is based on mTOR inhibition, decreasing cellular anabolic metabolism, growth, division and migration; fundamental pillars of restenosis. Recent studies have shown that there is a clear benefit when using everolimus eluting stents as compared to other BMS in regards to targeting lesion revascularization and the acquired results depending on the patient type and device used (38,39). Apart from the mTOR pathway inhibition, other strategies have been considered to avoid restenosis. For instance, potassium channels blockade, which are necessary for SMC activation (40). In fact, it has been shown that the inhibition of voltage-gated K+ 1.3 channels could reduce restenosis by targeting vascular SMC (41,42).

Anti-inflammatory drugs are used to inhibit the inflammatory response derived from stent implantation, reducing one of the main events that promote neointimal overgrowth. The promising use of Dexamethasone has been proved in preclinical models and in clinical studies (43,44). For instance, in porcine damaged coronary arteries to study drug delivery to the tissue, with effectivity being seen in the first 28 days (45), and in canine femoral arteries that showed a significant decrease in the neointimal hyperplasia (46). In other clinical studies, the dexamethasone-eluting stents have shown benefits compared to BMS, demonstrating a significant reduction of major adverse cardiac events at 12 months and in the restenosis/neointimal proliferation rates at 6 months (45–48). It is important to note that 10% of the patients treated with DES continue to suffer from restenosis and angina. Further, a delay in the healing of the injured vessel and very late thrombosis after DES implantation have been described (49,50). Therefore, there is still a necessity to develop less harmful stents/drug association to reduce their adverse effects (51).

Dual drug-eluting stents (Dual-DES) combine the beneficial effects of various compounds, while reducing individual dosage and associated adverse effects (52,53). For instance, the combined effect of sirolimus (antiproliferative)

and triflusal (antithrombotic) eluting stents, have been proven, *in vitro* and *in vivo,* to efficiently deliver both drugs, each in its appropriate dose, and to gain greater reduction of restenosis compared to the single drug-eluting stents (54). Other example are prednisolone plus sirolimus Dual-DES, in which, prednisolone enhances the effect of sirolimus, achieving a greater reduction of SMC proliferation, restenosis, fibrin expression and inflammation as well as an enhanced re-endothelialization (55). Furthermore, Byrne et al have proved that Dual-DES (rapamycin and probucol) in patients with CAD showed a higher reduction of restenosis than simple stents (56).

Bioabsorbable/biodegradable stents (BS) are another new generation of stents that try to reduce injury by naturally dissolving or being absorbed by the body (57). These types of stents can be composed of metals, such as magnesium (Mg) or zinc (Zn), or biodegradable polymers such as poli-D,L-lactic acid (PDLLA), poli-L-glycolic acid (PLGA) or poly(L-lactide–co-ε-caprolactone) (PLCL) that can be reabsorbed in the body after some time (58,59). BS present an advantage over other types of stents because they eradicate the factor of a foreign material remaining in the body permanently which is essential in avoiding immune reactions and having to remove the stent later on if necessary (60). Obviously, BS have to be made by biocompatible and non-toxic materials (61,62). In this respect, Mg and its alloys have been used in the development of the first metallic BS because Mg is highly biocompatible and also presents low thrombogenicity. In addition, in an aggressive chloride environment like the human body, its degradation is fast (63). Other metal alloys containing iron (Fe) or Zn have also been among the pioneer metals used to manufacture BS (64,65). In fact, Bowen et al described that Zn and its alloys had a slower degradation rate in comparison to Mg and Fe. Combining this with its good biocompatibility and mechanical properties, Zn and its alloys have been shown to be a safer choice to avoid issues associated with Mg and Fe BS since Mg has a faster corrosion rate than Zn and the corrosion of Fe produces non-bioresorbable iron oxides (66).

The second generation of BS was composed of biodegradable polymers that generate more innocuous products during their degradation than metallic oxides. One of the most frequently used biodegradable polymers is PLLA due to its high biocompatibility (67). In a period of 12 to 18 months, PLLA is metabolised into Carbon dioxide and water via the Krebs cycle with no toxic products resulting from the degradation. The first globally reported fully degradable stent was the PLLA-based BS by Igaki-Tamai et al (68). Down the road, there have been more advances in bioabsorbable polymeric stents in terms of their capability in drug deliverance and the way they are manufactured. Other polymers like PLGA, polyhydroxycarboxylic acids (PHCA), poly(3-hydroxybutyrate) (P3HB) (69) and PLCL are under study to assess their biocompatibility and functionality. Further examples of PLLA stents are Tissue Gen, ARTDIVA and Elixir, whose mechanical characteristics have been extensively studied (70–72). In fact, it was shown in the study about everolimus eluting PLLA stent Absorb-BVS-System that the mechanical strength of the stent rapidly deteriorated after the first 3 months following implantation (73). There have also been other polymers like poly(vinylidene fluoride)-hexafluoropropylene (PVDF-HFP) that have been used with second or third generation DES (65,74). Sirolimus and salicylic acid have also been combined with a poly-anyhydride ester to create a BS that has been found to have both anti-inflammatory and anti-proliferative characteristics (72). Although there are a number of polymers being used for medical purposes with properties that make them suitable for stent manufacturing, there are still some important issues to resolve, for example the generation of toxic products, poor mechanical properties and an unsatisfactory degradation rate (65). Taking all this into account, BS still do not present enough radial strength and stiffness in comparison with BMS and this can lead to fatigue and fracturing issues after the stent is implanted. In regards to this, research has been carried out to improve the mechanical properties of BS and it has been shown that plasticizing is an effective solution (75). The sterilisation techniques used on the stent must also be considered to avoid affecting the molecular weight and crystallinity of the material (76).

Bioresorbable drug eluting vascular scaffolds (BVS) are made up of polymers that will disappear after drug elution (76). Interestingly, several randomised trials have shown promising results when comparing BVS with traditional DES (77,78). Although the advantage of BVS is the transitivity of the scaffold, while presenting a longer period of time drug delivery, their efficacy and safety over time have not yet been proven due to limited data.

Nevertheless, it is important to remark that bioabsorbable stents have not represented a significant improvement clinically despite their initial promising results. To date, only two bioabsorbable stents are commercially available, Magnesium Magmaris (Biotronik) and polylactic Absorb (Abott) (79). In addition, a recent randomised meta-analysis study comparing the mid- and long-term clinical outcomes of both durable polymer drug-eluting stents (DP-DES) and bioabsorbable polymer drug-eluting stents (BP-DES) revealed no statistically significant differences in cardiac mortality, stent thrombosis, target lesion revascularization, target vessel failure or reinfarction rates (79).

In fact, a conducted trial to compare BVS versus metallic evorilimus-eluting stents (EES) did not produce conclusive results, since no significant differences were found between patients from either group in terms of cause of death after one year (80).

The first BVS to be put on the market and to be clinically used was the Absorb BVS (Abbott Vascular, Santa Clara, CA, USA) but even it had limitations in regards to scaffold recoil and thrombosis (81). A longer randomised study that tested the differences between BVS and DES was carried out in over 500 patients and there was a noted decrease in angina recurrence and deterioration in patients after 12 months (82). Nonetheless, after 3 years, there was in-stent loss and nitrate induced vasomotion observed. The original Absorb BVS had a strut thickness of 150 μm and this has been attributed to being the cause of the adverse reactions observed (82). Thus, this led to the development of second and third generation BVS to tackle the issues of the stent´s strut thickness. Therefore, a next generation of scaffolds with smaller strut thickness were developed, for example, Biolute BRS

with a strut thickness of 108 μm and MeRes and DESsolve, both with a strut thickness of 100 μm (81). The expected advantages from this new generation of BVS would be lessening the flow disturbances and in the long run, reducing platelet activation and thus the scaffold's thrombogenicity. Besides the strut thickness of the BVS, another promising research area is reducing the reabsorption time of the scaffold. In this regard, it has been demonstrated that DESsolve scaffolds are bio-absorbed and biodegraded within 1 to 2 years (81).

All this does not come without limitations and as mentioned before, this is a field that is promising but one that requires further research. Kozuma et al, further prove this with a 5 year follow up study they carried out to assess the long-term results of using BVS in comparison to DES that showed comparable results in regards to patient and device-based outcomes (83).

In short, there is still a need of more studies to prove the benefits of BVS in comparison to DES since studies carried out in the past 10 years showed BVS´s adverse effects and non-significant improvement in terms of patient´s outcomes in comparison to other types of stents (83,84).

Other strategies to improve stent hemocompatibility and reduce restenosis are based on the implementation of nanotechnology (70,94,95). The use of nanoparticles (NPs) to inhibit restenosis has been reviewed (86). In short, different types of NPs have been tested, such as liposomes, phospholipid-based micelles, polymeric nanoparticles, hydrogel nanospheres and magnetic nanoparticles leading to reduction of inflammation and angiogenesis (70,87).

Interestingly, similar to stents, the clearance of systemically inoculated nanoparticles by immune surveillance also represents a major barrier in the context of nanotherapy which has been extensively assessed (87). Thus, from a new perspective, the use of therapeutic nanoparticles, for instance directed against tumour cells, could be compared with the implantation of a stent. In both cases, foreign elements are recognized by the immune system and they cause rejection. Therefore, the advances related to the use of nanoparticles in other diseases can be applied to the field of stent engineering. In this regard, several strategies have been

designed in order to avoid immune reaction, for example, the classical nanoconstructs surface grafting with polyethylene glycol (PEG) polymers to reduce their recognition by the reticuloendothelial immune system (88). Nevertheless, the authors also highlighted the short-term perspectives of this approach due to the generation of anti-PEG antibodies by the host adaptive immune response. Furthermore, the functionalization of nanoplatforms with CD47, a self-recognition molecule, with the aim of avoiding or reducing their clearance by the innate immune system has also been shown (89).

On the other hand, significant advances in the last few years have led to the trend of cell membrane-coated nanoformulations with the ability to biomimic and efficiently evade their removal by phagocytosis (90). According to these data, it seems reasonable to suggest the translation of nanotherapy-associated advances, regarding immune evasion, to the development of new generation stents with reduced restenosis induction and improved biocompatibility. For instance, it may be interesting to look into the development of a CD47-homologous peptide-coated stent to increase its biocompatibility (91) or even cell membrane-coated stents. To this end, several cell membranes could be used, highlighting autologous endothelial cell-derived membranes in order to fabricate a kind of stent which may simulate a continuity of the host endothelial barrier. Obviously, this is a mere theoretical suggestion which should be experimentally assessed. In this respect, the importance of the cell-derived membrane orientation during the coating process should be remarked upon since it has been reported that the right-side-out orientation is the one which provides the appreciated immune-evasive properties (92).

1.3. Tissue-engineered vascular grafts (TEVGs)

Currently, the leading, long-term therapeutic strategy to treat severe, but not extremely urgent, obstructions in small-diameter vessels (lower than 6mm), highlighting coronary arteries, consists of performing bypass grafting using

autologous vasculature like the saphenous vein, radial arteries or internal thoracic artery (13). Nevertheless, bypass grafting involving autologous vessels may not be suitable in many cases due to a range of circumstances (i.e., inappropriate graft size, previous diseases, unfavourable operation history or the multiple nature of the vascular occlusion) (93,94). Additionally, other inconveniences should be highlighted, such as the vein graft failure due to stenosis (95). In a particular example, the internal mammary artery is commonly used for bypassing the left anterior descending artery in patients with CAD. In this regard, a clinical trial study revealed that the failure of this procedure had a statistical frequency near 10% and it was associated with some risk factors including vessel stenosis or the presence of additional bypass grafts in the diagonal branch (96). Indeed, the study established a link between the higher incidence of acute clinical events and an increased rate of repeat revascularization (probably derived from the traumatization of the body because of the external manipulation of autologous vessels) (96).

Large diameter synthetic grafts that are available on the market usually exhibit an acceptable long-term patency rate, however, small diameter synthetic vascular grafts present limited clinical application, in regards to poor mechanical properties and the appearance of infections, induction of intimal hyperplasia or thrombosis (97). In addition, there is no commercially available synthetic vascular graft for small diameter blood vessels. As an alternative, the replacement of the damaged vasculature by the development of tissue-engineered vascular grafts (TEVGs) which can be generally classified into scaffold-based TEVGs or self-assembled scaffold-free TEVGs has been proposed (98).

For instance, an alternative to constructed scaffolds is the use of natural scaffolds which already have all the properties that are needed, hence the use of decellularized vessels. The decellularization strategy relies on obtaining an acellular biological scaffold by removing donor tissue-resident cellular populations in order to minimise adverse host immune rejection, while simultaneously preserving the extracellular matrix and tri-dimensional structure. However, in order to have a practical clinical application as a biological scaffold, the decellularized

vessels must be previously re-cellularized, preferably, with the patient's own cells (99). As an example, the decellularization of porcine carotid arteries with the aim of obtaining biological vascular scaffolds feasible to be used as vascular grafts has been described (93,100,101). Nevertheless, small-diameter TEVGs based on allogenic or xenogenic decellularized scaffolds still have several limitations to be addressed before translation to clinical practice. For instance, the induction of host immune response against the vascular graft, inappropriate physical properties of the graft compared with native vessels which usually promotes the generation of aneurysms, graft-related thrombosis or graft-associated infections (99). In addition, developing an appropriate decellularization protocol still remains a matter of debate considering it should effectively remove not only host cells but also immunogenic antigens like α-Gal (102).

The development of techniques such as electrospinning and 3D printing have facilitated the fabrication of scaffolds that mimic natural vessels' structures (97). To date, *in vitro* and *in vivo* experiments have shown encouraging outcomes (103,104). Regarding the use of synthetic scaffolds, the development of a thermoplastic polyurethane synthetic vascular graft, using 3D printing technology, with improved mechanical and biocompatibility features compared with commercially available, non-biodegradable polytetrafluoroethylene grafts has been shown. In this study, the vascular graft was implanted into a rat abdominal aorta model using a patch technique. Despite it not being a full graft technique, the synthetic graft was shown to reduce calcification and thrombus formation in comparison to the standard polytetrafluoroethylene graft 30 days post-operation (105). However, biofabrication techniques, including bioprinting, still have some insurmountable limitations. Microfabrication technologies need to better mimic native vascular anatomy to minimise dimensional disparities at the anastomosis site and TEVG (103,106). Along with this, further research with large animals is needed, since only a few studies have been performed (107). To date, most of the studies have been conducted on rats and rabbits. Moreover, there is a need for

longer *in vivo* studies (>12 months) since the patency rate is still considerably lower than in autologous grafts (108).

In fact, self-assembled, scaffold-free vascular grafting is a novel strategy that takes advantage of recent advances in bioreactors and bioprinting technologies. In this line, Itoh and colleagues patented a procedure to create small-diameter, scaffold-free TEVGs by combining 3D bioprinting and bioreactor-based culturing approaches. First, tubular structures composed of human umbilical endothelial cells, human aortic smooth muscle cells and human dermal fibroblasts were generated using a 3D bioprinter. Then, these tubular structures were cultured in a perfusion system (bioreactor) and matured before their implantation in a rat model (109). Another alternative to constructed scaffolds is the use of natural scaffolds which already have all the properties that are needed, hence the use of decellularized vessels. The decellularization strategy relies on obtaining an acellular biological scaffold by removing donor tissue-resident cellular populations in order to minimise adverse host immune rejection, while simultaneously preserving the extracellular matrix and tri-dimensional structure. However, in order to have a practical clinical application as a biological scaffold, the decellularized vessels must be previously re-cellularized, preferably, with the patient's own cells (99). As an example, the decellularization of porcine carotid arteries with the aim of obtaining biological vascular scaffolds feasible to be used as vascular grafts has been described (93,100,101). Nevertheless, small-diameter TEVGs based on allogenic or xenogenic decellularized scaffolds still have several limitations to be addressed before translation to clinical practice. For instance, the induction of a host immune response against the vascular graft, inappropriate physical properties of the graft compared with native vessels which usually promotes the generation of aneurysms, graft-related thrombosis or graft-associated infections (99). In addition, developing an appropriate decellularization protocol still remains a matter of debate considering it should effectively remove not only host cells but also immunogenic antigens like α-Gal (102).

The development of a TEVG is still a challenge due to anatomical complexity. Additionally, the mechanical and biological functions of TEVGs need to be improved for TEVGs to make their way to clinical use (110).

Given the importance of developing cellularized- TEVGs, different cell sources "to dress" the grafts have been proposed. In this respect, autologous cells are the ideal candidate to minimise host immune reactions against the vascular grafts (98). However, using autologous cells, such as endothelial and smooth muscle cells, still present several hurdles like limited harvesting potential and the inability to reconstitute neo-tissues due to poor *ex vivo* differentiation and expansion rates. To overcome these limitations, the use of human skeletal myoblasts has been proposed as an alternative cell source, taking advantage of their high *in vitro* proliferative capacity (111). Another approach could rely on the generation of autologous induced pluripotent stem cells from peripheral blood-derived mononuclear cells and inducing their differentiation towards endothelial and smooth muscle cells to develop TEVGs. This approach can´t be translated clinically but it could be useful as a model of developing pathologic vessels with patient-specific cells to test novel therapeutic agents (112).

Another alternative to generate highly compatible autologous TEVGs consists of the subcutaneous implantation of tubular mandrels in the own host that will receive the future TEVG. This strategy takes advantage of the host immune reaction against the foreign body (tubular mandrel) around which a fibrotic capsule will be generated. After the maturation of the construct, the formed fibrotic conduit is extracted and used as a TEVG and this is very advantageous because of the short process duration (approximately 4 weeks) and the mechanical strength expressed by the resulting matrix *in vivo* (113). Nevertheless, the clinical translational potential of fibrotic capsule-based TEVGs may be deterred as it represents an invasive procedure highly dependent on either the host physiological characteristics (gender, age or pre-existing diseases like diabetes), the implant location or the implant features (i.e. chemical composition or topography). Additionally, it also requires an extensive period of time for its incubation (several

weeks) (114), so this therapeutic strategy remains mainly in the pre-clinical field. In fact, efforts are being made to shorten TEVGs production time to under 2 weeks in order to make them suitable for translation to clinical practice (115). Considering the high recurrence frequency of atherosclerosis, one strategy to reduce the time-limiting factor of TEVGs in the case of disease-recurrent patients could be their initiation at the time of the first therapeutic intervention, immediately after the recurrence of the vascular obstruction, as a preventive measure. Moreover, a second surgical intervention may increase post-operatory complications and significantly reduce patient quality of life (116). Considering the period between the application of the by-pass and noticeable reactions to it in the patient as a crucial clinical determinant, it seems reasonable to remark upon the importance of understanding the biological nature of atherosclerotic plaque formation. Such knowledge may provide us with relevant cues to develop specific drugs with the potential to promote atherosclerotic plaque reabsorption or, at least, mitigate its progression in order to gain time before obtaining an appropriate TEVG.

1.4. Inflammation leads to atheroma formation and tumour cell mass initiation

Traditional concepts in which a low-density lipoprotein is the sole cause of atherosclerosis are lately being questioned. The idea that atherosclerosis is a chronic inflammatory disease, with inflammation playing a central role in each stage of the atherosclerotic plaque life cycle has been gaining interest over the past few years (117). In fact, the use of inflammatory biomarkers has proven to be able to predict the risk of cardiovascular disease before any signals of the appearance of symptoms (118). Similarly, aberrant tumour cell growth is also supported by a background of chronic inflammation which represents a main risk factor for oncogenesis (119,120). In this respect, although atherosclerosis and cancer are two different diseases with distinct clinical management, and cancer is commonly associated with genetic mutations whereas atherosclerosis is rather related to environmental causative factors, there are still some similarities that are relevant to

remark (Figure 3). In fact, an interesting point of view relies on considering atheroma formation as a kind of local malignancy rising from the vascular wall (121), thus being a "wound that never heals" (122). The start point of atheroma formation involves the activation of endothelial cells from a quiescent-like phenotype towards a proliferative one (123) and interestingly, several stimuli can trigger this event through a pro-inflammatory signalling cascade, including LDL accumulation in the subendothelial space (124), blood flow disturbance at arterial curvatures or branch points (125).

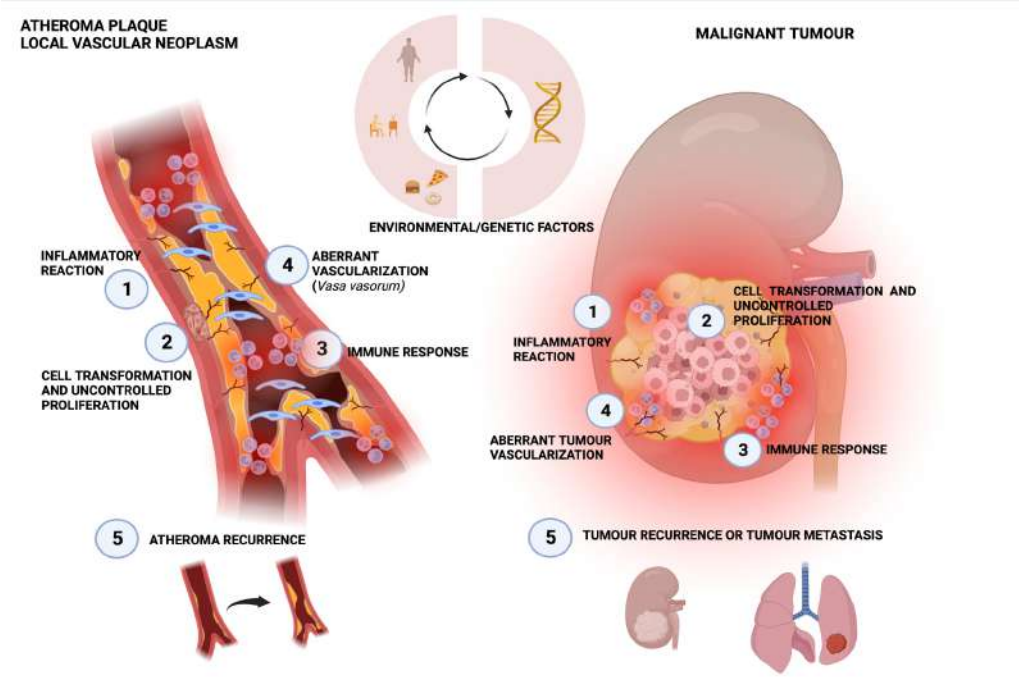


Figure 3. Similarities between atherosclerotic plaque and local neoplasia. The three circular arrows show that both diseases can be influenced by these same risk factors. Both processes are initiated by an inflammatory reaction (1), that leads to cell transformation and uncontrolled proliferation (2), which in turn triggers the immune response (3) and an aberrant vascularization (4). Finally, recurrence is frequently present for both atheroma and cancer (5).

Thus, the relevance of TNFα/TNFR1/NFkβ pro-inflammatory axis on a molecular level in the activation of endothelial cells to generate atherosclerotic lesions in response to changes in blood flow hemodynamics has been noted (126). Remarkably, a link between single-nucleotide genetic alterations and inflammation can be established by focusing on DNA-based deaminases like APOBEC3s family members or AID which induce the transition from Cytosine to Thymine and are enhanced by pro-inflammatory signals (127,128). Considering these facts and the documented association between DNA-based deaminases and carcinogenesis (129), it may be tempting to suggest the existence of an important role of deaminases in atherosclerosis.

Furthermore, the activated endothelium can undergo a process known as endothelial-to-mesenchymal transition (endMT) by which endothelial cells can acquire mesenchymal-like features partially losing their endothelial markers. In fact, the endMT process confers a remarkable phenotypic plasticity since it has been shown that endothelial cells can become smooth muscle-like cells and fibroblast-like cells during atherosclerosis (130). On a molecular level, the importance of the TGFβ/SMAD signalling pathway along with the TGFβ/Wnt2 axis in the progression of endMT, playing a key role in the increase of endothelial cells migration, invasion and neointimal formation has been reported (131). Moreover, it may be reasonable to propose that the activation of TGFβ and the endMT process may be early events in atherosclerosis since non-laminar blood flow and DNA methylation changes have been associated with the promotion of the endMT process and Wnt/β-catenin pathway in endothelial cells (132). Interestingly, the endMT process can be considered as the homologue of epithelial-to-mesenchymal transition (EMT) by which almost all types of tumours exhibit a more aggressive phenotype.

The uncontrolled proliferation of vascular SMC which migrate from the media to the intima vascular layer and contribute to neointimal hyperplasia and arterial remodelling during atherosclerotic plaque progression (133) could resemble oncogenesis. Specifically, vascular SMC undergo a phenotypic switch from a

quiescent and contractile phenotype towards a proliferative and migratory one with enhanced capacity to synthesise extracellular matrix components like collagen. Notably, the key role of miRNAs in such a dedifferentiation process by regulating intimal thickening has been reported, with the miR-146b-3p/PIK3CG axis as a representative example (134). Of note, the central role of transcription factor KLF4 in this phenotypic change triggered by several stimuli including the exposure of contractile vascular SMC to oxidised LDL has been highlighted (135). Parallelly, the phenotypic switching undergone by cancerous cells, from a well-differentiated phenotype towards a cancer stem-like one, which could also be termed as a "dedifferentiation process" has been widely described, with crucial roles during tumour progression of enhancing cellular aggressiveness and metastasis (136). Supporting the homology between vascular SMC and malignant cells, the clonal expansion of vascular SMC which have the potential to originate the majority of the cell types within atherosclerotic lesions has been confirmed (137). Interestingly, this fact may resemble the ability of cancer stem cells to generate all cancer subpopulations within a tumour mass. Indeed, the existence of a kind of "atherosclerotic stem cell" with an SMC lineage has already been suggested (138) which reflects a homologous potential to cancer stem cells.

In the same line, it has been revealed that vascular SMC phenotypic plasticity is far more complex than described above since SMC could even acquire distinct cellular fates, according to some single-cell analysis-based studies, such as myofibroblast-like (139) or macrophage-like phenotypes (140). More importantly, the recruitment of circulating innate immune cells, including monocytes/macrophages, into the intimal vascular layer in an early stage of atheroma biology has been confirmed. Specifically, the activation of endothelium triggered, for instance, by blood flow disturbances can lead to the expression of adhesion molecules, like ICAM1, in the surface of activated endothelial cells which promotes leukocyte extravasation (141). Therefore, these data may suggest the relevance of both fibroblast-like and macrophage-like cells in atherosclerotic occlusions. Similarly, the generation of a complex tumour microenvironment

around solid tumour masses, with cancer-associated fibroblasts (CAFs) and tumour-associated macrophages (TAMs) playing significant roles in the support of cancer progression has been widely observed (142). Focusing on plaque-associated macrophages (PAMs), it is relevant to note the distinct polarisation they can acquire within the plaque, with pro-inflammatory, M1-like PAMs being correlated with atherosclerosis progression and plaque instability/rupture whereas anti-inflammatory/regenerative, M2-like PAMs are correlated with disease regression, plaque stability and better prognosis (122). An equal bimodal polarisation can also be identified regarding TAMs but, intriguingly, with opposite roles since M1 TAMs have been associated with an anti-cancer behaviour while M2 TAMs have been classified as pro-tumorigenic cells (143). In any case, macrophage-like cell specific polarisation may be a factor of key importance in both disorders. Remarkably, M1-like polarisation in atherosclerotic plaque triggered by vascular SMC secretome has been shown to exhibit a diminished potential to recognize and remove opsonized diseased/unwanted cells, like proliferative vascular SMC (137). In other words, pro-atherosclerotic vascular SMC can modify immune cells like PAMs in order to escape immune surveillance, in a similar fashion to cancerous cells.

Next, it may be relevant to highlight that the intima-media thickening mentioned earlier, along with the accumulation of pro-inflammatory factors and chemokines in the adventitia vascular layer, could induce oxygen deficiency which may trigger abnormal vasa vasorum neoangiogenesis within atherosclerotic lesions (144). On the other hand, solid malignancies have also been characterised by strongly enhanced angiogenesis within the tumour mass. Indeed, it is thought that uncontrolled cellular proliferation and growth lead to local lack of oxygen and nutrients which may trigger the formation of abnormal intra-tumour vasculature characterised, for instance, by deficient pericyte coverage (145).

Traditionally, the main strategy to prevent/manage atherosclerosis relies on controlling its risk factors, i.e., blood LDL levels, hypertension or life habits like smoking. Although atherosclerotic occlusion can be removed by atherectomy, a

common recurrence of the disease within 2 years after the surgical intervention in up to 50% of patients has been noted (146). Interestingly, this fact may be similar to the well-known recurrence of malignant growth after the surgical removal of tumour mass. Thus, it is key to consider atherosclerotic plaque formation as a continuous process which may progress over time, so any effective therapeutic strategy may be a long-term treatment rather than a punctual procedure (147).

Considering the implication of the chronic inflammatory response in atherosclerosis, the administration of anti-inflammatory drugs like Omentin-1 has been proposed (148).

Recent clinical trials have shown that targeting inflammation can reduce cardiovascular events. The 'Canakinumab Anti-inflammatory Thrombosis Outcomes Study' (CANTOS) which was a randomised double-blind trial, targeting the interleukin-1β innate immunity pathway with the monoclonal antibody canakinumab concluded that the antiinflammatory therapy led to a significantly lower rate of recurrent cardiovascular events than the placebo, independent of lipid-level lowering (149). Although patients treated with canakinumab showed greater incidence of infections, one positive event was the reported highly significant reduction in incident and fatal lung cancer (150).

In addition, two trials using the natural anti-inflammatory factor Colchicine at different dosages have been conducted. The 'Colchicine Cardiovascular Outcomes Trial' (COLCOT) also showed improvements in reducing recurrent cardiovascular events after the development of acute coronary syndromes, but the incidence of pneumonia increased in the treated group. In the second trial, "Low Dose Colchicine 2" (LoDoCo2), the diminution in Colchicine administration also reported a reduction in recurrent events, similar to COLCOT, supporting the role of inflammatory pathways in the pathogenesis of atherosclerosis (151,152).

Following the rationale of relating atheroma biology with clinical approaches, it might also be tempting to specifically target activated endothelial cells in order to reverse the endMT process (131,153), or specifically focus on inhibiting the dedifferentiation of vascular SMC by promoting the recovery of their

contractile phenotype (154). Another potential therapeutic strategy could be the enhancing of efferocytosis, the removal of unwanted/pathogenic plaque-associated cells by own phagocytes, in order to induce plaque regression. In this regard, the neutralisation of CD47-expressing M1-like PAMs has been proposed with the aim of improving their sensibility to opsonized vascular SMC within atherosclerotic lesions (137). Furthermore, the modulation of PAMs phenotype could also be an interesting immunotherapy-based idea. In fact, enhancing M2 PAM polarisation and increasing M2/M1 PAM ratio within atherosclerotic occlusion may improve clinical outcomes and plaque regression (155).

Finally, it has been noted that the abnormal vasa vasorum neo-angiogenesis within atherosclerotic plaque usually leads to the establishment of weak vessels prone to rupture and induce intra-plaque haemorrhages and instability. Thus, the inhibition of vasa vasorum neo-angiogenesis and/or the promotion of the correct maturation of newly formed intra-plaque vasculature could be an interesting therapeutic approach with the aim of reinforcing plaque stability (144).

HYPOTHESIS

HYPOTHESIS

Vascular implants have the possibility of being developed using Tissue Engineering strategies with the pillars of tissue engineering being: i) cells; ii) scaffolding structures or “scaffolds”; iii) biocompatible molecules and iv) factors for the maintenance of cell differentiation and function.

A novel compact bioreactor can be developed for culturing cells to self-assemble in collagen-coated decellularized human vessels, with the specific characteristics, such as i) small size: to be easily used in a laminar flow cabinet and to be housed in a cell incubator, thus maintaining the sterility of the construction; ii) controlled medium influx and efflux into the artery and iii) controlled parameters like, temperature; rotation; chamber medium change; auto-cleaning, among others. Together with strategies that allow the maintenance of the endothelial phenotype, like the use of Sericin and Nodal, this approach could be a powerful tool for the recellularization of human arteries which would later be transferred to the clinical arena to alleviate the negative effects of current synthetic vascular grafts.

The novel strategy proposed could be key for Advanced Therapies in regenerative and personalised medicine for CVD.

OBJECTIVES

OBJECTIVES

Main objective:

To develop a vascular regeneration model for obtaining vascular grafts from decellularized vessels that present a viable endothelium.

Specific objectives

1. To develop a compact bioreactor for culturing cells to self-assemble in collagen-coated decellularized vessels, with the following characteristics:
   1.1. Size: to be easily used in a laminar flow cabinet and to be housed in a cell incubator, thus maintaining the sterility of the construction.
   1.2. Parameters: controlled medium influx and efflux into the artery; temperature; rotation; chamber medium change; auto-cleaning, among others.

2. Isolate endothelial progenitor cells (EPCs) from peripheral blood.
   2.1. Characterising the isolated and cultured EPCs by flow cytometry and immunocytochemistry of endothelial markers. Functionality tests like Dil-acLDL uptake.
   2.2. To prove the effect of Sericin and Nodal supplementation in the EPCs culture medium

3. Establish a vascular decellularization protocol from vessel samples of human origin
   3.1. Validation of the decellularization procedure by means of histological and immunocytochemical characterization showing the maintenance of the extracellular matrix to prove complete decellularization.

4. *In vitro* recellularization of human arteries using the novel bioreactor

4.1. Characterization of the recellularized arteries using immunofluorescence and histological assays.

MATERIAL AND METHODS

MATERIAL AND METHODS

2.1. Bioreactor design

2.1.1. USB serial communication

To connect the bioreactor with the PC, a USB 2.0 cable was used with a Type A male to Type B Mini male connector. Type A was the one that was connected to the USB port of the PC and Type B Mini was connected to the bioreactor controller.

The bioreactor's USB connector is located on the inner side (facing into the bioreactor) of the electronics leg/housing.

Figure 4. USB Type B mini to Type A cable

Figure 5. USB connection on the bioreactor (Type B mini)

2.1.2. Installation and configuration of the serial monitor

2.1.2.1. ARDUINO IDE Installation

The software was downloaded from the Arduino website through the following link:
https://www.arduino.cc/en/main/software

Figure 6. Arduino IDE download web view

2.1.2.2. Configuration of ARDUINO IDE

Once the program was opened, the configuration was then focused on two sections in the Tools tab:

- Selection of board and processor:

- Board: Arduino Nano
- Processor: ATMega328P

- Selection of the port: Before connecting the bioreactor to the PC, check all other ports that might be open, since the software can detect other devices connected by USB.

Once this is done, connect the bioreactor and recheck the ports. The new port that appears will be the one associated with the bioreactor. Select it.

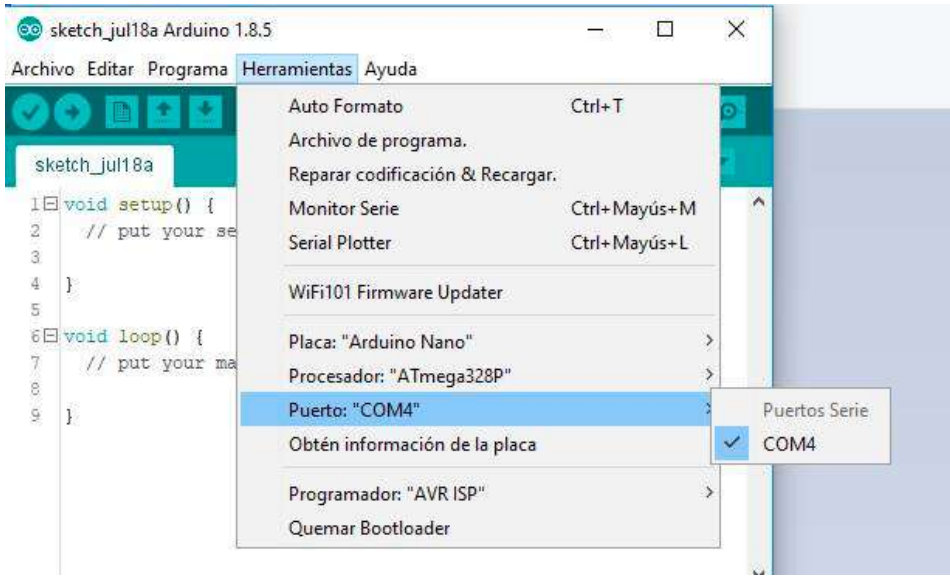


Figure 7. Tools tab and board, processor and port settings. Note: port value may vary

2.1.2.3. Configuration of the serial port

To achieve this, one had to click on the icon at the top right to open the serial monitor.

Once open, it was configured with the following options in the dropdowns:

- Both NL & CR
- 115200 baud

Autoscroll could be enabled so that the serial monitor showed the last messages sent by the bioreactor.

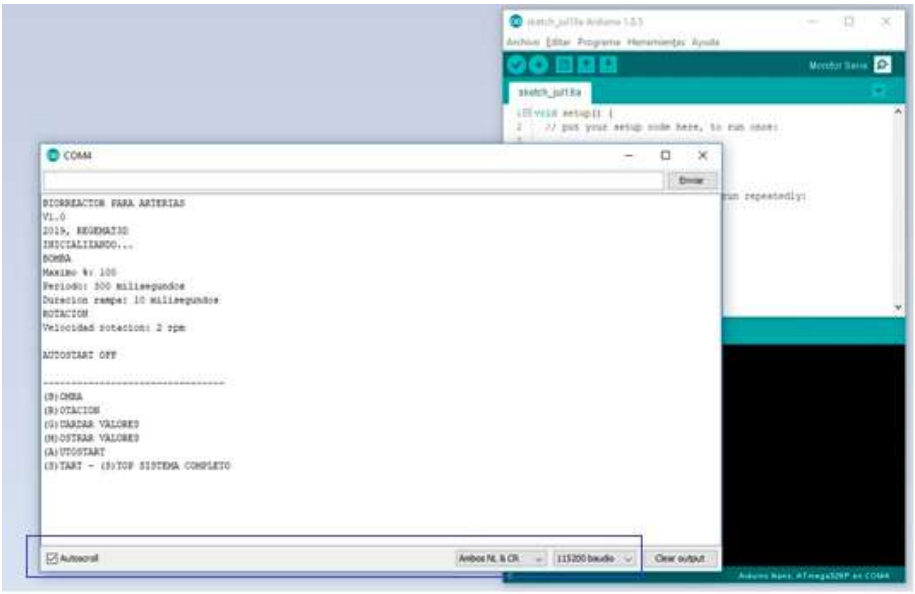


Figure 8. Serial monitor and option configuration

2.1.3. Serial interface

2.1.3.1. Main Menu

This is the initial system menu when the serial monitor is opened and it contains access to each configurable part of the bioreactor.

```
(B)OMBA
(R)OTACION
(G)UARDAR VALORES
(M)OSTRAR VALORES
(A)UTOSTART
(S)TART - (S)TOP SISTEMA COMPLETO
```

Figure 9. Main Menu

2.1.3.2. Pump and Rotation parameters

In this part, the current values of all parameters in the controller memory were saved. This permitted them to be kept even when the bioreactor is disconnected from the PC or turned off.

```
(B)OMBA
(R)OTACION
(G)UARDAR VALORES
(M)OSTRAR VALORES
(A)UTOSTART
(S)TART - (S)TOP SISTEMA COMPLETO

GUARDADO
```

Figure 10. Pump and Rotation parameters

2.1.3.3. Visualisation of values

The serial monitor shows the current values of all the parameters.
One has to note that the values shown are not those stored in memory. The values should be saved every time which is what was done in our trials.

```
VALORES ACTUALES
BOMBA
Maximo %: 100
Periodo: 300 milisegundos
Delta: 10 milisegundos
ROTACION
Velocidad rotacion: 2 rph

AUTOSTART OFF
```

Figure 11. Actual parameters used in the bioreactor

2.1.3.4. Auto Start

This tool is used to enable or disable the automatic system startup. It permitted the bioreactor to start working with the parameters it had in memory when powered by an electrical network, without the need to be connected to the PC.

NOTE: To connect the bioreactor to the PC and make parameter adjustments, it is recommended to have the Autostart option deactivated.

Figure 12. Autostart menu

2.1.3.5. Start – Stop Complete System

Activate/Deactivate both the pump and the rotation with the set parameters.

2.1.3.5.1. Pump control menu

The bioreactor pump functions by being controlled by a pattern of forward/backward pumping and braking movements mimicking the actions of blood flow in the heart. In this regard, there are a series of parameters that can be adjusted by the user (see graph):

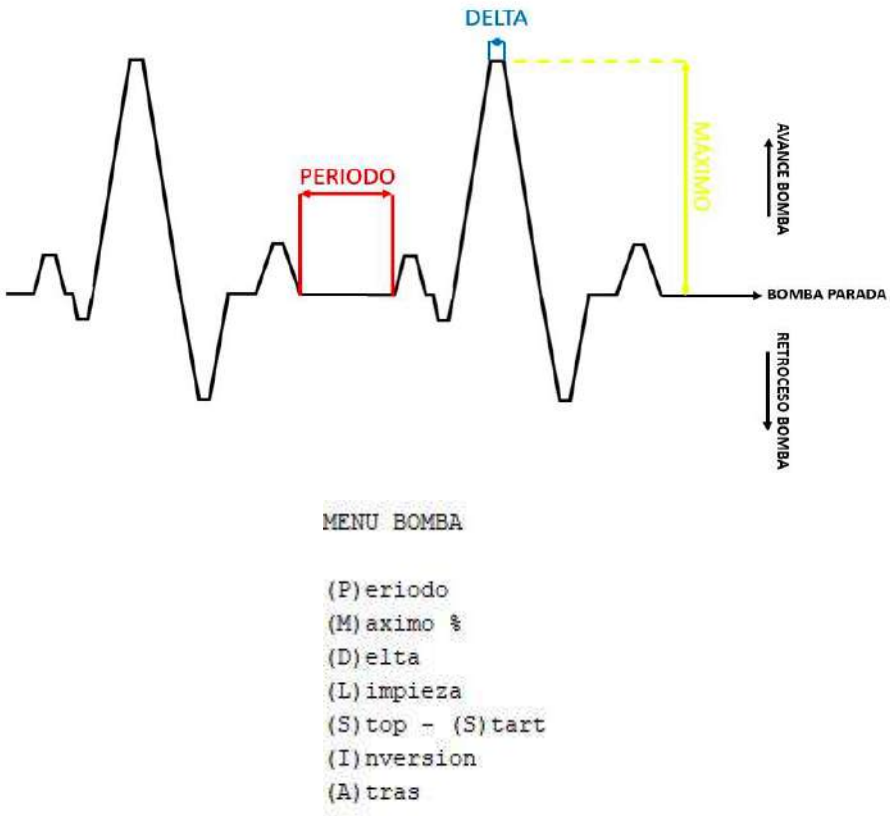


Figure 13. Graph showing the movement generated by the pump for the described parameters.

2.1.3.5.2. Pump parameters

Period

Interval, in milliseconds, between pulses.

Maximum

Percentage of maximum force applied by the pump. It has to be a value between 50 and 100.

Delta

Duration, in milliseconds, of each segment that makes up the pulse.

Cleaning

Activates the pump in one direction and keeps the fluid recirculating at maximum power. It can be used to clean/drain the circuit.

To stop the process, select Stop – Start.

Stop–Start

Stop/Start of the pump.

Inversion

Reverse the direction of fluid circulation.

Back

Back to the Main Menu.

2.1.3.5.3. Rotation control menu

The bioreactor chamber rotates one full turn in each direction. If you want to start the rotation with the chamber facing a certain position, rotate the chamber with the rotation off, and then turn the rotation on.

2.1.3.5.4. Rotation control menu parameters:

Speed

Rotation speed, in revolutions per hour.

Stop–Start

Stop/Start of the rotation motor.

Inversion

Change the direction of rotation of the chamber.

Back

Back to the Main Menu.

Figure 14. Parameters of the rotation control menu

2.2. Biological samples obtention

2.2.1. Peripheral blood collection

The blood was obtained from the Biobank of the Public Health System of Andalusia, Granada branch, in accordance with the regulations of the institution. After acquiring informed consent from healthy subjects, 40 ml of peripheral blood was obtained by venipuncture in the forearm from each patient. The blood was kept in vacutainer tubes with sodium heparin and was sent to the laboratory at a temperature of 4ºC.

2.2.2. Arteries of human origin obtention

The human arteries were obtained from the Biobank of the Public Health System of Andalusia, Biobank branch of the Reina Sofía University Hospital of Córdoba, in accordance with the institution's standards and were transported to the laboratory in dry ice and under sterile conditions.

2.3. Cell isolation and cell culture

2.3.1. Isolation and culture of endothelial progenitor cells (EPCs)

Fresh human blood, mixed with heparin was collected from the tubes and mixed with PBS in a 1:1 ratio. 25-30 ml of the blood mixture with PBS was then transferred to tubes with 15 ml of Histopaque 1007 (Sigma). After centrifugation at 500 g for 30 minutes (in a pendulum rotor), the mononuclear cell layer was collected and washed twice with PBS 2% FBS at 300 g for 5 minutes and a final wash at 200 g for 10 minutes. Next, the cell layer was resuspended in EGM-2 medium (Lonza), the number of cells were counted and 30-40 x 10^6 cells were seeded per well in a final volume of 2 ml in 6-well plates with type I collagen (BD). The medium was changed the following day and every 48h during the first week. From the second week and until the appearance of the colonies, the medium was replaced 3 times a week. Small colonies of cells appeared within 2-3 weeks. Once the well was confluent, they were transferred to 2 wells or a 25cm² vial of Type I Collagen (BD). The culture medium used was either EGM-2 (Lonza) or the medium designed with chimeric ligands. One cell passage was performed each time the cells reached 80% confluence. To carry out the passages, TrypLE Express (1X) (Lonza) was used.

2.3.2. Culture of human umbilical vein cells (HUVECs)

The HUVEC line was obtained from American Type Culture Collection (ATCC® CRL-1730TM) and cultured on 25T flasks previously coated with gelatine (Sigma) for 30 minutes at 37º and washed with PBS. The HUVECs were seeded with EBM-2 (Lonza) culture medium supplemented with EGM-2 single aliquots (Lonza) with 4% (v/v) FBS under standard cell culture conditions of 37ºC and 5% CO_2. The medium was changed every three days.

2.4. Cell characterization

2.4.1. Phenotypic and functional characterization

EPCs were characterized by flow cytometry and immunocytochemical techniques using the following antibodies: CD 31, CD 45, CD 133, KDR and CD146.

Functional characterization of EPCs was conducted by the ability of cells to uptake acetylated LDL (Dil-acLDL). For the Dil-Ac-LDL staining, the medium was removed and 100µl of fresh medium was added with 2.5µl of DiI-Ac-LDL (Cell applications, Inc) and left to incubate at 37º for 4 hours. As for the Ulex-lectin binding assay, the medium was removed and 200µl of fresh medium was added with FICT-labeled Ulex-lectin (Sigma-Aldrich) diluted 1:1000 and left to incubate at 37º during 45 minutes. Once completed incubation, cells were washed three times with PBS and fixed 20 minutes with PFA 4% at RT and nuclear staining was done with DAPI (Sigma-Aldrich). Cells were visualised under a Nikon Eclipse Tie-E A1 (USA) confocal microscopy and images analysed with ImageJ program™.

2.4.2. Colony morphology, count and size

To monitor morphology, cultures were viewed under an inverted microscope (magnification x40) on days 2, 4, 7, 14, 21 and 28 and photographs taken with Nikon Eclipse 50i (USA) microscope at 10x and 20x objective magnification. The counting of colonies was performed on days 2, 4, 7, 14, 21 and 28. 6 random colonies were later chosen and the diameter was analysed using the ImageJ program™.

2.4.3. Cell proliferation assay with Sericin

EPCs (5.000cells) were seeded in 96-well plates previously coated with fibronectin (2.5µg/cm2) (Sigma-Aldrich) and cultured under two conditions: EBM-2 medium (control) and EBM-2 medium with sericin 1% (Pure Sericin ™, Wako). On days 2, 5, 7 and 10 of culture, a cell proliferation assay was carried out using Alamarblue assay (Bio-Rad Laboratories, Inc.). Alamarblue solution was added to the medium and incubated at 37º. After 3 hours of incubation, the medium was removed and fluorescence intensity was read at an excitation wave of 530 nm and emission of 590 nm in a Microplate Reader MB-580/530 (Heales). The control included cell medium plus Alamarblue solution (Bio-Rad Laboratories, Inc.).

2.4.4. Immunofluorescent staining

EPCs and HUVECs were seeded on a µ-slide 8-well chamber (Ibidi AI-80826), washed twice with PBS and fixed with 4% paraformaldehyde (PFA) in PBS for 20 minutes at room temperature (RT). Fixed cells were blocked for 1 hour at RT with 5% BSA, 5% FBS in PBS and, then, incubated with primary antibodies, anti-VEGF and anti-Vimentin (Santa Cruz) diluted (1:100) in blocking buffer and incubated at 4º overnight. Cells were washed followed by the addition of secondary antibodies: Alexa 488 goat anti-rabbit IgG and Alexa 594 anti-mouse IgG (1:500) (Santa Cruz) at RT for 2 hours. Direct antibodies CD31-PE and CD133-APC (Milteny Biotec) were diluted (1:100) in the blocking buffer and incubated during 45 minutes at RT. Finally, cells were washed and incubated for 5 minutes with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Cells were observed and photographed under a Nikon Eclipse Ti-E A1 (USA) confocal microscopy and images were analysed using ImageJ program™.

2.5. Nodal induction

After 2 weeks of culture, 15.000-20.000 EPCs were seeded on µ-slide 8-well chambers (Ibidi AI-80826) and 100 ng/ml of nodal was added three times a

week during 14 days. For their characterization, cells were immunostained for VEGF, Vimentin (Santa Cruz), CD31-PE, CD133-APC (Milteny Biotec), Ulex-lectin binding (Sigma-Aldrich) and Di-Acl-LDL uptake (Cell applications, Inc). Cells were observed and photographed under a Nikon Eclipse Ti-E A1 (USA) confocal microscopy and cell fluorescence was measured with ImageJ program™ and finally, CTCF was calculated.

2.5.1. Wound healing assay

After 3 weeks of culture, 30.000-40.000 EPCs were seeded onto 6-well cell plates previously coated with fibronectin (5µg/cm2) (Sigma-Aldrich). 2 well plates were used for different concentrations of the Nodal, 50 ng/ml and 100 ng/ml, added to the cells and another well plate that would act as the control with no Nodal. 7 days later, after the cells had formed a monolayer, a wound healing assay was carried out to assess the effect of Nodal on the collective cell migration of the endothelial progenitor cells. The wound was created with the tip of a p20 pipette with the floating cells removed thereafter by a light wash with PBS. Images were then taken at different points in time over a 24-hour period using a phase contrast microscope with a 10x objective to view the rate of the gap closure and measure the cell free area. This was done after 5, 9.5 and 20 hours.

2.6. Artery decellularization

Human arteries of 10-15 cm in diameter were obtained from the Andalusian Tissue Bank and stored in cold PBS with 1% penicillin and streptomycin. The excess connective and adventitial tissue were removed and the artery washed with sterile PBS. Enzymatic digestion and detergent extraction were used for the decellularization of tissue samples following the protocol described by Sheridan et al. (156). In short, samples were immersed in deionized water for 24h at 4ºC and subsequently incubated during 1h at 37ºC in 0.05% Trypsin with 0.02% EDTA (Sigma Aldrich, St. Louis, MO, USA). After a short rinse in PBS to remove

the excess trypsin, samples were treated with a solution of 2% Triton X-100 and 0.8% ammonium hydroxide (Sigma) in deionized water for 72h at 4ºC. The solution was changed every 24 h to ensure the removal of all disrupted cellular material. Finally, a last wash was performed in de-ionized water by triplicate with a water change every 24 h to remove all chemical residues. All steps were carried out under continuous agitation except the trypsin incubation.

2.6.1. Histological assay

Histological analyses were performed to demonstrate the efficacy of the decellularization process. In this regard, samples were fixed in 4% PFA and embedded in paraffin in an automatic tissue processor (TP1020, Leica, Germany). Samples were cut into 5µm sections for their posterior staining with Hematoxylin and Eosin. Another method of tissue evaluation was used which involved the frozen sectioning of the arteries using an Optimum Cutting Temperature (OCT) compound to preserve the tissues that were later stained with H&A as well.

2.6.2. Immunofluorescence assay

For immunofluorescence sectioning, the arteries were fixed with 4 % paraformaldehyde in PBS for 20 min at room temperature (RT) and placed in 30 % sucrose overnight at 4 °C. Artery samples were embedded in optimal cutting temperature (OTC) compound. Blocks were stored at −80 °C, sectioned on a cryostat (CM1510S; Leica) into 8 µm-thick sections and placed on Polysine™ slides (Fisher Scientific). Sections were permeabilised and blocked for 1 h at room temperature with 5 % BS, washed in PBS and incubated with the primary antibody overnight at 4 °C. Primary antibodies used were: ColI (SC25974, Santa Cruz Biotechnology) and Elastin (sc-166543, SantaCruz Biotechnology). On the next day, samples were washed thrice with PBS, incubated with the secondary antibodies (Alexa) for 1 h at RT, washed thrice with PBS and mounted with

4',6-diamidino-2-phenylindole (DAPI)-containing mounting medium. Images were acquired with a Leica DM 5500B microscope.

2.7. Human Artery recellularization

The human arteries were recellularized using our novel bioreactor, cells used were HUVEC and EPCs. The decellularized artery was placed into the glass and metal chamber of the bioreactor and connected on either side to the input and output of the bioreactor internal flux. A peristaltic pump introduced culture medium into the artery at a controlled speed. The medium is then pushed through the lumen of the arteries to achieve trans-mural diffusion of solvents as well as adventitial bathing of the arteries before fluid recirculation. The bioreactor was calibrated beforehand to the required flow rate and caudal and set to Autostart so that it could start once plugged into an electric source.

The recellularization process was performed at room temperature with a flow rate of 0.5 L/min. First, 3 ml of medium containing $6x10^6$ cells was injected into the input tube and then into a 4 cm long artery that was previously coated with Collagen 1. This was all done while keeping the output closed. The bioreactor was then placed into the incubator at 37ºC and 5% CO_2. After 12 hours, the chamber was rotated and another 3 ml of medium with $6x10^6$ cells was injected into the artery to ensure the interior of the artery had sufficient cells for the recellularization process and the bioreactor was placed in an incubator overnight at 37ºC and 5% CO_2. The arteries were then incubated for another 12 hours in the bioreactor at 37ºC and 5% CO_2 with a continuous flow of cell/cell medium solution, in and out of the artery with a pulse every 3 hours.

After the recellularization processes in the bioreactor, cell viability was assessed using the LIVE/DEAD viability/cytotoxicity kit (Molecular Probes) following the manufacturer's instructions. Initially, cells were stained before being seeded into the bioreactor. Live cells were stained with calcein AM, while dead cells were labelled with EthD-1, a probe that indicates loss of plasma membrane

integrity (Invitrogen kit). Fluorescence microscopy (Nikon Eclipse Ti-E A1, USA) was utilised to capture images, and the analysis of images was performed using ImageJ software.

2.8. Statistical analysis

Results in this work are represented as mean ±SD. Differences between treatments were tested using the two tailed Student's T test. Results were considered significantly different at $p<0.05$(*) and $p<0.01$(**).

RESULTS

Bioreactor design and construction

RESULTS

3.1. Bioreactor design and construction

3.1.1. Bioreactor description

The REGEMAT bioreactor prototype was designed in PLA via 3D printing (Fig. 15). It was specifically designed with specific features that would simulate physiological arterial conditions. It possesses a programmable rotation system to ensure cellular adhesion to the arterial walls and it was also built with silicon tubes and keys that work hand in hand as a peristaltic pump to recreate the heartbeat.

Panel A of Fig 15 is a representation of the culture chamber embedded with a crystal window to visualise the artery. The culture chamber has an entrance through a tube with a screw cap that simulates a culture flask. This system allows the change of culture medium in the chamber so that the outer walls of the artery are always in contact with fresh medium. The blue box under the base was constructed to protect the electronic parts of the device.

The bioreactor was designed in such a way that it is connected to a pump via the two connectors that are shown in Fig 15B. The rotation system, made with gear wheels, is connected to the internal controller of the bioreactor, to be able to modify the rotation parameters.

The bioreactor chamber is shown in Fig. 16. The glass window can be removed to allow access to the chamber when introducing the artery. It is then fixed back using the screws that appear in the photograph, leaving the chamber sealed shut by a plastic frame that is fitted into the interior of the glass piece.

The bioreactor functions via a communication of USB cables that connect it to a computer using a USB 2.0 cable with a Type A connector to Type Mini B

connector. The Type A was connected to the laptop USB port and the Type Mini B was connected to the bioreactor's control panel. With the auto start function, this enabled the automatic activation and deactivation of the system. This allowed the bioreactor to function with predetermined parameters uploaded into its memory system beforehand, immediately it was plugged into an electric outlet without the necessity of it being connected to the laptop.

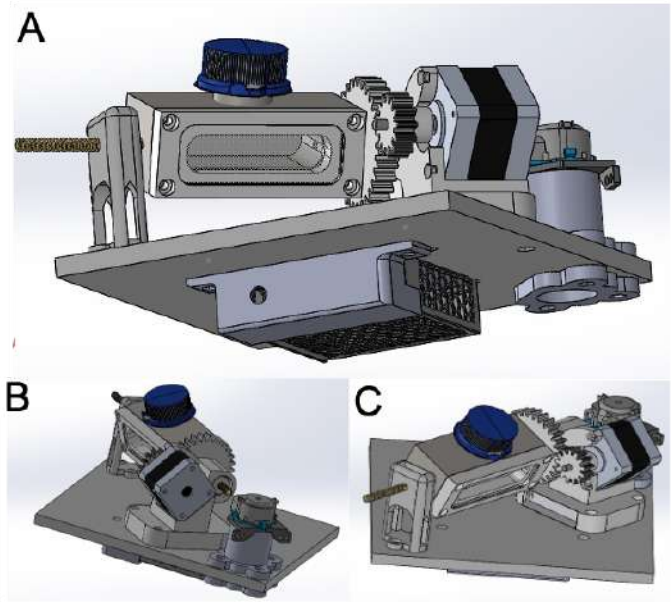


Figure 15. Graphic images of the bioreactor design. Three different views are shown

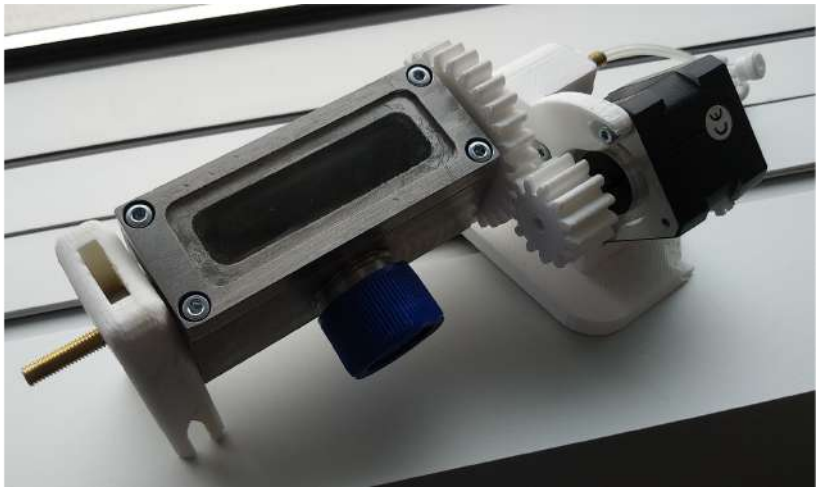


Figure 16. Photography of the bioreactor chamber

The use of decellularized human vessels in this bioreactor is an alternative to the use of other scaffolds as recellularization and revascularization therapeutic options because it prevents the occurrence of an immune response and this is one of the most important results sought in the construction of this bioreactor. Our design permitted the attachment of a human artery, in its native cylindrical form which would also facilitate its future possible use in translation to a future host where it may be the case. As previously mentioned, the cells used for the recellularization process were derived from human peripheral blood and their alignment and placement is a vital part of the vessel´s future functionality in regards to its strength and mechanical properties.

Additionally, the short length of the medium chamber, 6.6cm long, permitted the use of short pieces of the blood vessel which is an advantage when it comes to the high demand of small diameter bioengineered vascular options. There was also less manipulation of the blood vessel once it was attached to the keys which preserved its integrity and also further aided its sterility.

3.1.2. Cell injection system

The bioreactor possesses two silicon tubes that are the inlet and outlet of the cell/cell medium mixture, in and out of the vessel that is set up in the bioreactor.

Before the artery was attached to the bioreactor, various parameters were tested and the bioreactor was calibrated to values that properly mimicked the conditions of blood flow in a blood vessel and to also prevent the detachment of cells after the vessel had been recellularized. These different values were the retested with an artery in the bioreactor and the most suitable parameters were set for the recellularization process. The first of these values was the velocity with which the cell/cell medium mixture would be injected into the artery and it was tried at 50% and 100% of the total velocity the bioreactor was capable of. It was decided that 50% was the most ideal value. The time between the pulses as the medium was pumped into the bioreactor was 2000ms. The force of the pump was also calibrated to 50% with the Delta value oscillating between 10 and 200.

After the vessel had been attached to the keys on both ends of the bioreactor chamber, the bioreactor was filled with cell medium through the open tubular opening shown in Fig.15.

The chamber is then sealed off, with a plastic cap being placed onto the tubular entrance and the glass window being screwed back onto the chamber.

3.1.3. Continuous flow of cell/cell medium mixture

A 50mL falcon tube was filled with 48mL of a cell and cell medium mixture and both input and output tubes were placed into the tube, through 2 different holes made into the screw cap of the tube. The parameters were calibrated into the bioreactor system and it was then set to Autostart. The bioreactor, with its input and output tubes

attached to the 50 mL tube were all moved to the incubator. The bioreactor´s plug was then connected to an electrical socket and the Autostart process was initiated. The input tube absorbed the cell/cell medium mixture up into the artery and it then passed through the artery and out through the output tube and back into the falcon tube and the process was continued for 12 hours. This was carried out at different velocities, 50% and 100% and also with times in between the pulses, 0.5 minutes, 1 minute, 5 minutes, 15 minutes and 30 minutes. Another trial was carried out without any pulses as the medium mixture flowed in and out of the vessel. The recellularization rate of this method was not very high and it was believed that the continuous flow of medium and the pulses, when present, were both not very favourable for the reattachment of the cells onto the arterial walls. This was all done whilst the rotation option of the bioreactor was switched off. It was hypothesised that the continuous flow of the cell/cell medium culture with and without the pulse setting eroded the cells and prevented the attachment of said cells to the inner walls of the artery and it was concluded that a lower velocity and frequency of the pump would permit better results in regard to the recellularization. (Data not shown)

3.1.4. Unilateral cell/cell medium mixture flow

The cell/cell medium mixture is injected into the input tube using a 5mL syringe, gradually and with a low suction pressure; all whilst maintaining the output tube closed. The cells were then left to settle onto the bottom half of the artery for a period of 4 hours, 6 hours, 8 hours and 12 hours on different trial dates, after which the chamber was rotated automatically at 180^0, a rotation that was initially programmed into the bioreactor system. A new cell/cell medium mixture was injected into the input tube as before, and the cells were left to settle on this other surface of the artery that was initially at the top. This was also done for 4-hour, 6-hour and 12-hour periods. The cell/cell medium mixture was then allowed to flow in and out of the vessel at a velocity of 20% and a pulse every 3 hours for another 12 hours to mimic the human physiological conditions. This approach was more successful, with a high number of cells observed via immunofluorescence microscopy; and with an even higher number

of cells observed in the vessels whose cells were left to settle for more time, that is, 12 hours, which leads us to believe that the cells required a longer period of time to properly and homogeneously attach to the vessel walls.

An option to the programmed rotation after the specified period of time was continuous automatic rotation for 24 hours and this was tried out but the number of cells that were observed in the artery after the process was low in comparison to the cells observed after programmed rotation (Data not shown).

With all this in mind, the unilateral cell/cell mixture flow method, with programmed rotation every 12 hours for 24 hours, with a velocity of 50%, DELTA of 200, Caudal of 40m^3/s and then a 12 hour period of a continuous flow of the cell/cell medium mixture in and out of the artery with the pump calibrated at a velocity of 20% and pulses every 3 hours for another 12 hours, was the preferred system to use in our bioreactor with the most promising recellularization results as will be shown further ahead.

3.2. Key advantages of the novel bioreactor

- The size and shape of the bioreactor allow it to be fit into a culture hood and incubator (Fig. 17).
- The vessel is easy to mount into the chamber and the bioreactor on a whole is easy to assemble and use.
- Prevention of tissue contamination because of the ease to autoclave, which also provides the needed conditions for cell culturing in the vessels.
- Presents the needed conditions to mimic an In-vivo hemodynamic environment for example, the continuous flow of the medium that simulates blood flow.
- The bioreactor allows the full management of CO2, temperature and humidity levels to ensure ideal physiological conditions.
- There is complete access to the vessel which permits its monitoring in real time.

- The amount of medium needed is minimal making it cost effective.

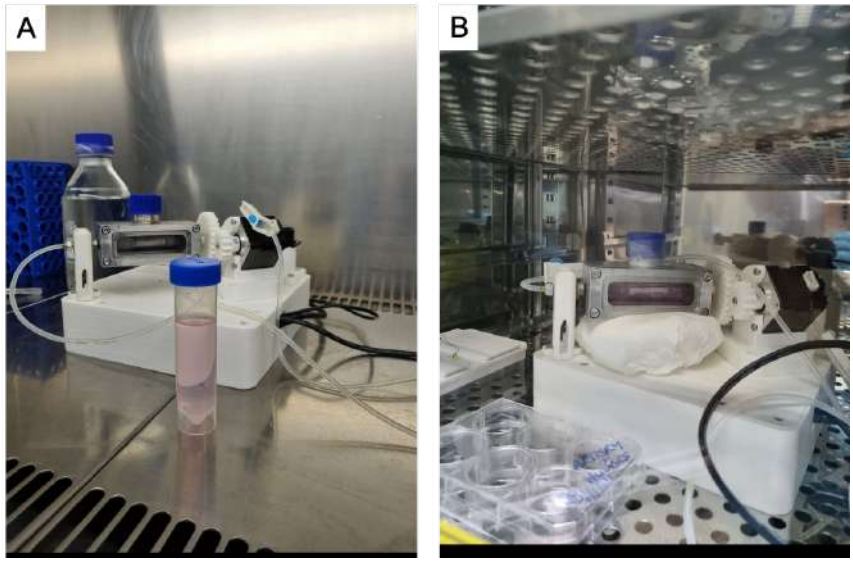


Figure 17. Photography of the bioreactor into the culture hood (A) and a culture stove (B).

3.2.1. Further applications of the novel bioreactor

- The model can be used as a trial prototype for the development of better and more advanced systems.
- The bioreactor can also be used to acquire controlled and relevant data over long periods of time in controlled test settings.
- The analysis of different recellularization protocols under different conditions
- Non-invasive option to animal testing

RESULTS

Improvements in EPCs culture: cell proliferation and EPC phenotype maintenance

3.1. EPCs isolation and culture

EPCs were isolated from human peripheral blood following the procedure described in the material and method section. Figure 18 provides a visual representation of the sequential steps employed in the isolation process, starting with the first step consisting in distributing the blood in 50 mL tubes (Fig 18A). The fresh peripheral blood was added, in an equal ratio, to sterile PBS in a 50mL falcon tube and properly homogenised (Fig 18B). 25mL of the blood/PBS mixture was carefully layered on top of 15mL of Histopaque 1007 which served as a density gradient solution in a 50mL tube (Fig 18C). This was then centrifuged at 500 x g without braking to successfully attain mononuclear cells resulting in an erythrocytes and granulocytes level (red), a mononuclear cell fraction (white layer), a plasma layer (yellow), and the density gradient solution (whitish) (Fig 18D). The cell layer was resuspended in EGM-2 medium and cultured in fibronectin coated dishes. The attached cells after 24 hours of culture are shown in Figure 19A. Fully formed colonies can be observed after 7 days in culture (Figure 19B).

.

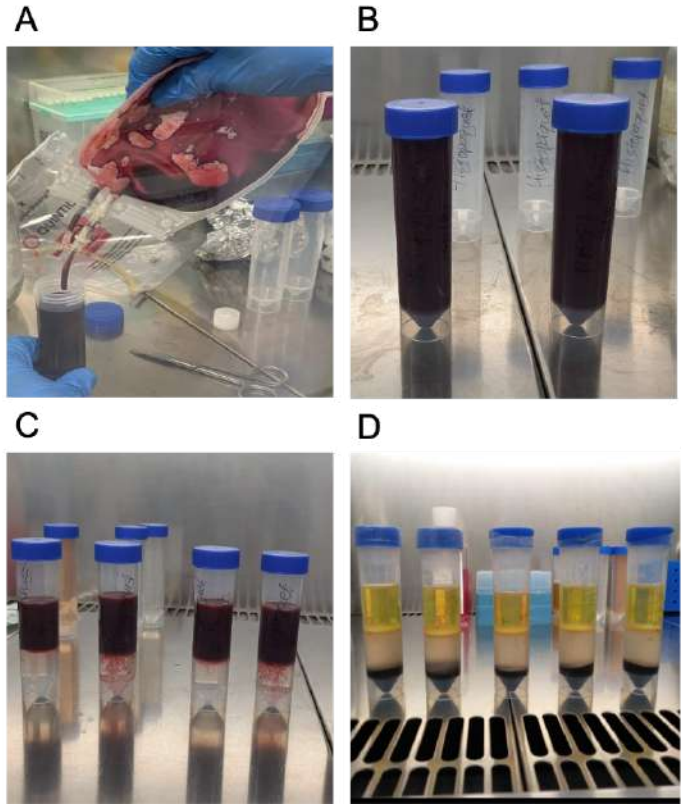


Figure 18. Visual representation of the sequential steps employed in the isolation of EPCs

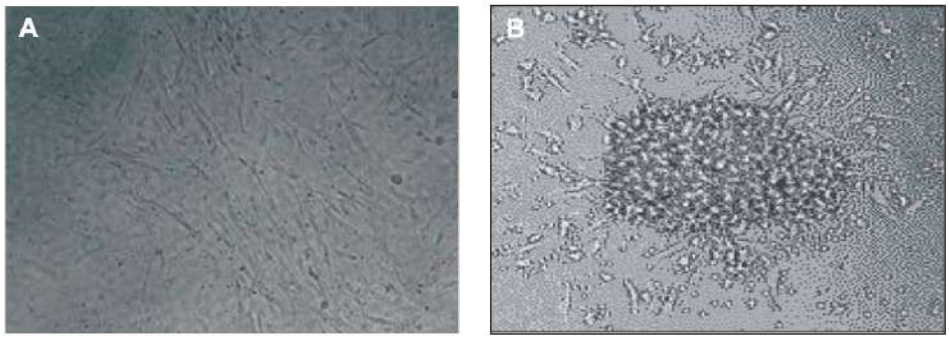


Figure 19. Microscopic image of EPCs isolated from human peripheral blood. Panel A shows a representative image of EPCs 24 hours after isolation. Panel B shows a typical EPC colony. Magnification for all panels: 10x.

3.2. EPCs characterization

3.2.1. Colonies characterization

Colonies were quantified and assessed at specific time points during the culture period. Initially, isolated peripheral blood mononuclear cells (PB MNCs) displayed a spherical morphology, and the emergence of colonies became evident after 2 days of culture. Between days 1 and 4, these colonies consisted of cells with monocytic characteristics, forming spherical clusters resembling balls (Figure 19 A). These clusters were distributed across the culture well, with a notable presence at both the peripheral and the central region. The appearance and development of early outgrowth cells (EOCs) exhibited variability among individual donors, spanning from days 5 to 10. EPCs were recognized as central clusters of rounded cells, encircled by sprouting spindle-shaped cells at the periphery, radiating outward from the core of the colony. Subsequent to 14 to 28 days of culture, the number of EOCs diminished (Figure 20A), while late outgrowth endothelial cells (OECs) increased in number. OECs were characterised by their cobblestone morphology (Figure 20A). Eventually, cells expanded and formed a monolayer.

During the initial week of culture, the number of clusters observed on day 2 amounted to 105 (±36.68), but this number decreased over time to 43 (±22.61) by day 7 (Figure 20B). Conversely, the size of the colonies increased over the course of the culture, with two prominent peaks observed at day 7 (50.11 ± 6.74 μm) and day 28 (54.04 ± 10.07 μm) (Figure 20C). Changes in colony diameter correlated with the formation and progression of EOCs and OECs colonies. On day 2, clusters exhibited an average diameter of 21.5 ± 8.16 μm, with a slight increase observed on day 4 (27.74 ± 7.11 μm) until well-defined EOCs, observed on day 7, displayed diameters exceeding 40 μm. As the culture progressed through days 14, 21, and 28, the number of colonies decreased to 35 (±13.03), 20 (±6.36), and 9 (±2.12), respectively (Figure 20B). Consequently, as EOCs expanded, colony sizes

decreased on days 14 and 21 (35.91 ± 8.35 µm and 34.24 ± 10.87 µm, respectively), and with the emergence of OECs, colony sizes increased, reaching their maximum diameter on day 28.

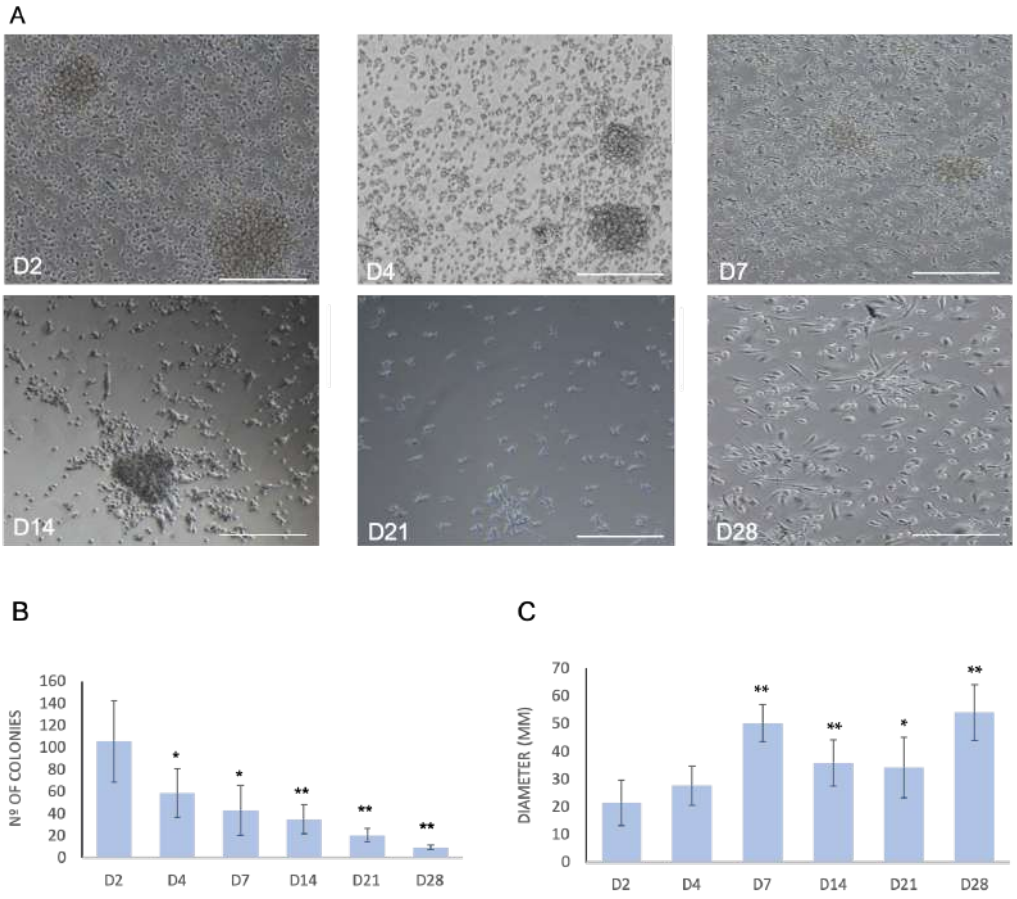


Figure 20. Colonies characterization. (**A**) Representative phase contrast microscope images of PBMN cells cultured during days 2, 4, 7, 14, 21 and 28 on collagen-I coated wells with EOC colonies at day 7 and OEC colonies at day 28. Magnification 10x. Scale bar = 50*µm* (**B**) Number of colonies formed in culture at different time periods (day 2, 4, 7, 14, 21, 28). Decrease in colony number was detected between days 2 to days 4 and forward. Statistically significant differences were found between days 2 to 4 (*p<0.05) and between day 2 to 7 and forward (**p<0.01). (**C**) Size (µm) of colonies at days 2, 4, 7, 14, 21 and 28. Statistically significant differences were found (*p<0.05) when comparing day 2 with day

21 and highly significant (**p<0.01) when comparing day 2 with day 7, 14, 28. No significant differences were found between days 2 and 4.

3.2.2. Cell characterization

Following a 4-week culture period, cells were subjected to immunostaining using various antibodies to identify specific surface markers commonly associated with endothelial progenitor cells (EPCs). EPCs consistently exhibited positive reactivity for established EPC surface antigens, including CD31, Ac-LDL uptake, and Ulex-lectin binding. Additionally, they expressed the stemness marker CD133 (see Figure 21).

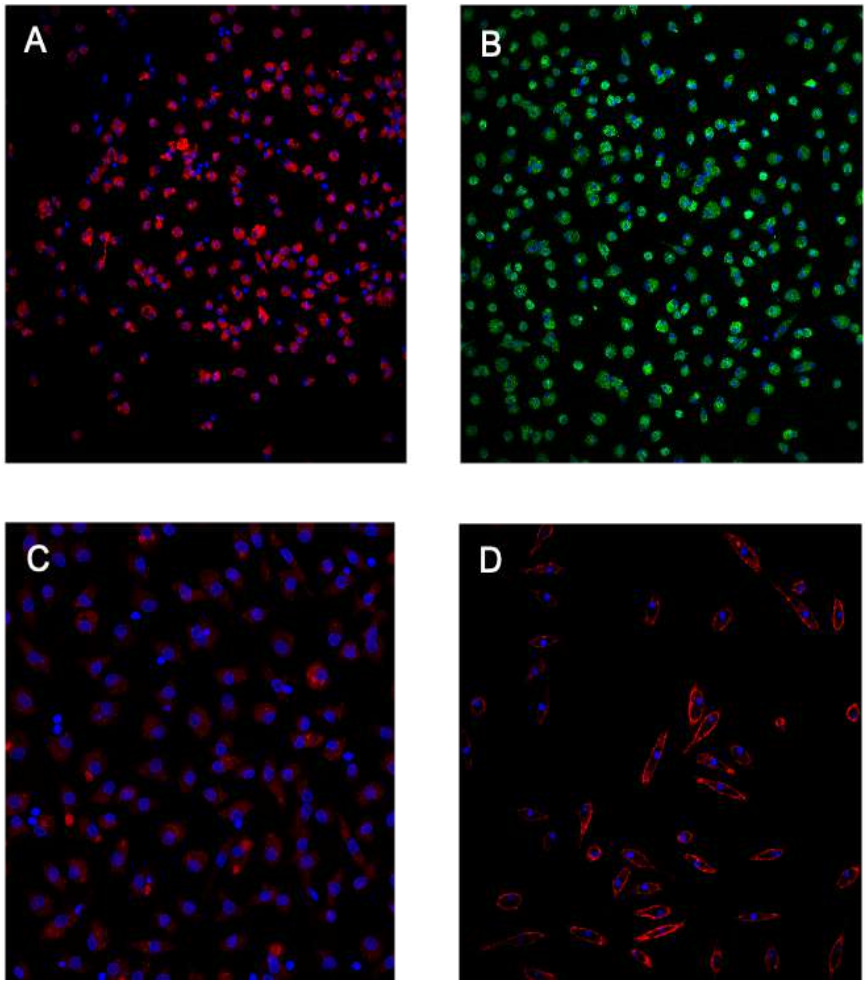


Figure 21. Immunostaining assay of isolated EPCs. The representative confocal microscope images depict EPCs cultured for 21 days and stained with various markers, including DAPI (blue, A), Di-AcLDL uptake (red, A), Ulex-lectin (green, B), CD31 (red, C), and CD133 (red, D). Magnification: 10x for A and B; 20x for C and D.

We evaluated the possible beneficial effect on cell growth of adding to the culture medium the protein silk protein Sericin. EPCs that were cultured in sericin-supplemented medium showed a slight increase, although significative, in cell proliferation, as can be appreciated in Figure 22.

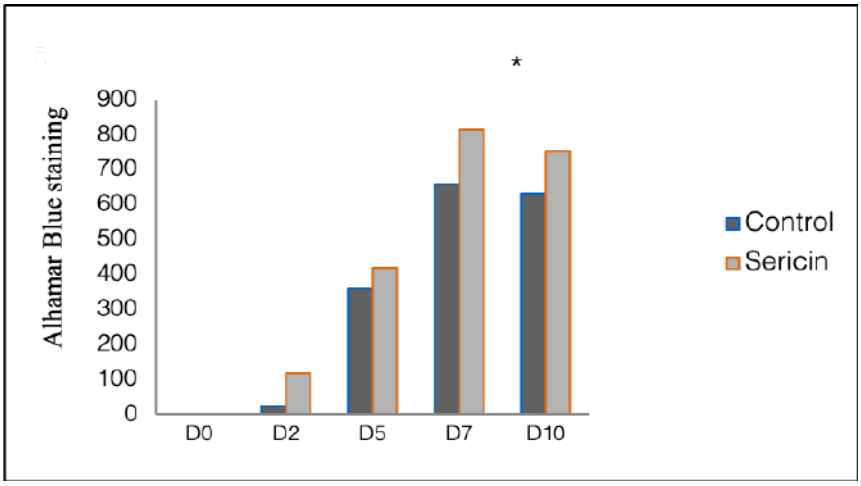
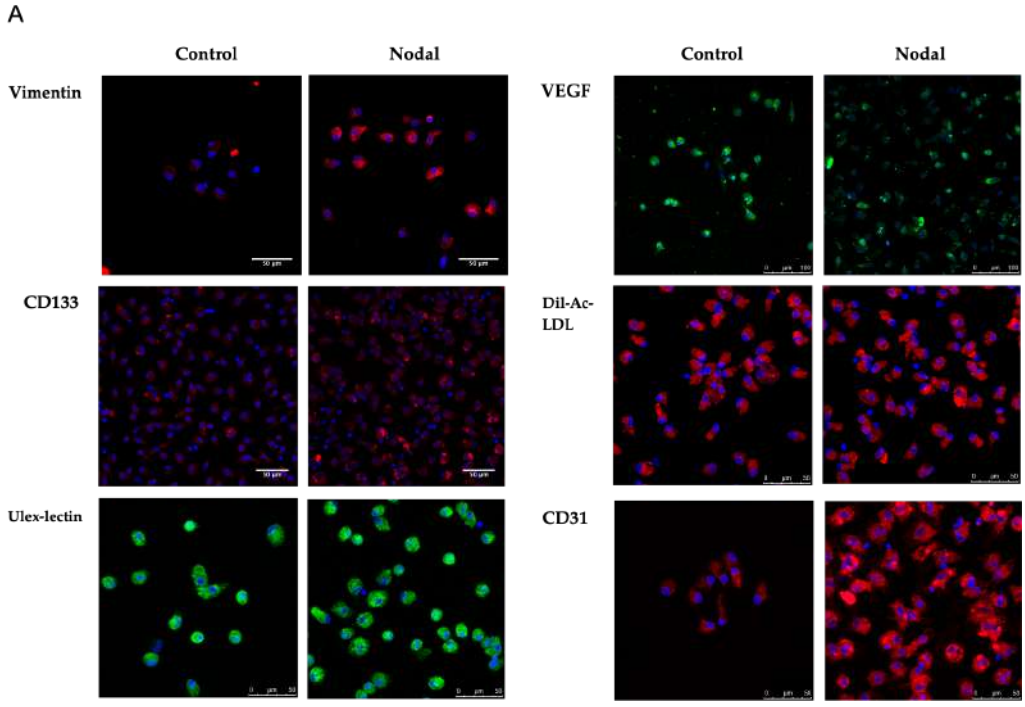


***Figure 22. Representative graphic of EPCs* cultured with sericin.** Alamar blue was used to quantify the proliferation and metabolic activity of EPCs at different time points during 10 days in culture.

3.3. Nodal induction in EPCs culture

Endothelial progenitor cells (EPCs), cultured for a duration of 2 weeks, underwent a 14-day treatment with Nodal. Subsequently, these cells were subjected to staining with antibodies targeting CD31, CD133, Vimentin, VEGF, as well as assays for Ulex-lectin binding and Di Acl-LDL uptake to assess potential alterations in cellular fluorescence intensity. Regarding Di-Acl-LDL, Ulex-lectin binding, and VEGF uptake, no statistically significant differences were observed in the corrected total cell fluorescence (CTCF) between the treated and untreated cells (Figure 23 A and B). However, noteworthy discrepancies in CTCF were evident concerning the expression of CD31, Vimentin, and CD133 (Figure 23 A and B). The observed increase in fluorescence intensity can be correlated with heightened expression of the aforementioned markers, thereby substantiating the proposition that Nodal exerts a positive influence on EPCs by enhancing the expression of canonical EPC markers and promoting their progenitor state.



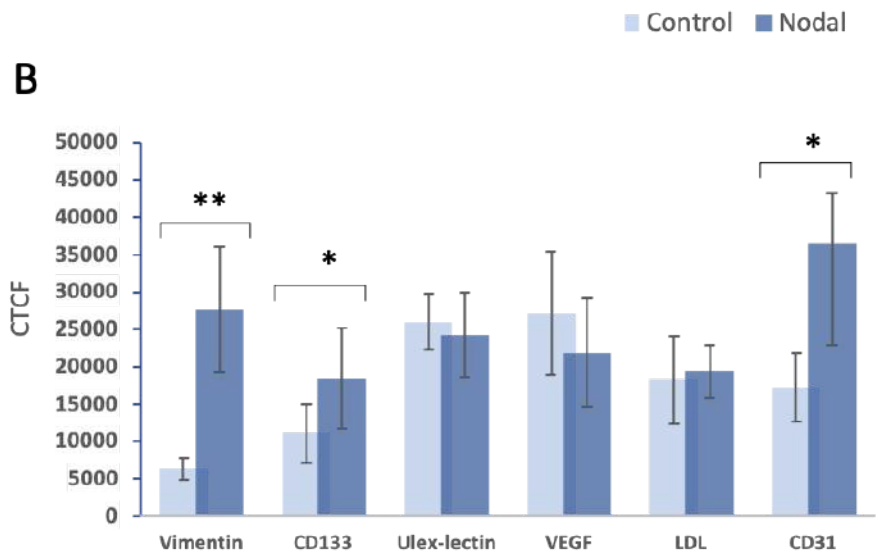


Figure 23. Nodal induction. (**A**) Representation from confocal microscopy of images of immunostained EPCs treated and untreated (control) with 100ng/ml of Nodal during 14 days. Magnification 10x. Scale bar = 50µm (**B**) Representation of cell fluorescence in Vimentin and CD133 in both control and Nodal treated cells. Y axis represents corrected total cell fluorescence (CTCF). After 14 days, a significant increase was observed in cell fluorescence for Vimentin (**$p<0.01$), CD133 (*$p<0.05$) and CD31 (*$p<0.05$).

3.4. Nodal enhances migration of EPCs

Cell migration was investigated using the wound healing assay in cultures of endothelial progenitor cells (EPCs) supplemented with Nodal. Two concentrations of Nodal, specifically 50 ng/ml and 100 ng/ml, were introduced into the culture medium and compared to a control group in which cells were maintained solely in the medium. Seven days following Nodal treatment, a defined wound was created using a p20 pipette tip, and the repopulation of cells within the generated gap was monitored at time intervals of 5, 9.5, and 20 hours, employing phase-contrast microscopy (see Figure 24). Notably, EPCs treated with 100 ng/ml of Nodal (Figure 24C) exhibited substantial cell migration, as evidenced by a coverage of

89.34 ± 6.00% of the cell-free area after 24 hours. In contrast, those treated with 50 ng/ml of Nodal (Figure 24B) displayed a lower migration rate, covering 62.81 ± 3.32% of the gap area. The control group exhibited the least migration, with only 29.85 ± 6.00% of the cell-free region populated by EPCs after the same duration. These findings underscore the influence of Nodal on the growth and migratory capacity of EPCs.

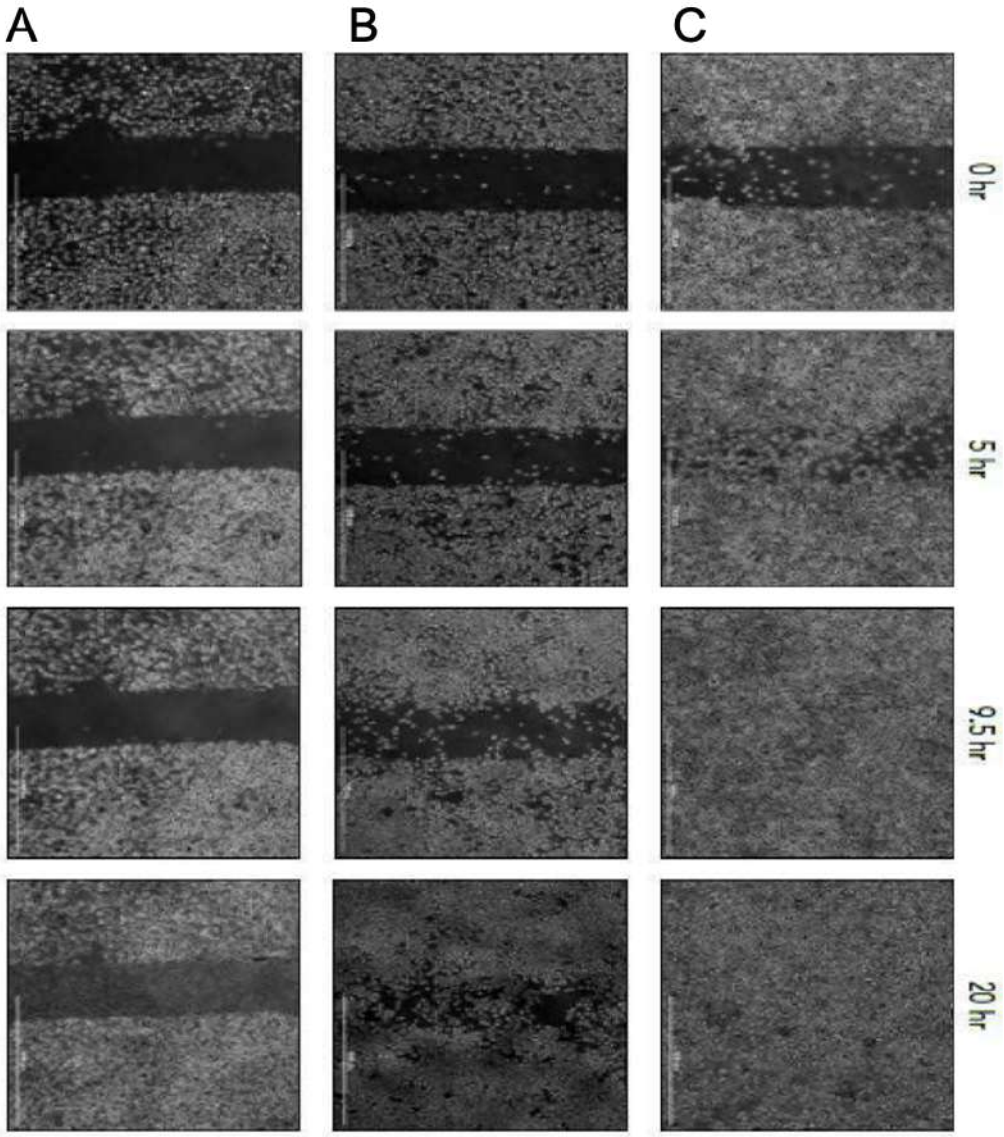


Figure 24. Representative images of EPCs monolayer after the wound healing assay.
Control cells (A); and EPCs treated with 500ng/ml of Nodal (B) or 100ng/ml of Nodal (C) .

RESULTS

Biological validation of the novel Bioreactor

3.3. Biological validation of the novel Bioreactor

3.3.1. Characterization of decellularized human arteries

Human arteries were decellularized following the protocol explained in the material and method section. To demonstrate the complete decellularization of the arteries, immunological and histological analyses were performed.

Figure 25 A and B show native arteries labelled with collagen (green); elastin (red) and Dapi (blue) where a correct distribution of the cells imbibed in the collagen fibres can be appreciated. Further, H&E staining (Fig 25 C and D) displayed a high cell density within the collagen fibres. On the other hand, decellularized arteries (Fig 25 E and F) did not show Dapi staining of the nuclei but a preserved ECM with intact collagen fibres. Further, H&E staining demonstrated the complete absence of cellular material after the decellularization process (stained pink) (Fig 25 G and H). Furthermore, it was observed that the ECM of the decellularized arteries was still intact with its defining elastin and collagen layers.

Thus, after the characterization, the efficacy of the decellularization process was confirmed.

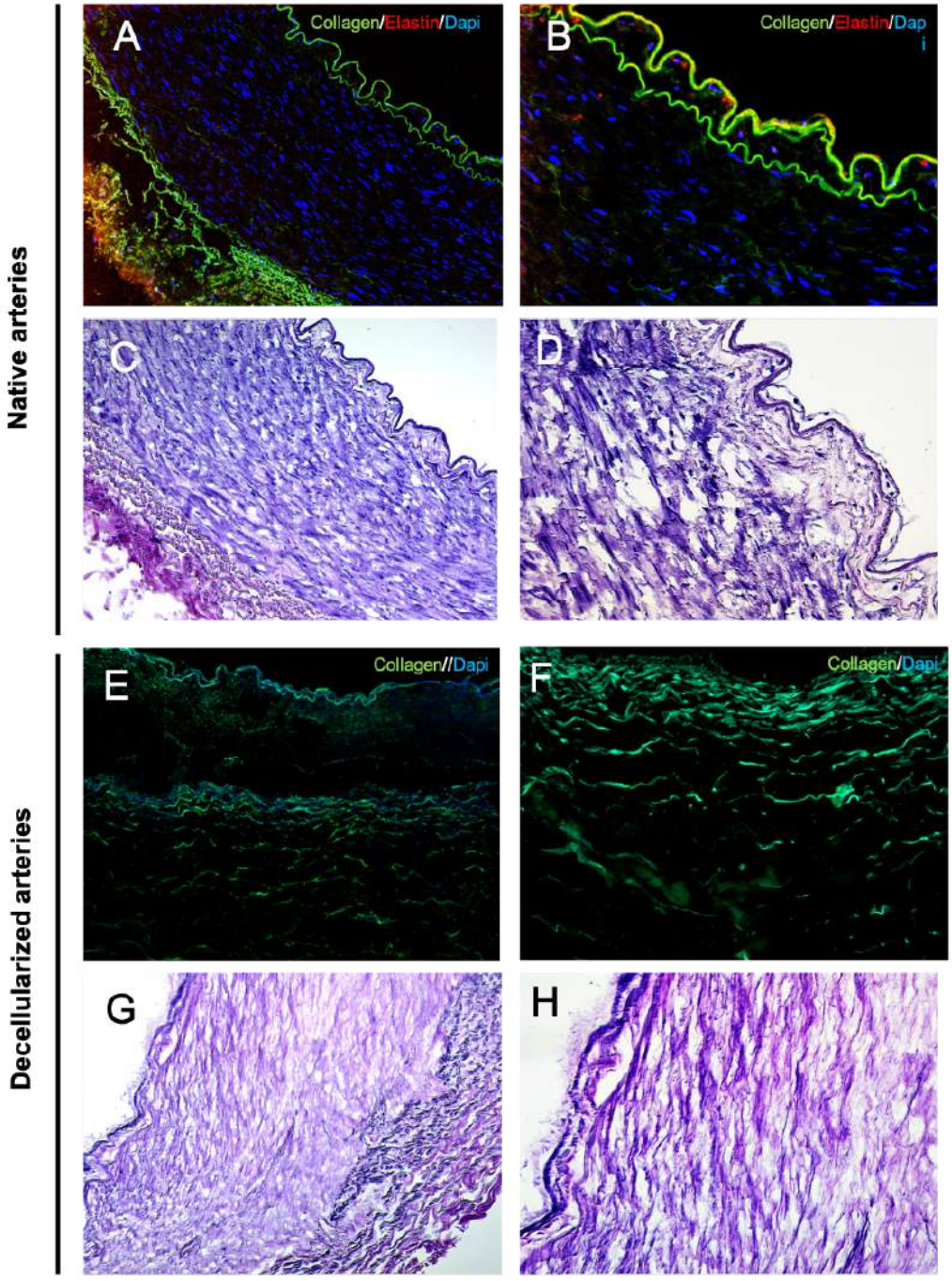


Figure 25. Representative images of native human arteries before the decellularization process (A-D) and after decellularization (E-H). Magnification 10x for A, C, E and G; and 20x for B, D, F and H.

3.3.2. Collagen coated vessels support endothelial cell growth

The ability of collagen to support the growth of endothelial cells was studied to be able to reconstruct the endothelial layer of the decellularized vessels.

The human arteries were coated with collagen. A homogeneous distribution of the coating on the internal membrane of the decellularized vessels was achieved by the dynamic system used of the bioreactor. The collagen was introduced via the bioreactor input, allowing the covering of the whole internal face of the vessel by the rotation of the artery within the chamber.

HUVECs seeded onto the internal membrane of decellularized and collagen-coated decellularized arteries are shown in Figure 26. Cell tracker green staining demonstrated the presence of live cells on the inner surface of the collagen-coated recellularized arteries.

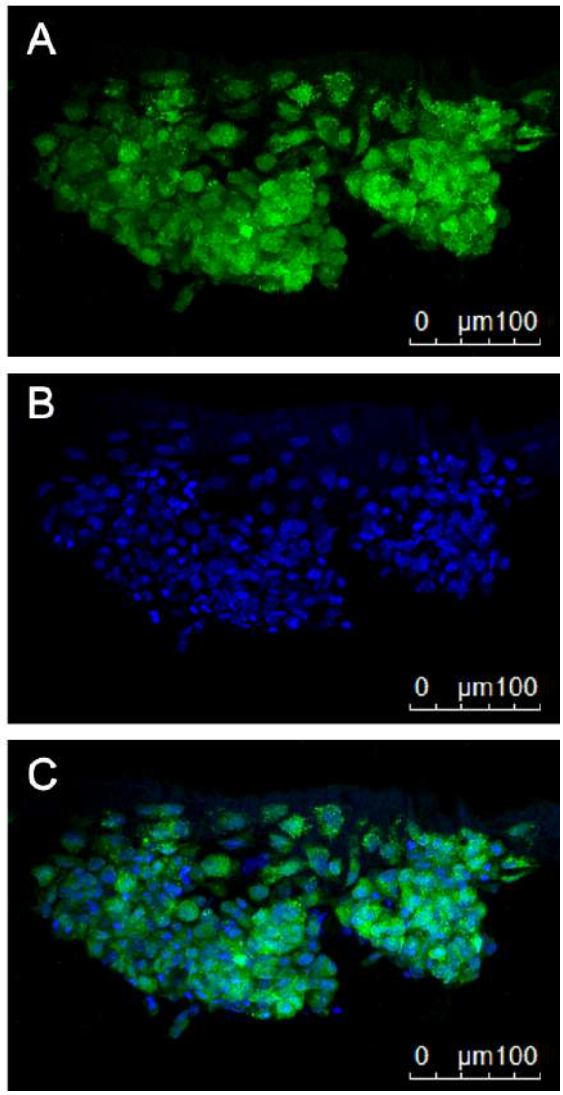


Figure 26. Representative imagines of HUVECs growth on native human vessel covered with collagen.

3.3.2. Recellularization of human vessels grown into the bioreactor

As mentioned above, the chosen recellularization method was the unilateral cell/cell mixture flow method, with programmed rotation every 12 hours for 24 hours, with a velocity of 50%, DELTA of 200, Caudal of 40m³/s and then

a 12-hour period of a continuous flow of the cell/cell medium mixture in and out of the artery with the pump calibrated at a velocity of 20% and pulses every 3 hours for another 12 hours.

The decellularized arteries were set up in the bioreactor, with 2 different injections of HUVEC or EPCs into the vessel; at the beginning of the procedure and 12 hours later. After 24 hours, the artery was removed from the bioreactor and the efficiency of the recellularization process assessed. Cell viability was analysed using the LIVE/DEAD viability kit. The recellularized arteries were then viewed with confocal microscopy which showed the presence of live cells (Figure 27 A and C). In addition, the presence of nuclei was notable in the immunofluorescence assays using DAPI (Figure 27 B and D). There was a noticeable cellular layer formed by the cells within the internal face of the artery, demonstrating the viability of the use of the bioreactor for recellularization of human arteries.

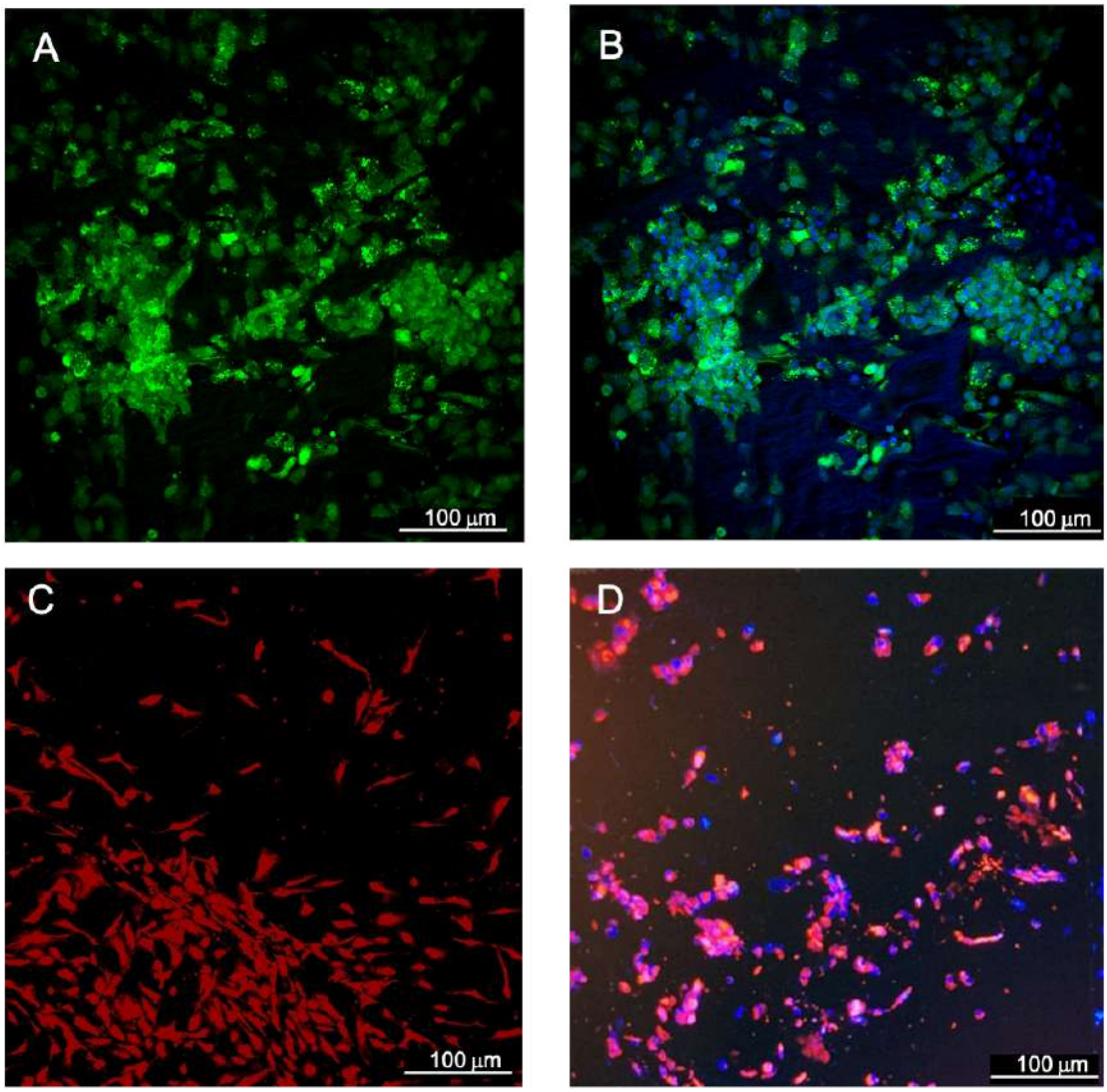


Figure 27. Representative images of native human arteries recellularized with HUVEC (A and B) and EPCs (C and D) growth into the bioreactor. Both cells were labelled with a viability kit, green for HUVEC and red for EPCS. B and D panels also show Dapi staining.

DISCUSSION

DISCUSSION

Vascular tissue engineering aims to develop grafts that mimic the structure and function of native tissue, enabling the replacement of damaged vessels. Decellularized arteries provide a unique approach by harnessing their inherent chemical and mechanical cues to facilitate cell adhesion, proliferation, and differentiation processes. However, there is an urgent requirement for an ex vivo model that accurately reflects human vascular physiology while sustaining the viability of recellularized vessels. In this sense, vascular bioreactors have emerged as promising options for the ex vivo cultivation of small vessels. Despite the great prospect of this technology in tissue bioengineering, the lack of standardised designs and inadequate protocols often hinder the reproducibility of experiments (157).

This work presents the development of a vascular regeneration model for obtaining vascular grafts from human decellularized vessels by the use of a novel bioreactor. Besides the creation of a bioreactor able to simulate the physiological conditions that vessels are exposed to in the human body, this study explored optimal culture conditions to preserve the characteristic features of EPCs. Furthermore, the potential application of these EPCs in the bioengineering of recellularized human arteries within the bioreactor was evaluated.

In TE approaches, cells with high plasticity and a strong proliferative capacity are highly desirable (158). Over the past decade, the identification of endothelial progenitor cells (EPCs) has provided an alternative strategy for developing vascular TE grafts. Since their isolation was first reported by Asahara et al. in 1997, EPCs have garnered significant attention. This discovery has challenged the notion that vasculogenesis is limited to embryonic development and has led to numerous studies highlighting the vast potential of EPCs (159,160). EPCs have demonstrated the ability to promote vascular repair

in ischemic tissues, facilitate neovascularization in various models of tissue injury, and play a significant role in tumor growth (10,11,12, 161-163).

In this study, EPCs were isolated from PB and characterised with several methods. First, the number, size, and morphology of the colonies formed after 28 days of culture were analysed. Additionally, the expression of cell surface markers (CD31, CD133, VEGF), the uptake of Di-Ac-LDL and the binding of Ulex-lectin were assessed. The results revealed that EOCs emerged as clusters of round cells with spindle-shaped cells expanding from the core during the first week of culture. Subsequently, after 2 to 4 weeks, OECs colonies developed and formed a monolayer with a characteristic cobblestone morphology. The initial cultures displayed a high number of round cell clusters, which decreased significantly over time. The colony diameter reached its maximum length at day 7 and 28, corresponding to the appearance and formation of EOCs and OECs, respectively. Notably, the colony size was significantly larger on days 7 and 28 compared to day 2. These observations align with previously reported findings in literature (164-167).

The characterization of EPCs has been a subject of controversy within the scientific community. Two distinct categories of EPCs have been established, namely H-EPCs and non-H-EPCs, referring to hematopoietic and non-hematopoietic origins, respectively (168). H-EPCs, derived from the bone marrow, are considered the primary source of EPCs due to their close developmental origin and expression of the hematopoietic stem marker CD34. These cells also express the immature marker CD133+ (168-170). On the other hand, non-H-EPCs originate from various sources such as adipose tissue, the blood vessel wall, liver, kidney, spleen, and intestine. Consequently, different subpopulations of non-H-EPCs have been identified, including circulating endothelial cells (CECs), circulating angiogenic cells (CACs), EOCs, colony-forming-unit endothelial cells (CFU-ECs), and OECs or endothelial colony-forming cells (ECFCs) (165, 170, 171).

There is currently no consensus regarding the specific markers for the identification of putative EPCs. Furthermore, the heterogeneity observed among the subpopulations of EPCs described in the literature, such as EOCs, OECs, CACs, and CECs, complicates the precise definition of these cells due to the shared expression of several surface markers between them and with other cell types including CD34, CD133, CD144, CD45, CD146, CXCR4, VEGF, KDR, vWF, eNOS, Ulex-lectin binding assays and Di Acl-LDL uptake (172,173). Nevertheless, certain distinguishing abilities have been observed that can aid in the identification of these cells. For instance, OECs demonstrate a high proliferative capacity and are capable of forming colonies when seeded at a density of 1 cell per well (174).

In our study, we observed positive expression of VEGF, Di-Acl-LDL uptake, and Ulex-lectin binding, which are also exhibited by mature endothelial cells. Additionally, the cells expressed the hematopoietic marker CD31 and the immature surface marker CD133, which are also found on some hematopoietic stem cells. When characterising EPC, all of the expressed markers mentioned are taken into consideration. Based on these results and the initial definition of EPCs as bone marrow CD34+ and/or CD133+ cells (161, 173), we confirm their presence in our cultures. EOCs are considered CD34+, CD133+, and KDR+ cells, capable of inducing neovascularization at ischemic sites in vivo (175), but they have low proliferation ability. On the other hand, Tura et al. (176) defined OECs as putative EPCs not derived from the bone marrow, confined to the CD34+, CD133-, and CD146+ cell fraction. Additionally, OECs have exhibited pro-angiogenic activity in vivo, they form perfused vessels, induce neoangiogenesis, and possess a highly proliferative capacity (174,175).

The isolation methodology and culture procedures play a critical role in the identification of EPC subpopulations (166, 177). However, the isolation and culture of EPCs still present challenges (178). To address these issues, various approaches have been developed, including surface coating with substances such as heparin-fibronectin, collagen II, 8g7, etc. (179-181), and the

introduction of growth factors in the culture medium to enhance cell proliferation and maintain the differentiated phenotype of EPCs. Among the growth factors, members of the transforming growth factor (TGF-β) superfamily have shown particular promise due to their crucial role in early embryonic development. For instance, Nodal, a TGF-β member, plays a critical role in the differentiation of the endoderm and mesoderm layers (182, 183). Nodal is also regulated by Notch signalling (specifically isoforms Notch-1 and Notch-4) and is involved in directing the differentiation of endothelial cells (ECs) into vascular networks. Previous in vitro studies on members of the TGF-β superfamily have demonstrated their importance in endothelial development and proliferation (184, 185). In this study, it was observed that Nodal-induced EPC cultures exhibited a significant increase in the expression of CD133 and Vimentin, indicating their potential role in promoting the differentiation and phenotype of EPCs. Nodal, a potent embryonic morphogen, is well-known for its role in maintaining pluripotency of stem cells and regulating mesodermal and endodermal induction (186). The maintenance of pluripotency is often associated with the increased expression of CD133. Additionally, Vimentin, a cytoskeletal filament, is known to be expressed in EPCs (187) and plays a crucial role in proper endothelial differentiation. Studies using knockout embryonic stem cells lacking Vimentin (Vim-/-) have shown that these cells develop smaller carotid arteries when cultured as embryoid bodies and exhibit spontaneous differentiation towards the endothelial lineage (188). Interestingly, aberrant expression of Nodal has been observed in several cancers, leading to increased VEGF secretion, enhanced cell proliferation, and promotion of angiogenesis (189). Therefore, it would be expected that our assay would demonstrate an increased fluorescence of VEGF. It is worth noting that signalling pathways involving VEGF, bone morphogenetic proteins (BMP), and Wnt have been shown to influence endothelial differentiation in vivo (188). These findings suggest that Nodal may play a role in maintaining the endothelial progenitor state of EPCs in vitro and contribute to the preservation

of their phenotype in long-term cultures. For future experiments, it would be interesting to evaluate the effects of combined cytokines, establish an optimal cytokine cocktail, or explore the use of Nodal/BMP chimeric ligands (190) to enhance EPC cultures.

On the other hand, the sericin protein derived from the silkworm cocoon has gained significant attention in recent years. This protein has demonstrated various beneficial properties, including enhancing cell attachment, exhibiting anticoagulating activity, and promoting cell growth in cultured cells. Due to these properties, sericin has been proposed as a potential substitute for foetal bovine serum (191,192). In this study, the promising properties of sericin in EPC cultures were also evaluated. It was found that sericin supplementation led to a significant increase in the proliferation rate of EPCs compared to the control group. These findings highlight the potential of sericin as a valuable component for enhancing EPC cultures and its therapeutic implications in promoting angiogenesis and tissue repair in ischemic conditions. A study conducted by Y. Song et al. further investigated the effects of sericin and revealed that it stimulates the expression of VEGF through ERK phosphorylation (193). Additionally, sericin was found to exert a protective effect on endothelial cells and cardiomyocytes by inhibiting the activation of caspase 3, thereby preventing apoptosis. The study also developed a sericin-genipin hydrogel, which when injected at ischemic sites in infarcted mouse models, demonstrated improved outcomes.

Autologous vascular transplants remain the gold standard treatment for patients with advanced cardiovascular disease. However, the limited availability and poor quality of donor sites pose a significant challenge (194). Currently, commonly used polymers for tissue-engineered vascular grafts (TEVGs) include polytetrafluoroethylene (PTFE), polyurethanes, and polyethylene terephthalate (PET). However, these polymers have several disadvantages, such as poor endothelialisation potential and difficulties in creating small-calibre arteries (195).

To address these challenges, an ideal scaffold for vascular tissue engineering should be composed of a biomaterial that promotes endothelialisation while inhibiting platelet attachment. Additionally, it should possess biomechanical properties similar to those of native vessels. TEVGs can be fabricated using synthetic materials, natural materials, or a combination of both, ensuring biocompatibility and minimising toxicity (196). A promising approach involves the use of decellularized arteries, which retain the natural chemical and mechanical cues necessary for cell adhesion, proliferation, and differentiation (194, 197).

Tissue decellularization is a process that involves the complete removal of cellular components from a tissue whilst preserving the ECM and the structural elements essential for blood vessel function, including collagen and elastin. This decellularized ECM serves as a natural scaffold with mechanical properties similar to those of the native tissue, making it an ideal substrate for tissue engineering applications (159). One of the significant advantages of decellularized matrices is their reduced immunogenicity compared to native tissue. By removing the majority of antigenic cellular components, the risk of immune rejection following transplantation is minimised. This feature makes decellularized matrices highly favourable for use as scaffolds in tissue transplantation procedures (160).

In this study, the use of a decellularized human artery seeded with EPCs in a rotary bioreactor is proposed as an effective strategy for developing a compatible vessel for small-diameter vascular grafts. For the process of tissue decellularization, it is crucial to ensure the careful removal of cellular materials, including nucleic acids and proteins, while preserving the integrity and functionality of the ECM components (198). Various techniques, such as chemical treatments, enzymatic digestion, and physical methods, can be employed to achieve efficient decellularization (199). The choice of method depends on the specific tissue type and desired properties of the decellularized matrix.

Among the various protocols described for the decellularization of vessels, enzyme-based techniques have been shown to cause ECM destruction (200). However, in the present study, a protocol previously reported by Seridan et al. was employed, which involved a brief trypsin treatment followed by treatment with Triton X-100 (201). The protocol used in this study aimed to effectively remove cellular components while preserving the integrity and structure of ECM. Trypsin treatment, which involves the enzymatic digestion of proteins, was applied for a short duration to facilitate cell removal. Subsequently, Triton X-100, a non-ionic detergent, was utilised to solubilize lipids and further aid in the decellularization process.

Microscopic techniques allowed the visualisation of the three distinct layers of the arterial wall (tunica intima, media, and adventitia). Comparing the microscopic images of native and decellularized samples, it can be observed that the protein composition of the ECM in these layers remains unchanged following the decellularization procedure. Previous research by Cai et al. demonstrated a decline in the biochemical properties of porcine carotid arteries after employing a 4-day decellularization protocol involving 1% Triton X-100 and 0.3% SDS solutions, resulting in reduced collagen content and a looser scaffold structure (93). The current study utilised classical histological staining techniques such as H&E and immunostainings, which confirmed the preservation of crucial ECM proteins like collagen. Furthermore, these findings align with previous studies utilising Triton X-100 as a detergent for artery decellularization, which similarly demonstrated effective preservation of the ECM (202, 203).

Following the decellularization process, we observed a successfully decellularized human vessel. Analysis of the ECM and basement membrane revealed no significant morphological alterations caused by the use of Triton. This observation holds significance as collagen contributes to vessel strength, while elastin provides distensibility and recoil properties (204). The preservation of ECM morphology in decellularized vessels is crucial for

maintaining the structural integrity and functionality necessary for their intended physiological role (180).

The decellularization of vascular scaffolds provides a promising approach for TE applications. However, achieving full endothelial cell repopulation on these scaffolds remains challenging due to complex microstructure, residual cellular remnants, and potential alterations in the ECM.

One of the main causes of vascular graft failure is the lack of a confluent endothelium due to the thrombogenicity of the graft (112, 205, 206). Acellular and decellularized TEVGs are commonly affected by thrombosis and thereby early failure (207). A variety of studies have focused on the issue of vascular regrowth after TEVG implantation due to its high complexity. To reduce the thrombogenicity of the TEVG, multiple solutions have been explored including the combination of synthetic and natural materials, the incorporation of anticoagulant molecules (e.g., heparin), or the functionalization with several drugs and factors (e.g., VEGF) to promote endothelialisation (104). Recently, a new magnetic approach to accelerate cell retention and improve cellular density which consists of the use of a magnetic hydrogel based on bacterial cellulose to target vascular cells has been proposed (208).

Nevertheless, the key to long-term thrombosis prevention is the formation of both the ECs and the SMCs layers. Both layers are critical to prevent intimal hyperplasia and for the functionality of native vessels (209). Moreover, the properties of the polymers must be carefully managed to avoid excessive migration and proliferation of cells which can lead to intimal or neointimal hyperplasia and stenosis, which typically reduces the patency of the vessel graft (210). From a clinical perspective, the proliferation and growth of endothelial cells need a precisely defined microenvironment that can be achieved through the incorporation of biomimetic components. In the case of ECs, creating a biomimetic microenvironment involves mimicking the natural physiological cues found in the native ECM to support their growth and function.

Surface modifications have been seen to improve cell attachment in Dacron grafts (211). Therefore, for the next experiments, collagen I coating was used as a possible way of achieving increased cellular repopulation. Indeed, collagen is used to improve the attachment and culture of EPCs in monolayer cultures as it is known that this extracellular matrix protein promotes cell attachment, spreading, and differentiation (212). Moreover, collagen is one of the primary structural components within the ECM of multiple tissues that demonstrates notable characteristics such as biodegradability, elevated biological functionality, and exceptional biocompatibility while maintaining a low inflammatory response (213).

To answer the question of whether endothelial cells may grow on decellularized matrices, HUVECs were manually seeded onto the endothelium surface of a decellularized artery coated with collagen I, cultured during 14 days, and finally, the viability was evaluated with CTG. Despite the fact that the native structure of the ECM provides an excellent environment for their attachment and the microstructure allows the setting of the cells and their proliferation, homogeneous repopulation of decellularized vascular scaffolds with endothelial cells was not achieved. These findings serve as a reminder that an incomplete recellularization and non-specific adhesion of non-EPCs remains a major concern as this can lead to problems such as restenosis, inflammation or hyperplasia (204).

Further research is needed to optimise cell seeding techniques and overcome these obstacles for the successful endothelialisation of decellularized vascular scaffolds. Confluent coverage of EPCs has shown proper function upon culturing them in a controlled environment and conditions (214). However, most studies just focus on biocompatibility or endothelialisation (215). In the present study, we intend to create a fully defined microenvironment and improve endothelialisation. Then, to face the challenge of orchestrating a controlled environment, a vascular bioreactor was designed.

The development of bioreactors holds great promise for advancing regenerative medicine and tissue engineering strategies, enabling the creation of functional and clinically relevant tissue constructs that can effectively integrate into the host tissue upon transplantation (216). Bioreactors play a major role in vascular tissue engineering, facilitating precise control over biochemical, physical, and mechanical environments, as well as monitoring the maturation of the tissues (215). It is of paramount importance that bioreactors are tailored and engineered for the targeted tissue, as an inadequately designed system may induce inappropriate cell signals, leading to impaired tissue proliferation and organisation, ultimately resulting in cellular dedifferentiation with an inappropriate structure (217).

In vascular tissue engineering, bioreactors can be applied in the development of vascular bypass grafts, heart valves, and stents (218). For vascular grafts, vessel bioreactors typically consist of fundamental modules which include a 3D tubular scaffold to support cellular growth, a mechanism for the exchange of media and nutrients essential for cell viability, a pumping module, and sensor arrays for continuous monitoring (219).

Vessel biology is difficult to replicate due to its intrinsic 3D structure with specific cell types at each layer, the multitude of physiological signalling factors, and parameters such as pressure, flow rate, and shear stress, which are critical but difficult to reproduce outside of the human body (215, 219).

In this study, we have developed a chamber bioreactor system designed to recellularize and sustain vessel constructs derived from human decellularized arteries. The bioreactor design incorporates a compartment to create a sterile microenvironment, allowing for the introduction of a small decellularized vessel and cells, as well as the medium for the culture maintenance and the control of the experiment.

For the development of novel bioreactor systems, characteristics such as ease of implementation, the reproducibility of the system, parameters, and culture conditions must all be taken into account. Furthermore, essential considerations

encompass easy monitoring capabilities, construct development, or the maintenance of sterility (215, 220). The overall dimensions and geometry of the bioreactor designed in this study are compatible with two culture flasks, enabling the use of a little amount of medium. In contrast with other bioreactors, the amount of medium needed is minimal making it cost effective (219). This chamber bioreactor system was configured to fit within a standard incubator ensuring the maintenance of consistent temperature and gas exchange conditions. These parameters are controlled due to the use of a filter cap on the culture chamber that simulates a culture flask and facilitates gas exchange.

There are other requirements such as accessibility and material compatibility that must also be considered when designing a bioreactor (220). Based on this idea, the bioreactor was designed to be compact, including a glass window on the chamber that allows complete access to the vessel while permitting its monitoring in real time.

The choice of materials must not exert detrimental effects on the tissue culture (221). In this regard, the selection of biocompatible or bioinert materials becomes imperative. Materials utilised should be amenable to sterilisation and conducive to operation in a humid environment at 37°C. The bioreactor system comprises a chamber reservoir made of glass and metal and sterilisation of the culture chamber can be achieved through autoclaving and alcohol, while non-sterilized components can be replaced after each use. Therefore, before implementation, the bioreactor system underwent sterilisation using an autoclave to ensure its cleanliness and suitability for the experimentation. No contamination was detected during the test period.

Besides minimising contamination, another benefit of the design is that it prioritises simplicity, enabling its easy assembly. Motors and pumps are compact, protected by a small box, able to work at 37°C, and capable of precise functioning with minimal forces. Most of the research in vascular bioreactors has been based on providing nutrient exchange to maintain cell viability (222). However, they lack in accurately reproducing essential physiological forces

such as mimicking arterial pressure flow rate, and shear stress in the same system (221). This deficiency often arises from the use of rudimentary pumping mechanisms. Consequently, there is a growing emphasis on advancing bioreactor development to incorporate the critical aspect of simulating physiological forces within the same system (215). This bioreactor system provides controlled flow conditions by mimicking physiological conditions, including mechanical stimulation and perfusion. The pump system of this study could be programmed at different pressures and experiments conducted under controlled conditions of continuous circulation. The bioreactor pump functions by being controlled by a pattern of forward/backward pumping and braking movements mimicking the actions of blood flow in the blood circulation. In this regard, there are a series of pump parameters that can be adjusted to establish the time between pulses and the force applied by the pump.

To assess the initial handling of the bioreactor and evaluate the sending parameters of the recellularization protocol, HUVEC cells were seeded into human arteries within the bioreactor. The results demonstrated that collagen enhanced superficial cell attachment, with cells exhibiting a homogeneous distribution. This observation suggests that the bioreactor employed in this study played a pivotal role in achieving homogeneous cell coverage.

The bioreactor was then validated by seeding the decellularized human vessels with human EPCs as the long-term target is to translate this scaffold clinically. Confocal images showed a high number of viable cells under the bioreactor culture conditions, together with a more homogeneous distribution when compared to the traditional culture protocol. Vascular bioreactors face the challenge of keeping the cells dispersed throughout the vessel given the inherent gravitational force's tendency to concentrate them to the bottom (223). The bioreactor was designed to be able to rotate the chamber controlling the speed and the direction of rotation in opposition to the force of gravity. With this system, it is possible to keep a medium and cell solution evenly distributed throughout the vessel until the cells adhere sufficiently to the surface and ensure

attachment to the tunica layer. The application of mechanical cues generated by a rotary bioreactor plays a pivotal role in the dynamic cell culture system established in this study (224, 225). This system aims to facilitate the homogeneous distribution of cells in the ECM scaffold, ultimately leading to the development of a functional vascular graft with physiological relevance.

Perfusion bioreactor systems, such as the rotary bioreactor employed in this study, are widely recognized for their significant benefits in vascular tissue engineering (219). In this study, the programmable rotation system was also built with silicon tubes and keys that work hand in hand with a peristaltic pump to recreate the dynamic physiological environment of blood vessels. These tubes are connected to the vessel inside the chamber and the media circulating through them ensures the provision of glucose, oxygen, and essential nutrients to the cells adhered to the intima layer, and at the same time facilitates the removal of metabolic waste products.

This system is able to expose vascular cells to controlled flow and shear stress conditions, which have been found to have a profound impact on various cellular responses, arterial formation, and maintenance, thereby enhancing the maturation and functionality of the engineered vascular tissue construct (219, 226).

The use of the designed bioreactor and the combination of human decellularized vessels and EPCs isolated from human peripheral blood cultured with Nodal holds great promise for advancing the field of vascular tissue engineering. Moreover, the use of this model offers the potential to contribute to the development of functional and durable vascular grafts for therapeutic applications or pharmaceutical research.

However, to gain a comprehensive understanding of the potential applications of this bioreactor, further investigations are needed. Longer in vitro studies are needed to explore the fate and behaviour of endothelial cells cultured on these human decellularized scaffolds by assessing aspects such as proliferation, differentiation, and functional characteristics.

Human cells were selected for this investigation owing to the ultimate goal of implementing this scaffold into clinical applications. Nevertheless, to facilitate the translation of this approach for animal experimentation and eventual clinical deployment, autologous human cells will need to be isolated and introduced into the distinct layers of the vessel.

Additionally, in vivo studies are crucial to evaluate the performance and biocompatibility of the seeded vessels in an animal model, evaluating aspects such as tissue integration, neovascularization, and long-term functionality.

CONCLUSIONS

CONCLUSIONS

1. The three main components of tissue engineering (cells, growth factors and scaffolds) can be manipulated in vitro to form replacement vessels suitable for vascular repair.

2. Pluripotent endothelial cells (EPCs) can be isolated from the patient's peripheral blood. EPC culture conditions improve by adding Nodal and Sericin to the medium.

3. The addition of Nodal to the EPC culture medium allows the maintenance of the endothelial progenitor cell phenotype in culture.

4. Arteries of human origin can be successfully decellularized and are suitable supports for use as a vascular graft.

5. A new bioreactor is presented with the characteristics of being small, suitable for installation in the culture hood and incubator; the possibility of autoclaving its components; possessing a controlled intravascular flow and a rotational chamber with medium supply.

6. The use of the bioreactor developed in this study permits the recellularization of decellularized human arteries, with EPCs obtained from peripheral blood.

7. The strategy to recellularize human arteries through the use of a bioreactor could help improve the production of vascular grafts.

BIBLIOGRAPHY

1. Roth, G.A., Johnson, C., Abajobir, A., Abd-Allah F., Abera S.F., Abyu, G., et al. (2017). Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol*. 70(1),1–25. doi: 10.1016/j.jacc.2017.04.052
2. Kubota, Y., Evenson, K.R., Maclehose, R.F., Roetker, NS., Joshu, CE., Folsom, A.R.ET et al. (2017). Physical Activity and Lifetime Risk of Cardiovascular Disease and Cancer. *Med Sci Sports* Exerc.49(8),1599–605. doi: 10.1249/MSS.0000000000001274
3. Tappia, P.S. and Blewett, H.. (2020). Nutrition and Cardiovascular Health. Vol. 21, International journal of molecular sciences. doi: 10.3390/ijms21072284
4. Libby, P. (2021). The changing landscape of atherosclerosis. *Nature* 592(7855),524–33. doi: 10.1038/s41586-021-03392-8
5. Herrington, W., Lacey, B., Sherliker, P., Armitage and J., Lewington, S. (2016). Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease .*Circ Res*. 118(4),535–46. doi: 10.1161/CIRCRESAHA.115.307611
6. Tibaut, M., Mekis, D. and Petrovic, D. (2017). Pathophysiology of Myocardial Infarction and Acute Management Strategies. *Cardiovasc Hematol Agents Med Chem.* 14(3),150–9. doi: 10.2174/1871525714666161216100553
7. Crea, F. and Libby, P. (2017). Acute Coronary Syndromes.The Way Forward From Mechanisms to Precision Treatment. *Circulation*. 136(12),1155–66. doi: 10.1161/CIRCULATIONAHA.117.029870
8. Martín-Timón, I., Sevillano-Collantes, C., Segura-Galindo, A. and Del Cañizo-Gómez, F.J. (2014). Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World J Diabetes*. 5(4),444–70. doi: 10.4239/wjd.v5.i4.444

9. Hsich, E.M. (2016). Matching the Market for Heart Transplantation. *Circ Heart Fail*. 9(4):e002679. doi: 10.1161/CIRCHEARTFAILURE.115.002679
10. Sekuli,c M., Zacharias, M. and Medalion, B. (2019). Ischemic Cardiomyopathy and Heart Failure. *Circ Heart Fail*. 12(6),e006006. doi: 10.1161/CIRCHEARTFAILURE.119.006006
11. Gaba, P, Gersh, B.J, Ali, Z.A, Moses, J.W and Stone, G.W.(2021). Complete versus incomplete coronary revascularization: definitions, assessment and outcomes. *Nat Rev Cardiol*. 18(3),155–68. doi: 10.1038/s41569-020-00457-5
12. Keulards, D.C.J., Karamasis, G .V., Alsanjari, O., Demandt, J.P.A., Van't Veer, M., Zelis, J.M., et al. (2020). Recovery of Absolute Coronary Blood Flow and Microvascular Resistance After Chronic Total Occlusion Percutaneous Coronary Intervention: An Exploratory Study. *J Am Heart Asso*c. 9(9),e015669. doi: 10.1161/JAHA.119.015669
13. Sánchez, P.F., Brey, E.M., and Briceño, J.C. (2018) Endothelialization mechanisms in vascular grafts. J Tissue Eng Regen Med. 12(11),2164–78. doi: 10.1002/term.2747
14. Melly, L., Torregrossa, G., Lee, T., Jansens, J.L. and Puskas J.D. (2018). Fifty years of coronary artery bypass grafting. *J Thorac Dis*.10(3),1960–7. doi: 10.21037/jtd.2018.02.43
15. Marx, SO., Totary-Jain, H. and Marks, A.R. (2011). Vascular smooth muscle cell proliferation in restenosis. *Circ Cardiovasc Interv*.4(1),104–11. doi: 10.1161/CIRCINTERVENTIONS.110.957332
16. Xie, Q., Huang, J., Zhu, K. and Chen, Q. (2021). Percutaneous coronary intervention versus coronary artery bypass grafting in patients with coronary heart disease and type 2 diabetes mellitus: Cumulative meta-analysis. *Clin Cardiol*. 44(7),899–906. doi: 10.1002/clc.23613
17. MacRae, J.M., Dipchand, C., Oliver, M., Moist, L., Lok, C., Clark, E., et al. (2016). Arteriovenous Access Failure, Stenosis, and Thrombosis. *Can J kidney Heal Dis*. 2016;3:2054358116669126. doi:

10.1177/2054358116669126

18. Carrabba, M. and Madeddu, P. (2018). Current Strategies for the Manufacture of Small Size Tissue Engineering Vascular Grafts. *Front Bioeng Biotechnol*. 6,41. doi: 10.3389/fbioe.2018.00041

19. Palmerini, T., Serruys, P., Kappetein, A.P., Genereux, P., Riva, D.D., Reggiani, L.B. et al. (2017). Clinical outcomes with percutaneous coronary revascularization vs coronary artery bypass grafting surgery in patients with unprotected left main coronary artery disease: A meta-analysis of 6 randomized trials and 4,686 patients. *Am Heart J.* 190,54-63. doi: 10.1016/j.ahj.2017.05.005.

20. Karacsonyi, J. and Brilakis, E.S. (2017). Bypass Graft Failure: Changing What We Can, Accepting What We Cannot Change, and Developing the Science to Know the Difference. Vol. 10, Circulation. *Cardiovascular interventions*. United States. doi: 10.1161/CIRCINTERVENTIONS.117.005212

21. Naegeli, KM., Kural, M.H., Li, Y., Wang, J., Hugentobler, E.A., Niklason, L.E., et al . (2022). Bioengineering Human Tissues and the Future of Vascular Replacement. *Circ Res*.131(1),109–26. doi: 10.1161/CIRCRESAHA.121.319984

22. Manubolu, V.S. and Budoff, M.J. (2022). Achieving coronary plaque regression: a decades-long battle against coronary artery disease. *Expert Rev Cardiovasc Ther*.20(4),291–305. doi: 10.1080/14779072.2022.2069559

23. Libby P. (2021) The changing landscape of atherosclerosis. *Nature.* 592(7855):524-533. doi: 10.1038/s41586-021-03392-8.

24. Tapia-Vieyra, J.V., Delgado-Coello, B. and Mas-Oliva, J. (2017). Atherosclerosis and Cancer; A Resemblance with Far-reaching Implications. *Arch Med Res*.48(1),12–26. doi: 10.1016/j.arcmed.2017.03.005

25. Shlofmitz, E., Iantorno, M. and Waksman, R. (2019) Restenosis of Drug-Eluting Stents:

A New Classification System Based on Disease Mechanism to Guide

Treatment and State-of-the-Art Review. *Circ Cardiovasc Interv.* 12(8):e007023.
doi: 10.1161/CIRCINTERVENTIONS.118.007023.

26. Dai , X., Wiernek, S., Evans, J.P. and Runge, M.S. (2016) Genetics of coronary artery disease and myocardial infarction. *World J Cardiol.* 26;8(1):1-23. doi: 10.4330/wjc.v8.i1.1.
27. Sajadian, M., Alizadeh, L., Ganjifard, M, Mardani, A., Ansari, M.A. and Falsoleiman H. (2018). Factors Affecting In-stent Restenosis in Patients Undergoing Percutaneous Coronary Angioplasty. *Galen Med J*. 7,e961. doi: 10.22086/gmj.v0i0.961
28. King, S.B., Iii. (2021). How Does Angioplasty Work? Vol. 24, *Cardiovascular revascularization medicine* : including molecular interventions. United States. p. 89–90. doi: 10.1016/j.carrev.2020.12.008
29. Denktas, A.E. (2010). Primary percutaneous coronary intervention in ST-segment elevation myocardial infarction is more effective than fibrinolysis at reducing the composite outcome of death or reinfarction after 8 years. *Evid Based Med*.15(5),145–6. doi: 10.1136/ebm1108
30. Sigwart, U., Puel, J., Mirkovitch, V., Joffre, F., Kappenberger, L. (1987). Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. *N Engl J Med*. 316(12),701–6. doi: 10.1056/NEJM198703193161201.
31. Iqbal, J., Gunn, J., Serruys, P.W. (2013). Coronary stents: historical development, current status and future directions. *Br Med Bull*.106:193–211. doi: 10.1093/bmb/ldt009
32. Cornelissen, A., Vogt, F.J. (2019). The effects of stenting on coronary endothelium from a molecular biological view: Time for improvement? *J Cell Mol Med*. 23(1),39–46. doi: 10.1111/jcmm.13936
33. Buccheri, D., Piraino, D., Andolina, G., Cortese, B. (2016). Understanding and managing in-stent restenosis: a review of clinical data, from pathogenesis to treatment. *J Thorac Dis.*8(10),E1150–62. doi:

10.21037/jtd.2016.10.93

34. Drozd, J., Wójcik, J., Małek, R., Korona, B., Zapolski, T., Wysokiński, A., et al. (2010). Acute coronary syndrome - a frequent clinical manifestation of bare metal in-stent restenosis. *Kardiol Pol*.68(6),637–45.

35. Iwata, H., Osborn, E.A., Ughi, G.J., Murakami, K., Goettsch, C., Hutcheson, J.D., et al. (2021). Highly selective pparα (Peroxisome proliferator-activated receptor α) agonist pemafibrate inhibits stent inflammation and restenosis assessed by multimodality molecular-microstructural imaging. *J Am Heart Assoc.* 10(20). doi: 10.1161/JAHA.121.020834

36. Bønaa, K.H., Mannsverk, J., Wiseth, R., Aaberge, L., Myreng, Y., Nygård, O., et al. (2016). Drug-Eluting or Bare-Metal Stents for Coronary Artery Disease. *N Engl J Med.*375(13),1242–52. doi: 10.1056/NEJMoa1607991

37. Brugaletta, S., Gomez-Lara, J., Ortega-Paz, L., Jimenez-Diaz, V., Jimenez ,M., Jiménez-Quevedo, P., et al. (2021). 10-Year Follow-Up of Patients With Everolimus-Eluting Versus Bare-Metal Stents After ST-Segment Elevation Myocardial Infarction. *J Am Coll Cardiol*.77(9),1165–78. doi: 10.1016/j.jacc.2020.12.059

38. Brener, S.J., Kereiakes, D.J., Simonton ,C.A., Rizvi, A., Newman, W., Mastali, K., et al. (2013). Everolimus-eluting stents in patients undergoing percutaneous coronary intervention: final 3-year results of the Clinical Evaluation of the XIENCE V Everolimus Eluting Coronary Stent System in the Treatment of Subjects With de Novo Native Coronary Artery. *Am Heart J*. 166(6),1035–42. doi: 10.1016/j.ahj.2013.08.030

39. Sakurai, R., Inajima, T., Kaneda ,H., Nagai, R. and Hashimoto, H. (2013). Sirolimus-eluting stents reduce long-term mortality compared with bare metal stents in ST-segment elevation myocardial infarction: a meta-analysis of randomized controlled trials. *Int J Cardiol.* 2013 Jul,167(1):162–7. doi: 10.1016/j.ijcard

40. Lasch, M., Caballero Martinez, A., Kumaraswami, K., Ishikawa-Ankerhold ,H., Meister, S., Deindl, E., et al. (2020). Contribution of the Potassium

Channels K(V)1.3 and K(Ca)3.1 to Smooth Muscle Cell Proliferation in Growing Collateral Arteries. *Cells*. 9(4). doi: 10.3390/cells9040913.

41. Ye, M., Guo, X., Wang, H., Wang ,Y., Qian, X., Deng, H., et al. (2020). Mutual regulation between β-TRCP mediated REST protein degradation and Kv1.3 expression controls vascular smooth muscle cell phenotype switch. *Atherosclerosis*. 313,102–10 doi: 10.1016/j.atherosclerosis.2020.08.018
42. Bobi, J., Garabito, M., Solanes, Nú., Cidad, P., Ramos-Pérez, V., Ponce, A., et al. (2020). Kv1.3 blockade inhibits proliferation of vascular smooth muscle cells in vitro and intimal hyperplasia in vivo. *Transl Res.* 224,40–54. doi: 10.1016/j.trsl.2020.06.002
43. Radke, P.W., Weber, C., Kaiser, A., Schober, A. and Hoffmann, R. (2004). Dexamethasone and restenosis after coronary stent implantation: new indication for an old drug? *Curr Pharm Des.*10(4),349–55. doi: 10.2174/1381612043453324.
44. Braga, S.F., Neves, J.R., Ferreira, J., Carrilho ,C., Simões ,J.C., Mesquita ,A., et al. (2019). Neointimal Hyperplasia. *Rev Port Cir cardio-toracica e Vasc orgao Of da Soc Port Cir Cardio-Toracica e Vasc*. 26(3),213–7.
45. Lincoff, A.M., Furst, J.G., Ellis, S.G., Tuch, R.J. and Topol, E.J. (1997). Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J Am Coll Cardiol*. 29(4),808–16. doi: 10.1016/s0735-1097(96)00584-0
46. Strecker, E.P., Gabelmann ,A., Boos, I., Lucas, C., Xu, Z., Haberstroh, J., et al. (1998). Effect on intimal hyperplasia of dexamethasone released from coated metal stents compared with non-coated stents in canine femoral arteries. *Cardiovasc Intervent Radiol*.21(6),487–96. doi: 10.1007/s002709900309
47. Park ,Y.M., Han, S.H., Lee, K., Suh, S.Y., Oh, P.C., Chung ,W.J., et al. (2013). Dexamethasone-eluting stents had sustained favorable ischemic driven target lesion revascularization rates over 5 years: a randomized controlled prospective study. Vol. 165, *International journal of cardiology.*

Netherlands.p. 359–62. doi: 10.1016/j.ijcard.2012.08.033

48. Pesarini, G., Ferrero, V., Tomai, F., Paloscia, L., De Cesare, N., Tamburino, C., et al. (2009). Steroid-eluting stents in patients with acute coronary syndromes. Angiographic results of DESIRE: Dexamethasone-Eluting Stent Italian REgistry. *J Invasive Cardiol*. 21(3),86–91.

49. van Beusekom, H.M.M., Saia ,F., Zindler, J.D., Lemos ,P.A., Swager-Ten Hoor ,S.L., van Leeuwen, M.A.H., et al. (2007). Drug-eluting stents show delayed healing: paclitaxel more pronounced than sirolimus. *Eur Heart J*. 28(8),974–9. doi: 10.1093/eurheartj/ehm064

50. Taniwaki ,M., Radu, M.D., Zaugg, S., Amabile, N., Garcia-Garcia ,H.M., Yamaji, K., et al. (2016). Mechanisms of Very Late Drug-Eluting Stent Thrombosis Assessed by Optical Coherence Tomography. *Circulation*. 133(7),650–60. doi: 10.1161/CIRCULATIONAHA.115.019071

51. Wang, X., Chen, X., Sun, W., Tian, T., Zhou, S., Zhang, Z., et al. (2019). Very Late Stent Thrombosis in Drug-Eluting Stents New Observations and Clinical Implications. *Cardiol Rev*. 27(6),279–85. doi: 10.1097/CRD.0000000000000283

52. Zhang, B., Zheng, B., Wang, X., Shi, Q., Jia, J., Huo, Y., et al. (2017). Polymer-free dual drug-eluting stents evaluated in a porcine model. *BMC Cardiovasc Disord*. 17(1),222. doi: 10.1186/s12872-017-0654-7.

53. Cha, J.J., Kim, G.C., Hur, S.H., Bae, J.H., Choi, J.W., Jin, D.K., et al. (2020). Efficacy and Safety of Dual-Drug-Eluting Stents for de Novo Coronary Lesions in South Korea-The Effect Trial. *J Clin Med*. 10(1). doi: 10.3390/jcm10010069

54. Huang, Y., Venkatraman, S.S., Boey, F.Y.C., Lahti, E.M., Umashankar, P.R., Mohanty, M., et al. (2010). In vitro and in vivo performance of a dual drug-eluting stent (DDES). *Biomaterials*.31(15),4382–91. doi: 10.1016/j.biomaterials.2010.01.147.

55. Lee, S.Y., Bae, I.H., Sung Park, D., Jang. E.J., Shim, J.W., Lim, K.S., et al. (2016). Prednisolone- and sirolimus-eluting stent: Anti-inflammatory

approach for inhibiting in-stent restenosis. *J Biomater Appl*.31(1),36–44. doi: 10.1177/0885328216630498

56. Byrne, R.A., Mehilli, J., Iijima, R., Schulz, S., Pache, J., Seyfarth, M., et al. (2009). A polymer-free dual drug-eluting stent in patients with coronary artery disease: a randomized trial vs. polymer-based drug-eluting stents. *Eur Heart J*. 30(8),923–31. doi: 10.1093/eurheartj/ehp044
57. Erne, P., Schier, M. and Resink, T.J. (2006). The road to bioabsorbable stents: reaching clinical reality? *Cardiovasc Intervent Radiol*. 29(1),11–6. doi: 10.1007/s00270-004-0341-9
58. Jinnouchi, H., Torii, S., Sakamoto, A., Kolodgie, F.D., Virmani, R., Finn, A.V., et al. (2019). Fully bioresorbable vascular scaffolds: lessons learned and future directions. *Nat Rev Cardiol.* 16(5),286–304. doi: 10.1038/s41569-018-0124-7
59. Beshchasna, N., Saqib, M., Kraskiewicz, H., Wasyluk, Ł., Kuzmin, O., Duta, O.C., et al. (2020). Recent Advances in Manufacturing Innovative Stents. *Pharmaceutics*. 12(4). doi: 10.3390/pharmaceutics12040349.
60. Borhani, S., Hassanajili, S., Ahmadi Tafti, S.H., Rabbani, S. (2018) Cardiovascular stents: overview, evolution, and next generation. Prog Biomater. 7(3):175-205. doi: 10.1007/s40204-018-0097-y.
61. Zhang, Z.Q., Yang, Y.X, Li, J.A., Zeng, R.C., Guan. S.K. (2021). Advances in coatings on magnesium alloys for cardiovascular stents - A review. *Bioact Mater*. 6(12),4729–57. doi: 10.1016/j.bioactmat.2021.04.044
62. Joner, M., Ruppelt, P., Zumstein, P., Lapointe-Corriveau, C., Leclerc, G., Bulin, A., et al. (2018). Preclinical evaluation of degradation kinetics and elemental mapping of first- and second-generation bioresorbable magnesium scaffolds. *EuroIntervention J Eur Collab with Work Gr Interv Cardiol Eur Soc Cardio*l. 14(9),e1040–8. doi: 10.4244/EIJ-D-17-00708
63. Gu, X., Zheng, Y., Cheng, Y., Zhong, S., Xi, T. (2009). In vitro corrosion and biocompatibility of binary magnesium alloys. *Biomaterials*. 30(4),484–98.

doi: 10.1016/j.biomaterials.2008.10.021

64. Bowen, P.K., Shearier, E.R., Zhao, S., Guillory, R.J 2nd., Zhao, F., Goldman, J., et al. (2019). Biodegradable Metals for Cardiovascular Stents: from Clinical Concerns to Recent Zn-Alloys. *Adv Healthc Mater*. 5(10),1121–40. doi: 10.1002/adhm.201501019.

65. McKavanagh, P., Zawadowski, G., Ahmed, N., Kutryk, M. (2018) The evolution of coronary stents. *Expert Rev Cardiovasc Ther*.16(3),219–28. doi: 10.1080/14779072.2018.1435274.

66. Bowen, P.K., Drelich, J., Goldman, J. (2013). Zinc exhibits ideal physiological corrosion behavior for bioabsorbable stents. *Adv Mater*. 25(18),2577–82. doi: 10.1002/adma.201300226

67. Xiao, J., Feng, G., Kang, G., Lan, Z., Liao, T., Kislauskis, E. et al. (2015). 6-Month Follow-Up of a Novel Biodegradable Drug-Eluting Stent Composed of Poly-L-Lactic Acid and Amorphous Calcium Phosphate Nanoparticles in Porcine Coronary Artery. *J Biomed Nanotechnol*. 11(10),1819–25. doi: 10.1166/jbn.2015.2102

68. Tamai ,H., Igaki, K., Kyo, E., Kosuga, K., Kawashima ,A., Matsui, S., et al. (2000). Initial and 6-month results of biodegradable poly-l-lactic acid coronary stents in humans. *Circulation*. 102(4),399–404 doi: 10.1161/01.cir.102.4.399

69. Williams, S.F., Rizk, S., Martin, D.P. (2013). Poly-4-hydroxybutyrate (P4HB): a new generation of resorbable medical devices for tissue repair and regeneration. *Biomed Tech (Berl)*. 58(5),439–52. doi: 10.1515/bmt-2013-0009

70. Zhao, K., Li, D., Shi, C., Ma, X., Rong, G., Kang, H., et al. (2016). Biodegradable Polymeric Nanoparticles as the Delivery Carrier for Drug. *Curr Drug Deliv*.13(4),494–9. doi: 10.2174/1567201813041605210046O9

71. Wang, Y., Li, G., Yang, L., Luo, R., Guo, G., (2022). Development of Innovative Biomaterials and Devices for the Treatment of Cardiovascular Diseases. *Adv Mater.* e2201971. doi: 10.1002/adma.202201971

72. Charpentier, E., Barna, A., Guillevin. L., Juliard, J.M. (2015). Fully bioresorbable drug-eluting coronary scaffolds: A review. *Arch Cardiovasc Dis*.108(6–7),385–97. doi: 10.1016/j.acvd.2015.03.009

73. Bil, J., Gil, R.J. (2016). Bioresorbable vascular scaffolds-what does the future bring? *J Thorac Dis*. 8(8),E741-5. doi: 10.21037/jtd.2016.05.80.

74. Waksman, R. (2017). A new generation of drug-eluting stents: Indications and outcomes of bioresorbable vascular scaffolds. *Cleve Clin J Med.* 84(12 Suppl 4),e20–4. doi: 10.3949/ccjm.84.s4.05

75. Grabow ,N., Schlun, M., Sternberg, K., Hakansson, N., Kramer, S., Schmitz, K.P.(2005). Mechanical properties of laser cut poly(L-lactide) micro-specimens: implications for stent design, manufacture, and sterilization. *J Biomech Eng* 127(1),25–31. doi: 10.1115/1.1835349.

76. Weir, N.A, Buchanan, F.J, Orr, J.F, Farrar, D.F, Boyd, A.(2004). Processing, annealing and sterilisation of poly-L-lactide. *Biomaterials.* 2004 25(18),3939–49. doi: 10.1016/j.biomaterials.2003.10.076

77. Nogic, J., McCormick, L.M., Francis, R., Nerlekar, N., Jaworski, C., West, N.E.J., Brown, A.J. (2018). Novel bioabsorbable polymer and polymer-free metallic drug-eluting stents. *J Cardiol.* 71(5),435-443. doi: 10.1016/j.jjcc.2017.12.007

78. Ellis, S.G., Kereiakes, D.J, Metzger, D.C, Caputo, R.P., Rizik D.G. Teirstein P.S., et al. ABSORB III Investigators. (2015). Everolimus-eluting bioresorbable scaffolds for coronary artery disease. *N Engl J Med.* 373(20),1905-1915. doi: 10.1056/NEJMoa1509038

79. Mridha, N., Subhaharan, D., Niranjan, S., Rashid, M.K., Psaltis, P. and Singh, K. (2019). A meta-analysis of randomized controlled trials to compare long-term clinical outcomes of bioabsorbable polymer and durable polymer drug-eluting stents. *Eur Heart J Qual Care Clin Outcomes.* 1-5(2),105-113. doi: 10.1093/ehjqcco/qcy036.

80. Tamburino, C., Capranzano, P., Gori, T., Latib, A., Lesiak, M., Nef, H., et al. (2016). 1-Year outcomes of everolimus-eluting bioresorbable scaffolds

versus everolimus-eluting stents: a propensity-matched comparison of the GHOST-EU and XIENCE V USA registries.*JACC Cardiovasc Interv.* 9(5),440-449. doi: 10.1016/j.jcin.2015.10.042.

81. Seo, J., Ahn, J.M., Hong, S.J, Kang, D.Y, Hong, S.J, Her, A.Y., et al. (2020). Bioresorbable Vascular Scaffolds Versus Drug-Eluting Stents for Diffuse Long Coronary Narrowings. *Am J Cardiol.* 125(11),1624-1630. doi: 10.1016/j.amjcard.2020.02.031
82. Caiazzo G, Kilic ID, Fabris E, Serdoz R, Mattesini A, Foin N, et al. (2015). Absorb bioresorbable vascular scaffold: What have we learned after 5 years of clinical experience? *Int J Cardiol.*201,129-36 doi: 10.1016/j.ijcard.2015.07.101
83. Serruys, P.W., Chevalier, B., Dudek, D., Cequier, A., Carrié, D., Iniguez, A., et al. (2015). A bioresorbable everolimus-eluting scaffold versus a metallic everolimus-eluting stent for ischaemic heart disease caused by de-novo native coronary artery lesions (ABSORB II): an interim 1-year analysis of clinical and procedural secondary outcomes from a randomised controlled trial. *Lancet.* 385(9962),43-54 doi: 10.1016/S0140-6736(14)61455-0
84. Kozuma, K., Tanabe, K., Hamazaki, Y., Okamura, T., Ando, J., Ikari, Y., et al. ABSORB Japan Investigators. (2020). Long-Term Outcomes of Absorb Bioresorbable Vascular Scaffold vs. Everolimus-Eluting Metallic Stent - A Randomized Comparison Through 5 Years in Japan. *Circ J.* 84(5),733-741. doi: 10.1253/circj.CJ-19-1184
85. Sharifkazemi, M., Zafar, H., Sharif, R. Wyns, W., and Sharif, F. (2022). Do Bioresorbable Vascular Scaffold Stents Reduce Very Late Stent Thrombosis? A Systematic Review. *SN Compr. Clin.* Med. 4, 64 https://doi.org/10.1007/s42399-022-01147-8
86. Karimi, M., Zare, H., Nik, A.B., Yazdani, N., Hamrang, M., Mohamed, E., et al. (2016). Nanotechnology in diagnosis and treatment of coronary artery disease. *Nanomedicine (Lond).* 11(5),513–30. doi: 10.2217/nnm.16.3
87. Hu, B., Boakye-Yiadom, K.O., Yu, W., Yuan, Z.W., Ho, W., Xu, X., et al.

(2020). Nanomedicine Approaches for Advanced Diagnosis and Treatment of Atherosclerosis and Related Ischemic Diseases. *Adv Healthc Mater.* (16),e2000336 doi: 10.1002/adhm.202000336

88. Senti, M.E., de Jongh, C.A., Dijkxhoorn, K., Verhoef, J.J.F., Szebeni, J., Storm, G., et al. (2022). Anti-PEG antibodies compromise the integrity of PEGylated lipid-based nanoparticles via complement. Vol. 341, *Journal of Controlled Release*. p. 475–86. doi: 10.1016/j.jconrel.2021.11.042
89. Pham, L.M., Poudel, K., Phung, C.D., Nguyen, T.T., Pandit, M., Nguyen, H.T, et al. (2021) Preparation and evaluation of dabrafenib-loaded, CD47-conjugated human serum albumin-based nanoconstructs for chemoimmunomodulation. *Colloids Surfaces B Biointerfaces*.112093. doi: 10.1016/j.colsurfb.2021.112093.
90. Cai, J.X., Liu. J.H., Wu, J.Y., Li, Y.J., Qiu, X.H., Xu, W.J., et al. (2022) Hybrid Cell Membrane-Functionalized Biomimetic Nanoparticles for Targeted Therapy of Osteosarcoma. *Int J Nanomedicine*. Volume 17.837–54. doi: 10.2147/IJN.S346685
91. Inamdar, V.V., Fitzpatrick, E.G., Alferiev, I.S., Levy, R.J., Stachelek, S.J., and Fishbein, I. (2020) Mitigation of Blood Borne Cell Attachment to Metal Implants through CD47-Derived Peptide Immobilization. *J Vis Exp*. JoVE. (166),1–15. doi: 10.3791/61545
92. Bu, Y., Zhang, X., Zhu, A., Li, L., Xie, X.,and Wang, S.(2021) Inside-out Oriented Cell Membrane Biomimetic Magnetic Nanoparticles for High-Performance Drug Lead Discovery. *Anal Chem.* 93(22),7898–907. doi: 10.1021/acs.analchem.1c00567.
93. Cai, Z., Gu, Y., Xiao, Y., Wang, C., and Wang, Z. (2021) Porcine carotid arteries decellularized with a suitable concentration combination of Triton X-100 and sodium dodecyl sulfate for tissue engineering vascular grafts. *Cell Tissue Bank*. 22(2),277–86 doi: 10.1007/s10561-020-09876-7
94. Georgakarakos, E., Tasopoulou, K., Koutsoumpelis, A., and Argyriou, C., (2021). The “Pull, Cast, and Fix” Technique for Bypass in the Midpopliteal

(P2) Arterial Segment in Chronic Femoropopliteal Occlusions. Case Reports *Ann Vasc Surg.* 71,523–7. doi: 10.1016/j.avsg.2020.08.153.

95. Botelho, F.E., Cacione, D.G., Leite, J.O., and Baptista-Silva, J.C. (2021) Endoluminal interventions versus surgical interventions for stenosis in vein grafts following infrainguinal bypass. *Cochrane Database Syst Rev.* 28;4(4):CD013702. doi: 10.1002/14651858.CD013702.pub2.

96. Harskamp, R.E., Alexander, J.H., Ferguson, T.B. Jr., Hager, R., Mack, M.J., Englum, B., et al. (2016) Frequency and Predictors of Internal Mammary Artery Graft Failure and Subsequent Clinical Outcomes: Insights From the Project of Ex-vivo Vein Graft Engineering via Transfection (PREVENT) IV Trial. *Circulation.* 12;133(2):131-8. doi: 10.1161/CIRCULATIONAHA.115.015549.

97. Weekes, A., Bartnikowski, N., Pinto, N., Jenkins, J., Meinert, C., and Klein, T.J. (2022). Biofabrication of small diameter tissue-engineered vascular grafts. *Acta Biomater.* 15(138),92–111. doi: 10.1016/j.actbio.2021.11.012

98. Chen, S.G., Ugwu, F., Li W.C., Caplice, N.M, Petcu, E., Yip, S.P., et al. (2021). Vascular Tissue Engineering: Advanced Techniques and Gene Editing in Stem Cells for Graft Generation. *Tissue Eng*. 27(1),14–28. doi: 10.1089/ten.TEB.2019.0264.

99. Lin, C.H., Hsia, K., Ma, H., Lee, H., Lu, and J.H. (2018). In vivo performance of decellularized vascular grafts: A review article. *Int J Mol Sci.* 19(7). doi: 10.3390/ijms19072101.

100. López-Ruiz, E., Venkateswaran, S., Perán, M., Jiménez, G., Pernagallo, S., Díaz-Mochón, J.J., et al. (2017). Poly(ethylmethacrylate-co-diethylaminoethyl acrylate) coating improves endothelial re-population, bio-mechanical and anti-thrombogenic properties of decellularized carotid arteries for blood vessel replacement. *Sci Rep.* (1),407. doi: 10.1038/s41598-017-00294-6.

101. Heng, J.W., Yazid, M.D., Abdul Rahman, M.R. and Sulaiman, N. (2021) Coatings in Decellularized Vascular Scaffolds for the Establishment of a

Functional Endothelium: A Scoping Review of Vascular Graft Refinement. *Front Cardiovasc Med.* 29;8:677588. doi: 10.3389/fcvm.2021.677588.

102. Jin, X., Kang, R., Deng, R., Zhao, X., Wang, Z., Rong, W., et al. (2022) Fabrication and characterization of an acellular annulus fibrosus scaffold with aligned porous construct for tissue engineering. *J Biomater Appl.* 36(6),985–95. doi: 10.1177/08853282211041956

103. Fazal, F., Raghav, S., Callanan, A., Koutsos, V. and Radacsi, N. (2021) Recent advancements in the bioprinting of vascular grafts. *Biofabrication.* 28;13(3). doi: 10.1088/1758-5090/ac0963.

104. Rickel, A.P., Deng, X., Engebretson, D. and Hong Z. (2021) Electrospun nanofiber scaffold for vascular tissue engineering. *Mater Sci Eng C Mater Biol Appl.* 129:112373. doi: 10.1016/j.msec.2021.112373.

105. Sohn, S.H., Kim, T.H., Kim, T.S., Min, T.J., Lee, J.H., Yoo, S.M., et al. (2021).

Evaluation of 3d templated synthetic vascular graft compared with standard graft in a rat model: Potential use as an artificial vascular graft in cardiovascular disease. *Materials (Basel).* 2021 Mar 5;14(5):1239. doi: 10.3390/ma14051239.

106. Henry, J. J. D., Yu, J., Wang, A., Zhang, Y., Li, X. S., Guex, A. G., et al. (2017). A compliant and biomimetic three-layered vascular graft for small blood vessels. *Biofabrication.* 9:025010. doi: 10.1088/1758-5090/aa6bae.

107. Fang, S., Ellman, D.G., and Andersen, D.C. (2021) Review: Tissue Engineering of Small- Diameter Vascular Grafts and Their In Vivo Evaluation in Large Animals and Humans. *Cells.* 23;10(3):713. doi: 10.3390/cells10030713.

108. Horakova, J., Mikes, P., Lukas, D., Saman, A., Jencova, V., Klapstova, A., et al. (2018) Electrospun vascular grafts fabricated from poly(L-lactide-co-ε-caprolactone used as a bypass for the rabbit carotid artery. *Biomed. Mater.* 13, 065009 doi: 10.1088/1748-605X/aade9d.

109. Itoh, M., Nakayama, K., Noguchi, R., Kamohara, K., Furukawa, K.,

Uchihashi, K., et al. (2015) Scaffold-free tubular tissues created by a bio-3D printer undergo remodeling and endothelialization when implanted in rat aortae. *PLoS One*. 10(9),1–15. doi: 10.1371/journal.pone.0136681

110. Konig, G., Mcallister, T. N., Dusserre, N., Garrido, S. A., Iyican, C., Marini, A., et al. (2010). Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. *Biomaterials* 30, 1542–1550. doi: 10.1016/j.biomaterials.2008.11.011
111. Saito, J., Yokoyama, U., Nakamura, T., Kanaya, T., Ueno, T., Naito Y, et al. (2021) Scaffold-free tissue-engineered arterial grafts derived from human skeletal myoblasts. Artif Organs. 45(8),19–32. doi: 10.1111/aor.13930
112. Generali, M., Casanova, EA., Kehl, D., Wanner, D., Hoerstrup, SP., Cinelli, P., et al. (2019). Autologous endothelialized small-caliber vascular grafts engineered from blood-derived induced pluripotent stem cells. *Acta Biomater*. 97,333–43. doi: 10.1016/j.actbio.2019.07.032
113. Qiu, X., Li-Ping Lee, B., Wong, S.Y., Ding, X., Xu, K., Zhao, W., et al. (2021). Cellular remodeling of fibrotic conduit as vascular graft. *Biomaterials.* 1–24. doi: 10.1016/j.biomaterials.2020.120565
114. Geelhoed, W.J., Moroni, L., and Rotmans, J.I., (2017). Utilizing the Foreign Body Response to Grow Tissue Engineered Blood Vessels in Vivo. *J Cardiovasc Transl Res.* 10(2),167–79. doi: 10.1007/s12265-017-9731-7
115. Von Bornstädt, D., Wang, H., Paulsen, M.J., Goldstone, A.B., Eskandari, A., Thakore, A., et al. (2018) Rapid self-assembly of bioengineered cardiovascular bypass grafts from scaffold-stabilized, tubular bilevel cell sheets. *Circulation.* 138(19),2130–44. doi: 10.1161/CIRCULATIONAHA.118.035231
116. Bianco, V., Kilic, A., Gleason, T.G., Aranda-Michel, E., Habertheuer, A., Humar, R., et al. (2021) Long-term Outcomes After Reoperative Coronary Artery Bypass Grafting. *Ann Thorac Surg.* 111(1),150–8. doi: 10.1016/j.athoracsur.2020.04.092

117. Libby, P. (2021) Inflammation during the life cycle of the atherosclerotic plaque. *Cardiovasc Res.*117(13),2525–36. doi: 10.1093/cvr/cvab303

118. Ridker, P.M., Koenig, W., Kastelein, J.J., Mach, F., and Lüscher, T.F. (2018) Has the time finally come to measure hsCRP universally in primary and secondary cardiovascular prevention? *Eur Heart J.* 7;39(46):4109-4111. doi: 10.1093/eurheartj/ehy723.

119. Neufert, C., Heichler, C., Brabletz, T., Scheibe, K., Boonsanay, V., Greten, F.R., et al. (2021) Inducible mouse models of colon cancer for the analysis of sporadic and inflammation-driven tumor progression and lymph node metastasis. *Nat Protoc*. 16(1),61–85. doi: 10.1038/s41596-020-00412-1

120. Liu, C.H., Chen, Z., Chen, K., Liao, F.T, Chung C-E, Liu X, et al. (2021) Lipopolysaccharide-Mediated Chronic Inflammation Promotes Tobacco Carcinogen-Induced Lung Cancer and Determines the Efficacy of Immunotherapy. *Cancer Res*. 81(1),144–57. doi: 10.1158/0008-5472.CAN-20-1994

121. Fasehee, H., Fakhraee, M., Davoudi, S., Vali, H., and Faghihi, S. (2019) Cancer biomarkers in atherosclerotic plaque: Evidenced from structural and proteomic analyses. *Biochem Biophys Res Commun*. 509(3),687–93. doi: 10.1016/j.bbrc.2018.12.160.

122. Garcia-Sabaté, A., Mohamed, W.K.E., Sapudom, J., Alatoom, A., Al Safadi, L., and Teo, J.C.M. (2020). Biomimetic 3D models for investigating the role of monocytes and macrophages in atherosclerosis. *Bioengineering.* 7(3),1–20. doi: 10.3390/bioengineering7030113

123. da Luz, P. L., Chagas, A. C. P., Dourado, P. M. M., Laurindo, F. R. M. (2018) Endothelium in Atherosclerosis: Plaque Formation and Its Complications. *Endothelium and cardiovascular diseases: vascular biology and clinical syndromes: Elsevier.* p.493-512

124. Guo, J., Li, J., Zhang, J., Guo, X., Liu, H., Li, P., et al. (2021) LncRNA PVT1 knockdown alleviated ox-LDL-induced vascular endothelial cell injury and atherosclerosis by miR-153-3p/GRB2 axis via ERK/p38 pathway.

Nutr Metab Cardiovasc Dis. 31(12),3508–21. doi: 10.1016/j.numecd.2021.08.031

125. Chien, C.S., Li, J.Y.S., Chien, Y., Wang, M.L., Yarmishyn, A.A., Tsai, P.H., et al. (2021) METTL3-dependent N6-methyladenosine RNA modification mediates the atherogenic inflammatory cascades in vascular endothelium. *Proc Natl Acad Sci U S A*. 118(7),1–12. doi: 10.1073/pnas.2025070118
126. Singh, B., Kosuru, R., Lakshmikanthan, S., Sorci-Thomas, MG., Zhang, D.X., Sparapani, R., et al. (2021). Endothelial Rap1 (Ras-Association Proximate 1) Restricts Inflammatory Signaling to Protect from the Progression of Atherosclerosis. *Arterioscler Thromb Vasc Biol.* 1,638–50. doi: 10.1161/ATVBAHA.120.315401
127. Petljak, M., Alexandrov, L.B., Brammeld, J.S., Price, S., Wedge, D.C., Grossmann, S., et al. (2019). Characterizing Mutational Signatures in Human Cancer Cell Lines Reveals Episodic APOBEC Mutagenesis. *Cell.* 176(6),1282-1294.e20. doi: 10.1016/j.cell.2019.02.012
128. Zong, C., Kimura, Y., Kinoshita, K., Takasu, S., Zhang, X., Sakurai, T., et al. (2019). Exposure to 1,2-Dichloropropane Upregulates the Expression of Activation-Induced Cytidine Deaminase (AID) in Human Cholangiocytes Co-Cultured with Macrophages. *Toxicol Sci.* 168(1),137–48. doi: 10.1093/toxsci/kfy280
129. Nishikori, A., Nishimura, Y., Shibata, R., Ohshima, K.I., Gion, Y., Ikeda, T., et al. (2021) Upregulated Expression of Activation-Induced Cytidine Deaminase in Ocular Adnexal Marginal Zone Lymphoma with IgG4-Positive Cells. *Int J Mol Sci*. 22(8). doi: 10.3390/ijms22084083
130. Zhao, G., Lu, H., Liu, Y., Zhao, Y., Zhu, T., Garcia-Barrio, M.T., et al. (2021). Single-Cell Transcriptomics Reveals Endothelial Plasticity During Diabetic Atherogenesis. *Front Cell Dev Biol.* 9,1–14. doi: 10.3389/fcell.2021.689469
131. Zhang, J., Rojas, S., Singh, S., Musich, P.R., Gutierrez, M., Yao, Z., et al. (2021). Wnt2 Contributes to the Development of Atherosclerosis. *Front*

Cardiovasc Med. 8,1–9. doi: 10.3389/fcvm.2021.751720

132. Björck, H.M., Du, L., Pulignani, S., Paloschi, V., Lundströmer, K., Kostina, A.S., et al. (2018). Altered DNA methylation indicates an oscillatory flow mediated epithelial-to-mesenchymal transition signature in ascending aorta of patients with bicuspid aortic valve. *Sci Rep.* 8(1),1–15. doi: 10.1038/s41598-018-20642-4

133. An, W., Luong, L.A., Bowden, N.P., Yang, M., Wu, W., Zhou, X., et al. (2022). Cezanne is a critical regulator of pathological arterial remodelling by targeting β-catenin signalling. *Cardiovasc Res.* 118(2),638–53. doi: 10.1093/cvr/cvab056

134. Zhuang, X., Gao, F., Shi, L., Liu, W., Wang, W., He, X., et al. (2021). MicroRNA-146b-3p regulates the dysfunction of vascular smooth muscle cells via repressing phosphoinositide-3 kinase catalytic subunit gamma. *Bioengineered.* 12(1),2627–38. doi: 10.1080/21655979.2021.1937904

135. Wang, Z.Y., Cheng, J., Liu, B., Xie, F., Li, C.L., Qiao, W., et al. (2021). Protein deglycase DJ-1 deficiency induces phenotypic switching in vascular smooth muscle cells and exacerbates atherosclerotic plaque instability. *J Cell Mol Med.* 25(6),2816–27. doi: 10.1111/jcmm.16311

136. Sandiford, O.A., Donnelly, R.J., El-Far, M.H., Burgmeyer, L.M., Sinha, G., Sri Pamarthi, et al. (2021). Mesenchymal Stem Cell–Secreted Extracellular Vesicles Instruct Stepwise Dedifferentiation of Breast Cancer Cells into Dormancy at the Bone Marrow Perivascular Region. *Cancer Res.* 81(6),567–82. doi: 10.1158/0008-5472.CAN-20-2434

137. Wang, Y., Nanda, V., Direnzo, D., Ye, J., Xiao, S., Kojima, Y., et al. (2020). Clonally expanding smooth muscle cells promote atherosclerosis by escaping efferocytosis and activating the complement cascade. *Proc Natl Acad Sci U S A.* 117(27),15818–26. doi: 10.1073/pnas.2006348117

138. Direnzo, D., Owens, G.K. and Leeper, N.J. (2017). “Attack of the Clones” Commonalities Between Cancer and Atherosclerosis. *Circ Res.* 120(4),624–6. doi: 10.1161/CIRCRESAHA.116.310091

139. Wirka, R.C., Wagh, D., Paik, D.T., Pjanic, M., Nguyen, T., Miller, C.L., et al. (2019). Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis. *Nat Med.* 25(8),1280–9. doi: 10.1038/s41591-019-0512-5

140. Brandt, K., Burger, F., Baptista, D., Roth, A., Fernandes, Da Silva, R., Montecucco, F., et al. (2022). Single-cell analysis uncovers osteoblast factor Growth Differentiation Factor 10 as mediator of vascular smooth muscle cell phenotypic modulation associated with plaque rupture in human carotid artery disease. *Int J Mol Sci.* 23(3),1796. doi: 10.3390/ijms23031796

141. Murphy, P.A., Butty, V.L., Boutz, P.L., Begum, S., Kimble, A.L., Sharp, P.A., et al. (2018). Alternative RNA splicing in the endothelium mediated in part by rbfox2 regulates the arterial response to low flow. *Elife*. 7,1–21. doi: 10.7554/eLife.29494

142. Tang, P.C.T., Chung, J.Y.F., Xue, V.W., Xiao, J., Meng, X.M., Huang, X.R., et al. (2022). Smad3 Promotes Cancer-Associated Fibroblasts Generation via Macrophage–Myofibroblast *Transition. Adv Sci.* 9(1),1–14. doi: 10.1002/advs.202101235

143. Hernández-Camarero, P., López-Ruiz, E., Marchal. J.A. and Perán, M. (2021). Cancer: a mirrored room between tumor bulk and tumor microenvironment. *J Exp Clin Cancer Res.* 40(1),217. doi: 10.1186/s13046-021-02022-5

144. Li, M., Qi, Z., Zhang, J., Zhu, K. and Wang, Y. (2021). Effect and Mechanism of Si-Miao-Yong-An on Vasa Vasorum Remodeling in ApoE−/− Mice with Atherosclerosis Vulnerable Plague. Front Pharmacol. 12,1–12. doi: 10.3389/fphar.2021.634611

145. Xu, Z., Guo, C., Ye, Q., Shi, Y., Sun, Y., Zhang, J., et al. (2021). Endothelial deletion of SHP2 suppresses tumor angiogenesis and promotes vascular normalization. *Nat Commun.* 12(1),1–15. doi: 10.1038/s41467-021-26697-8

146. Bath, J., Lawrence, P.F., Neal, D., Zhao, Y., Smith, J.B., Beck, A.W., et al. (2021). Endovascular interventions for claudication do not meet minimum

standards for the Society for Vascular Surgery efficacy guidelines. *J Vasc Surg.* 73(5),1693–700. doi: 10.1016/j.jvs.2020.10.067

147. Botts, S.R., Fish, J.E. and Howe, K.L. (2021). Dysfunctional Vascular Endothelium as a Driver of Atherosclerosis: Emerging Insights into Pathogenesis and Treatment. *Front Pharmacol.* 1,1–18. doi: 10.3389/fphar.2021.787541

148. Lin, S., Li, X., Zhang, J. and Zhang, Y. (2021). Omentin-1: Protective impact on ischemic stroke via ameliorating atherosclerosis. *Clin Chim Acta.* 517,31–40. doi: 10.1016/j.cca.2021.02.004

149. Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., et al. (2017) Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med.* 21;377(12):1119-1131. doi: 10.1056/NEJMoa1707914.

150. Ridker, P.M., MacFadyen, J.G., Thuren, T., Everett, B.M., Libby, P., Glynn, R.J. et al. (2017) Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet.* 21;390(10105):1833-1842. doi: 10.1016/S0140-6736(17)32247-X.

151. Tardif, J.C., Kouz, S., Waters, D.D., Bertrand, O.F., Diaz, R., Maggioni, A.P., et al. (2019) Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *N Engl J Med.* 26;381(26):2497-2505. doi: 10.1056/NEJMoa1912388.

152. Nidorf, S.M., Fiolet, A.T.L., Mosterd, A., Eikelboom, J.W., Schut, A., Opstal, T.S.J., et al. (2020) Colchicine in Patients with Chronic Coronary Disease. *N Engl J Med.* 5;383(19):1838-1847. doi: 10.1056/NEJMoa2021372.

153. Huang, Q., Gan, Y., Yu, Z., Wu, H. and Zhong, Z. (2021). Endothelial to Mesenchymal Transition: An Insight in Atherosclerosis. *Front Cardiovasc Med.* 8,1–9. doi: 10.3389/fcvm.2021.734550

154. Shi, X., Zhang, Y., Han, J., Peng, W., Fang, Z., Qin, Y., et al. (2021).

Tryptanthrin Regulates Vascular Smooth Muscle Cell Phenotypic Switching in Atherosclerosis by AMP-Activated Protein Kinase/Acetyl-CoA Carboxylase Signaling Pathway. *J Cardiovasc Pharmacol.* 77(5),642–9. doi: 10.1097/FJC.0000000000001008

155. Zhang, Q., He, J., Xu, F., Huang, X., Wang, Y., Zhang, W., et al. (2021). Supramolecular copolymer modified statin-loaded discoidal rHDLs for atherosclerotic anti-inflammatory therapy by cholesterol efflux and M2 macrophage polarization. *Biomater Sci.* 9(18),6153–68. doi: 10.1039/d1bm00610j

156 Sheridan, W.S., Duffy, G.P. et al. (2012) Mechanical characterization of a customized decellularized scaffolds for vascular tissue engineering. *J. Mech. Behav. Biomed. Mater*. 8:,58-70, doi.10.1016/j.jmbbm.2011.12.003

157 Mitchell, T.C, Feng, N.L, Lam, Y.T, Michael, P.L, Santos, M, Wise SG. (2023). Engineering Vascular Bioreactor Systems to Closely Mimic Physiological Forces In Vitro Tissue Engineering. *Tissue Eng Part B Rev*. 29(3),232-243. doi: 10.1089/ten.TEB.2022.0158.

158 Yao R, Zhang R, Li J, et al. Engineering vascular tissue with functional endothelial cells derived from human pluripotent stem cells. Cell Physiol Biochem. 2021;55(1):73-88. doi:10.33594/000000353

159 Badylak, S. F., Freytes, D. O. & Gilbert, T. W. (2009). Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomaterialia 5, 1–13.

160 Arenas-Herrera, J. E., Ko, I. K., Atala, A. & Yoo, J. J. (2013). Decellularization for whole organ bioengineering. Biomed. Mater 8, 14106–9.

161 Balistreri, R.C. 2017. Endothelial progenitor cells. A new real hope?. UNIPA Springer Series

162 Tagawa, S.; Nakanishi, C. et al. Determination of early and late endothelial progenitor cells in peripheral circulation and their clinical association with coronary artery disease. Int J Vasc Med. 2015; ID 674213,

doi:10.1155/2015/674213

163 Asahara, T.; Kawamoto, A. Concise review: circulating endothelial progenitor cells for vascular medicine. Stem Cells 2011; 29(11):1650–1655, doi:10.1002/stem.745.

164 Mead, L.E.; Prater, D. et al Isolation and characterization of endothelial progenitor cells from human blood. Curr Protoc Stem Cell Biol. 2008. Chapter 2: Unit 2C.1, doi:10.1002/9780470151808.sc02c01s6

165 Marchetti, V.; Peters, C. Endothelial progenitor cells and endothelial cells. 2015

166 Khzam, L.B.; Bouchereau, O. et al. Early outgrowth cells versus endothelial colony forming cells functions in platelet aggregation. J. Transl. Med. 2015; 13:353, doi:10.1186/s12967-015-0723-6

167 López-Ruiz, E., Perán, M. et al. Cardiomyogenic differentiation potential of human endothelial progenitor cells isolated from patients with myocardial infraction. Cytotherapy, 2014; 16:1229-1237, doi: 10.1016/j.jcyt.2014.05.012

168 Balistreri, C.R.; Buffa, S. et al. Are Endothelial Progenitor Cells the Real Solution for Cardiovascular Diseases? Focus on Controversies and Perspectives. Biomed Res Int. 2015, 2015: Article ID 835934 / doi:10.1155/2015/835934

169 Yoder, M.C. Human endothelial progenitor cells. Cold Spring Harb Perspect Med 2012; 2:a006692

170 Yoder, M.C. Defining human endothelial progenitor cells. Journal of Thrombosis and Haemostasis, 7 (Suppl. 1): 49-52, doi:10.1111/j.1538-7836.2009.03407.x

171 Parham, K.A.; Pitson, S.M.; et al. Regulation of EPCs: the gateway to blood vessel formation. New Journal of Science, 2014; doi:10.1155/2014/972043

172 Sen, S., McDonald, S.P. et al. Endothelial progenitor cells: novel biomarker and promising cell therapy for cardiovascular disease. Clinical Science, 2011; 120: 263-283, doi:10.1042/CS20100429

173 Pelosi, E., Castelli, G. et al. Endothelial progenitors. Blood Cells, Molecules and Diseases 2014; 186-194, doi:10.1016/j.bcmd.2013.11.004

174 Alphonse, R. S.; Vadivel, A. et al. The isolation and culture of endothelial colony-forming cells from human and rat lungs. Nat. Prot. 2015; 10(11): 1697-1708, doi: 10.1038/nprot.2015.107

175 Resch, T., Pircher, A. et al. Endothelial progenitor cells: current issues on characterization and challenging clinical applications. Stem Cell Rev. and Rep., 2012; 8: 926-939, doi:10.1007/s12015-011-9332-9

176 Tura, O., Skinner, E.M. et al. Late outgrowth endothelial cells resemble mature cells and are note derived from bone marrow. Stem Cells, 2013; 31: 338-348, doi: 10.1002/stem.1280

177 Bueno-Betí, C.; Novella, S. et al. An affordable method to obtained cultured endothelial cells from peripheral blood. J. Cell. Mol. Med. Vol 17, No 11, 2013 pp. 1475-1483

178 Marchal, J.A.; Picón, M. et al. Purification and long-term expansion of multipotent endothelial-like cells with potential cardiovascular regeneration. Stem cells and development, 2012; 21(4):562-74, doi:10.1089/scd.2011.0072

179 Relou, I.A.M., Damen, C.A. et al. Effect of culture conditions on endothelial cell growth and responsiveness. Tissue & Cell, 1998; 30(5): 525-530

180 López-Ruiz, E., Venkateswaran, S. et al. Poly(ethylmethacrylate-co-diethylaminoethyl acrylate) coating improves endothelial re- population, bio-mechanical and anti-thrombogenic properties of decellularizedcarotid arteries for blood vessel replacement. Sci Rep. 2017; 7(1):407, doi:10.1038/s41598-017-00294-6.

181 Zhou, M., Liu, Z. et al. Tissue engineering of small-diameter vascular grafts by endothelial progenitor cells seeding heparin-coated decellularized scaffolds. Res. Part. B, 2012; 100B:111-120, doi:10.1002/jbm.b.31928

182 Watabe, T.; Miyazono, K. et al. Roles of TGF-β family signaling in stem cell renewal and differentiation. Cell Res. 2009; 19:103-115, doi:10.1038/cr.2008.323

183 Takenaga, M., Fukumoto, M. et al. Regulated Nodal signaling promotes differentiation of the definitive endoderm and mesoderm form ES cells. J. Cell Science, 2007; 120(2): 2078-2090. doi:10.1242/jcs.004127

184 Suzuki, Y., Montagne, K. et al. BMPs promote differentiation and migration of endothelial cells via stimulation of VEGF-A/VEGFR2 and angiopoietin-1/Tie2 signalling. J. Biochem, 2008; 143:199-206, doi:10.1093/jb/mvm215

185 Kim, J., Kim, M. et al. BMP9 induces cord blood-derived endothelial progenitor cell differentiation and ischemic neovascularization via ALK1. ArteriosclerThrombVascBiol, 2015; 35:2020-2031, doi:10.1161/ATVBAHA.115.306142

186. Lee, C.C., Jan, H.J. et al. Nodal promotes growth and invasion in human gliomas. Oncogene, 2010; 29: 3110-3123, doi:10.1038/onc.2010.55

187 Lin, Y., Wei J. et al. Proteomic analysis of endothelial progenitor cells exposed to oxidative stress. International Journal of Molecular Medicine, 2013; 32: 607-614, doi:10.3892/ijmm.2013.1419

188 Boraas, L.C. & Ahsan, T. Lack of Vimentin impairs endothelial differentiation of embryonic stem cells. Scientific Reports, 2016; 6:30814, doi: 10.1038/srep30814

189. Hueng, D.Y., Lin, G.J. et al. Inhibition of Nodal suppresses angiogenesis and growth of human gliomas. J. Neurooncol., 2011; 104: 21-31, doi:10.1007/s11060-010-0467-3

190 Esquivies, L., Blackler, A. et al. Designer Nodal/BMP2 chimeras mimic nodal signaling, promote chondrogenesis and reveal a BMP2-like structure. J. Biol. Chem., 2014; 289: 1788-1797, doi: 10.1074/jbc.M113.529180

191 Tsubouchi, K.; Igarashi, Y. et at. Sericin enhances attachment of cultured human skin fibroblasts. Bioscience, Biotechnology, and Biochemistry, 69:2, 403-405, doi:10.1271/bbb.69.403

192 Liu, L., Wang, J. et al. Systematic evaluation of sericin protein as a substitute for fetal bovine serum in cell culture. Scientific Reports, 2016; 31516 doi:10.1038/srep31516

193 Song, Y., Zhang, C. et al. An injectable silk sericin hydrogen promotes cardiac function recovery after ischemic myocardial infraction. Acta biomaterialia, 2016; doi: 10.1016/j.actbio.2016.05.039

194 G, N.; Tan, A. et al. Tissue engineering vascular grafts a fortiori: looking back and going forward. Expert Opin. Biol. Ther. 2015; 15(2):231-244, doi:10.1517/14712598.2015.980234

195 Chlupáč, J.; Filová, E. et al. Blood vessel replacement: 50 years of development and tissue engineering paradigms in vascular surgery. Physiol. Res. 2009; 58 (Suppl. 2): S119-S139

196 De Isla, N., Huseltein, C. et al. Introduction to tissue engineering and application for cartilage engineering. Bio-Medical Materials and Engineering, 2010; 20:127–133, doi:10.3233/BME-2010-0624

197 Garreta, E., Oria, R. et al. Tissue engineering by decellularization and 3D bioprinting. Materials today, 2017; 20 (4): 166-178, doi:10.1016/j.mattod.2016.12.005

198 Stoltz JF, Zhang L, Ye JS, De Isla N. Organ reconstruction: Dream or reality for the future. Biomed Mater Eng. 2017;28(s1):S121-S127. doi: 10.3233/BME-171633. PMID: 28372287

199 Neishabouri A, Soltani Khaboushan A, Daghigh F, Kajbafzadeh AM, Majidi Zolbin M. Decellularization in Tissue Engineering and Regenerative Medicine: Evaluation, Modification, and Application Methods. Front Bioeng Biotechnol. 2022 Apr 25;10:805299. doi: 10.3389/fbioe.2022.805299.

200 Meyer, S. R. et al. Comparison of aortic valve allograft decellularization techniques in the rat. J. Biomed. Mater. Res. - Part A 79, 254–262 (2006).

201 Sheridan, W. S., Duffy, G. P. & Murphy, B. P. Mechanical characterization of a customized decellularized scaffold for vascular tissue engineering. J. Mech. Behav. Biomed. Mater. 8, 58–70 (2012).

202 Zou, Y. & Zhang, Y. Mechanical evaluation of decellularized porcine thoracic aorta. J. Surg. Res. 175, 359–368 (2012).

203 Mancuso, L., Gualerzi, a, Boschetti, F., Loy, F. & Cao, G. Decellularized ovine arteries as small-diameter vascular grafts. Biomed. Mater. 9, 45011 (2014).

204 Xu J, Shi GP. Vascular wall extracellular matrix proteins and vascular diseases. Biochim Biophys Acta. 2014 Nov;1842(11):2106-2119. doi: 10.1016/j.bbadis.2014.07.008. Epub 2014 Jul 18. PMID: 25045854; PMCID: PMC4188798.

205. Weekes, A., Bartnikowski, N., Pinto, N., Jenkins, J., Meinert, C., and Klein, T.J. (2022) Biofabrication of small diameter tissue-engineered vascular grafts. *Acta* Biomater. 15;138:92-111. doi: 10.1016/j.actbio.2021.11.012.

206. Joseph. J., Domenico Bruno, V., Sulaiman, N., Ward, A., Johnson, T.W., Baby, H.M., et al. (2022) A novel small diameter nanotextile arterial graft is associated with surgical feasibility and safety and increased transmural endothelial ingrowth in pig. J Nanobiotechnology. 2022 Feb 8;20(1):71. doi: 10.1186/s12951-022-01268-1.

207. Owens, C.D., Gasper, W.J., Rahman, A.S., and Conte, M.S. (2015) Vein graft failure. J Vasc Surg. 61(1):203-16. doi: 10.1016/j.jvs.2013.08.019.

208. Arias, S.L., Shetty, A., Devorkin, J., and Allain, J.P. (2018) Magnetic targeting of smooth muscle cells in vitro using a magnetic bacterial cellulose to improve cell retention in tissue-engineering vascular grafts. Acta Biomater. 1;77:172-181.doi: 10.1016/j.actbio.2018.07.013.

209. Ju,Y.M., Ahn, H., Arenas-Herrera, J., Kim, C., Abolbashari, M., Atala, A., et al. (2017) Electrospun vascular scaffold for cellularized small diameter

blood vessels: A preclinical large animal study. Acta Biomater. 2017 Sep 1;59:58-67. doi: 10.1016/j.actbio.2017.06.027.

210. Leal, B.B.J., Wakabayashi, N., Oyama, K., Kamiya, H., Braghirolli, D.I., and Pranke, P. (2021) Vascular Tissue Engineering: Polymers and Methodologies for Small Caliber Vascular Grafts. Front Cardiovasc Med. 1;7:592361. doi: 10.3389/fcvm.2020.592361. eCollection 2020.

211 Pärsson H, Jundzill W, Johansson K, Jonung T, Norgren L. Healing characteristics of polymer-coated or collagen-treated Dacron grafts: an experimental porcine study. Cardiovasc Surg. 1994 Apr;2(2):242-8. PMID: 8049955.

212 Dalsing MC, Kevorkian M, Raper B, Nixon C, Lalka SG, Cikrit DF, Unthank JL, Herring MB. An experimental collagen-impregnated Dacron graft: potential for endothelial seeding. Ann Vasc Surg. 1989 Apr;3(2):127-33. doi: 10.1016/S0890-5096(06)62005-0. PMID: 2527547.

213 Wu HC, Wang TW, Kang PL, Tsuang YH, Sun JS, Lin FH. Coculture of endothelial and smooth muscle cells on a collagen membrane in the development of a small-diameter vascular graft. Biomaterials. 2007 Mar;28(7):1385-92. doi: 10.1016/j.biomaterials.2006.11.012. Epub 2006 Dec 4. PMID: 17141865.

214 Peters EB. Endothelial Progenitor Cells for the Vascularization of Engineered Tissues. Tissue Eng Part B Rev. 2018 Feb;24(1):1-24. doi: 10.1089/ten.TEB.2017.0127. Epub 2017 Jul 3. PMID: 28548628; PMCID: PMC5797330.

215 Jafarihaghighi F, Ardjmand M, Mirzadeh A, Hassani MS, Parizi SS. Current challenges and future trends in manufacturing small diameter artificial vascular grafts in bioreactors. Cell Tissue Bank. 2020 Sep;21(3):377-403. doi: 10.1007/s10561-020-09837-0. Epub 2020 May 15. PMID: 32415569.

216 Hansmann J, Groeber F, Kahlig A, Kleinhans C, Walles H. Bioreactors in tissue engineering - principles, applications and commercial constraints.

Biotechnol J. 2013 Mar;8(3):298-307. doi: 10.1002/biot.201200162. Epub 2012 Nov 19. PMID: 23161827.

217 Castro N, Ribeiro S, Fernandes MM, Ribeiro C, Cardoso V, Correia V, Minguez R, Lanceros-Mendez S. Physically Active Bioreactors for Tissue Engineering Applications. Adv Biosyst. 2020 Oct;4(10):e2000125. doi: 10.1002/adbi.202000125. Epub 2020 Sep 13. PMID: 32924326.

218 Paez-Mayorga J, Hernández-Vargas G, Ruiz-Esparza GU, Iqbal HMN, Wang X, Zhang YS, Parra-Saldivar R, Khademhosseini A. Bioreactors for Cardiac Tissue Engineering. Adv Healthc Mater. 2019 Apr;8(7):e1701504. doi: 10.1002/adhm.201701504. Epub 2018 May 8. PMID: 29737043.

219 Mitchell TC, Feng NL, Lam YT, Michael PL, Santos M, Wise SG. Engineering Vascular Bioreactor Systems to Closely Mimic Physiological Forces In Vitro. Tissue Eng Part B Rev. 2023 Jun;29(3):232-243. doi: 10.1089/ten.TEB.2022.0158. Epub 2022 Dec 8. PMID: 36274223.

220 Akintewe OO, Roberts EG, Rim NG, Ferguson MAH, Wong JY. Design Approaches to Myocardial and Vascular Tissue Engineering. Annu Rev Biomed Eng. 2017 Jun 21;19:389-414. doi: 10.1146/annurev-bioeng-071516-044641. Epub 2017 May 3. PMID: 28471698.

221 Devillard CD, Marquette CA. Vascular Tissue Engineering: Challenges and Requirements for an Ideal Large Scale Blood Vessel. Front Bioeng Biotechnol. 2021 Oct 4;9:721843. doi: 10.3389/fbioe.2021.721843. PMID: 34671597; PMCID: PMC8522984.

222 Couet F, Mantovani D. Perspectives on the advanced control of bioreactors for functional vascular tissue engineering in vitro. Expert Rev Med Devices. 2012 May;9(3):233-9. doi: 10.1586/erd.12.15. PMID: 22702253.

223 Nasseri BA, Pomerantseva I, Kaazempur-Mofrad MR, Sutherland FW, Perry T, Ochoa E, Thompson CA, Mayer JE Jr, Oesterle SN, Vacanti JP. (2003). Dynamic rotational seeding and cell culture system for vascular tube formation. Tissue Eng 9(2):291–299

224 Stefani I, Asnaghi M, Cooper-White J, Mantero S. (2018). A double chamber rotating bioreactor for enhanced tubular tissue generation from human mesenchymal stem cells: a promising tool for vascular tissue regeneration. J Tissue Eng Regen Med 12(1):e42–e52

225 L'Heureux N (2012) Developing a completely biological tissue engineered blood vessel for clinical use. The FASEB J. 26(1_supplement):191–198.

226 Syedain, Z. H., L. A. Meier, J. W. Bjork, A. Lee, and R. T. (2011). Tranquillo. Implantable arterial grafts from human fibroblasts and fibrin using a multi-graft pulsed flow-stretch bioreactor with noninvasive strength monitoring. Biomaterials 32:714–722.

frontiers | Frontiers in Cell and Developmental Biology

TYPE Review
PUBLISHED 16 March 2023
DOI 10.3389/fcell.2023.1148768

OPEN ACCESS

EDITED BY
Daniela Quaglino,
University of Modena and Reggio Emilia, Italy

REVIEWED BY
Wen Zeng,
Army Medical University, China
Daria Shishkova,
Research Institute for Complex Issues of Cardiovascular Diseases, Russia

*CORRESPONDENCE
Elena López-Ruiz,
elruiz@ujaen.es
Macarena Perán,
mperan@ujaen.es

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Molecular and Cellular Pathology, a section of the journal Frontiers in Cell and Developmental Biology

RECEIVED 20 January 2023
ACCEPTED 06 March 2023
PUBLISHED 16 March 2023

CITATION
Barungi S, Hernández-Camarero P, Moreno-Terribas G, Villalba-Montoro R, Marchal JA, López-Ruiz E and Perán M (2023), Clinical implications of inflammation in atheroma formation and novel therapies in cardiovascular diseases.
Front. Cell Dev. Biol. 11:1148768.
doi: 10.3389/fcell.2023.1148768

Clinical implications of inflammation in atheroma formation and novel therapies in cardiovascular diseases

Shivan Barungi[1†], Pablo Hernández-Camarero[1†], Gerardo Moreno-Terribas[2], Rafael Villalba-Montoro[3], Juan Antonio Marchal[4,5,6,7], Elena López-Ruiz[1,7]* and Macarena Perán[1,4,7]*

[1]Department of Health Sciences, University of Jaén, Jaén, Spain, [2]San Cecilio University Hospital of Granada, Granada, Spain, [3]Tissue Establishment, Centro de Transfusión de Tejidos y Células, Córdoba, Spain, [4]Centre for Biomedical Research (CIBM), Biopathology and Regenerative Medicine Institute (IBIMER), University of Granada, Granada, Spain, [5]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain, [6]Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain, [7]Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain

Cardiovascular diseases (CVD) are the leading causes of death and disability in the world. Among all CVD, the most common is coronary artery disease (CAD). CAD results from the complications promoted by atherosclerosis, which is characterized by the accumulation of atherosclerotic plaques that limit and block the blood flow of the arteries involved in heart oxygenation. Atherosclerotic disease is usually treated by stents implantation and angioplasty, but these surgical interventions also favour thrombosis and restenosis which often lead to device failure. Hence, efficient and long-lasting therapeutic options that are easily accessible to patients are in high demand. Advanced technologies including nanotechnology or vascular tissue engineering may provide promising solutions for CVD. Moreover, advances in the understanding of the biological processes underlying atherosclerosis can lead to a significant improvement in the management of CVD and even to the development of novel efficient drugs. To note, over the last years, the observation that inflammation leads to atherosclerosis has gained interest providing a link between atheroma formation and oncogenesis. Here, we have focused on the description of the available therapy for atherosclerosis, including surgical treatment and experimental treatment, the mechanisms of atheroma formation, and possible novel therapeutic candidates such as the use of anti-inflammatory treatments to reduce CVD.

KEYWORDS

cardiovascular diseases, coronary artery disease, stents, atherosclerosis, cancer, nanotechnology, vascular tissue engineering

1 Introduction

Cardiovascular diseases (CVD) are the leading causes of death and disability in the world, with coronary artery disease (CAD) being the most common (Roth et al., 2017). Current high-income countries' lifestyles, like low physical activity and diets with high fat and glucose content (Kubota et al., 2017; Tappia and Blewett, 2020), contribute to the formation of an atherosclerotic plaque in low diameter vessels such as coronary arteries that supply blood to the myocardium (Libby, 2021a). Atherosclerosis progressively occludes blood vessels and, in certain situations, the atheroma could break after the complete occlusion and form a clot that collapses the vessel (Herrington et al., 2016). In both cases, the patient will suffer a myocardial infarction (MI) that can cause death as a result of the defective irrigation of cardiac muscle by the affected vessels if it is not quickly and properly treated (Tibaut et al., 2017).

Current treatments for atherosclerosis consist of repairing the vascular lumen of the affected vessels to allow a normal blood flow to the compromised muscle (Crea and Libby, 2017). Complementary pharmacological treatments may contribute to a decrease in atherosclerosis progression, for example, by controlling the cholesterol and glucose blood levels as well as trying to prevent thrombosis (Martín-Timón et al., 2014). If the pathology is sustained during a long period without treatment or if the patient suffers MI, the cardiac cell death and adverse cardiac remodelling can lead to terminal heart failure (THF) (Hsich, 2016; Sekuli et al., 2019). The recovery of normal blood flow can be performed percutaneously, by angioplasty, or surgically, by coronary artery bypass (CAB) (Gaba et al., 2021) Figure 1. In the first case, the procedure is minimally invasive, a coronary catheter with a balloon is introduced until the stenotic area, the balloon is inflated and the artery recovers its normal lumen after which the balloon is deflated and removed (Keulards et al., 2020). In most cases, a stent is implanted when the balloon is inflated to keep the vessel open. In the second case, the coronary bypass implies a surgery that uses other healthy autologous vessels, whose replacement is innocuous (usually mammary and saphenous veins or the radial artery (Sánchez et al., 2018), to connect the aorta to a point of the coronary artery distal to the stenotic area. Therefore, this surgical intervention may allow the "by-passing" of the atheroma and the recovery of normal irrigation in the compromised myocardium (Melly et al., 2018). While MI requires a quick recovery of the blood flow to reduce necrosis at maximum, angina represents a non-critical situation which can be solved with slower procedures implying surgical interventions. Thus, angioplasty can be used for both angina and MI whereas CAB is usually reserved for angina (Melly et al., 2018). Even so, these procedures still have important drawbacks to consider. On one hand, stent implantation stimulates vascular wall cells proliferation which promotes re-occlusion of the vessel through a process known as restenosis (Marx et al., 2011). To overcome restenosis and to provide a long-lasting solution, new stent designs have been

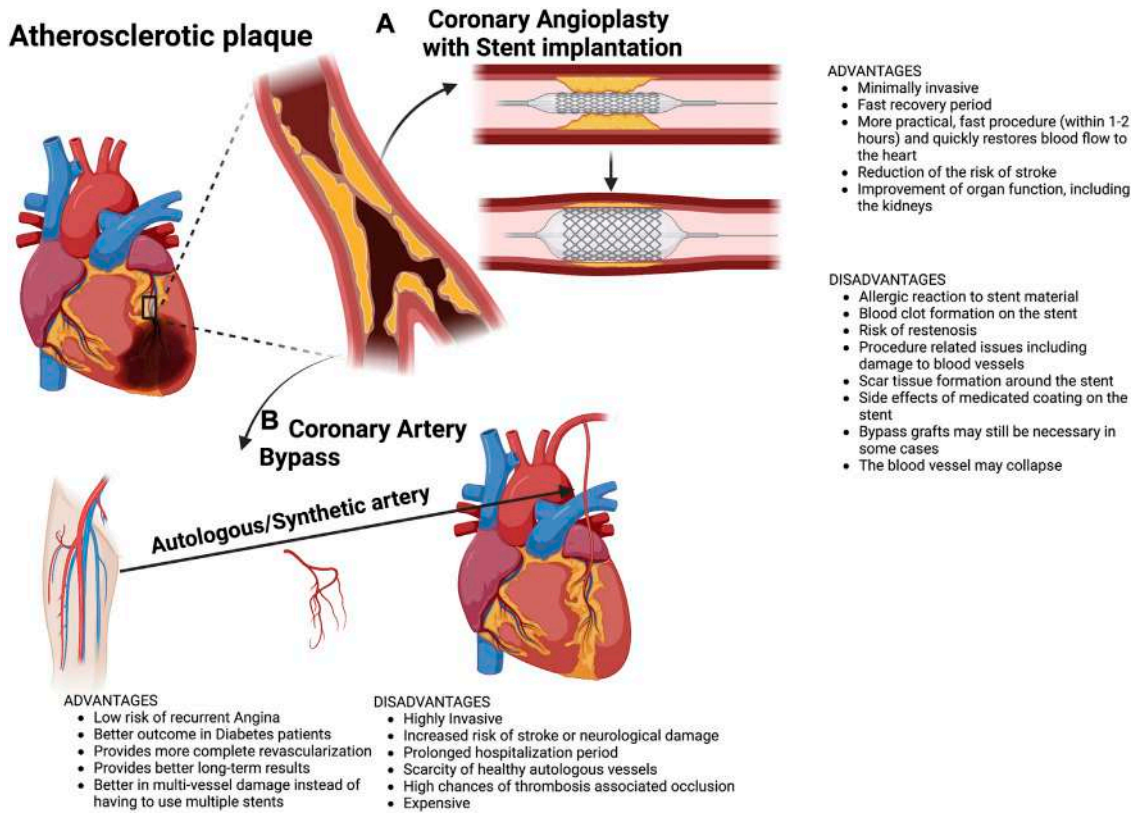


FIGURE 1
Common clinical procedures to treat coronary occlusion by atherosclerosis. **(A)** Coronary angioplasty with stent implantation and **(B)** Coronary artery bypass, either with an autologous artery or synthetic artery. Main advantages and disadvantages of both techniques are listed.

developed which include drug-eluting stents or bioresorbable stents. On the other hand, although CAB surgery has been demonstrated to be more efficient than angioplasty (Xie et al., 2021), it is still an expensive and invasive procedure and in 5%–40% of cases, the graft can collapse from thrombosis-associated occlusion (MacRae et al., 2016). Moreover, healthy autologous vessels are not always available and there are additional difficulties such as the absence of commercially available small-diameter (<6 mm) vascular conduits needed for coronary artery replacement (Carrabba and Madeddu, 2018).

Comparison of clinical outcomes between percutaneous coronary revascularization vs. coronary artery bypass grafting has revealed that both procedures resulted in similar rates of mortality, myocardial infarction, or stroke (Palmerini et al., 2017).

Because of the current limitations in CVD treatments, revascularization therapies have been focused on biological approaches with the aim of restoring, improving and maintaining tissue function over prolonged periods of time (Karacsonyi and Brilakis, 2017). Some of these novel strategies include the use of nanotherapy to prevent in-stent restenosis, or advances in vascular tissue engineering to develop tissue-engineered vascular grafts (TEVGs). A variety of approaches such as electrospinning and 3D printing have been employed to fabricate TEVGs while others strategies are based on the use of allogenic or xenogenic decellularized scaffolds (Naegeli et al., 2022). Parallelly, developing new therapeutic drugs with the potential to promote the atherosclerotic plaque reabsorption or, at least, mitigate its progression to gain time before obtaining an appropriate TEVG may represent a complementary approach. In this line, it is of key importance to understand the biological nature of the atherosclerotic plaque formation (Manubolu and Budoff, 2022).

Current evidence supports the role of inflammation in the initiation and evolution of plaque, with promotion of cell migration and proliferation that lead to lesion progression (Libby, 2021a). In this respect, atheroma or plaque formation has been described as a kind of neoplasm of vascular smooth muscle origin, establishing similarities between atherosclerosis and cancer (Tapia-Vieyra et al., 2017). Thus, stent implantation represents an external agent that could cause restenosis, but other intrinsic agents such as patients' genetic alterations could increase the probabilities of developing the atheroma (Dai. et al., 2016; Shlofmitz et al., 2019).

Here we review common CVD treatments and novel therapeutic approaches focusing on the use of nanotherapy and tissue-engineering strategies. Furthermore, atherosclerosis progression and molecular/physiological events that support the correlation between inflammation and atherosclerosis are highlighted in order to open new avenues and opportunities to develop efficient drugs which may prevent and treat atherosclerosis.

2 Coronary angioplasty and stent implantation

Coronary angioplasty, developed by Dr.Gruntzig in 1977, was the first method described to perfuse stenotic vessels by recovering the vascular lumen through the inflation of a catheter-guided balloon to the atheroma (Sajadian et al., 2018). The procedure is minimally invasive, relatively non-expensive, effective, and the patient, in most cases, can leave the hospital the same day (King and.ii, 2021). Nevertheless, it has been shown that after a while, angioplasty vessels progressively return to their occluded status in between 30%–40% of the cases (Denktas, 2010) thus, to avoid the abrupt vessel collapse after angioplasty, a little expandable coil or stent is implanted (Sigwart et al., 1987).

It was in 1986 when the first human coronary artery implant was carried out using WALLSTENT® (Schneider AG). This was a structure made of a stainless steel wire-mesh that was self-expanding (Iqbal et al., 2013). Non-etheless, it was taken off the market a few years later due to existing limitations in the stent delivery system which limited its clinical utility. Over the years, many more stents were developed like Multi-link® (Advanced Cardiovascular Systems), Micro® (Applied Vascular Engineering) and Wiktor® (Medtronic) to mention but a few. This was a major advance in the field of coronary angioplasty but it did not come without a number of drawbacks as most of the early stents were bulky and hard to manage technically due to their high metallic density which ultimately led to a high rate of sub-acute thrombosis (Iqbal et al., 2013). Moreover, the mechanical stimuli over the cell wall provoked cytokine release that stimulates cell proliferation and migration to the injury. This phenomenon is named in-stent restenosis and may provoke coronary artery re-occlusion through an inflammatory and proliferative response against the foreign body (Buccheri et al., 2016; Cornelissen and Vogt, 2019). Hence, despite there being reduced restenosis in comparison to the plain old balloon angioplasty, the occurrence of in-stent restenosis was still high because of the migration and proliferation of cells within the stents (Iqbal et al., 2013). In fact, in-stent restenosis was associated to a high mortality and morbidity rate (Drozd et al., 2010). According to these data, restenosis and the immune response against the stent are two of the main hurdles in this therapeutic approach. Remarkably, both events are characterized by a pro-inflammatory response (Iwata et al., 2021), so it seems reasonable to assume that the immune reaction against the stent may be deeply involved in the promotion of in-stent restenosis. With the aim to solve in-stent restenosis associated complications, a new generation of stents including drug-eluting stents (DES) and bioresorbable stents (BS) have been developed (Buccheri et al., 2016). Figure 2 schematically shows the implantation of the stent and the subsequent appearance of restenosis together with different type of stents.

Regarding DES, different compounds have been used to cover bare metal stents (BMS) to target the proliferation of vascular smooth muscle cells (SMC), platelet activation, inflammation and thrombosis. Examples of DES are heparin-coated BMS, used to prevent thrombosis, and BMS loaded with phosphorylcholine which imitates the cell membrane, but the benefits have been limited (Iqbal et al., 2013). DES can also be coated with bioactive compounds that avoid vascular SMC activation and proliferation, reducing restenosis development and also modifying the healing process after implantation (Bønaa et al., 2016; Brugaletta et al., 2021). Everolimus, sirolimus and paclitaxel have been shown as antineoplastic agents with a high potential to reduce neointimal overgrowth, maintaining a bigger vascular lumen. Their mechanism of action is based on mTOR inhibition, decreasing cellular anabolic metabolism, growth, division and migration; fundamental pillars of restenosis. Recent studies have shown that there is a clear benefit when using everolimus eluting stents as compared to other BMS in

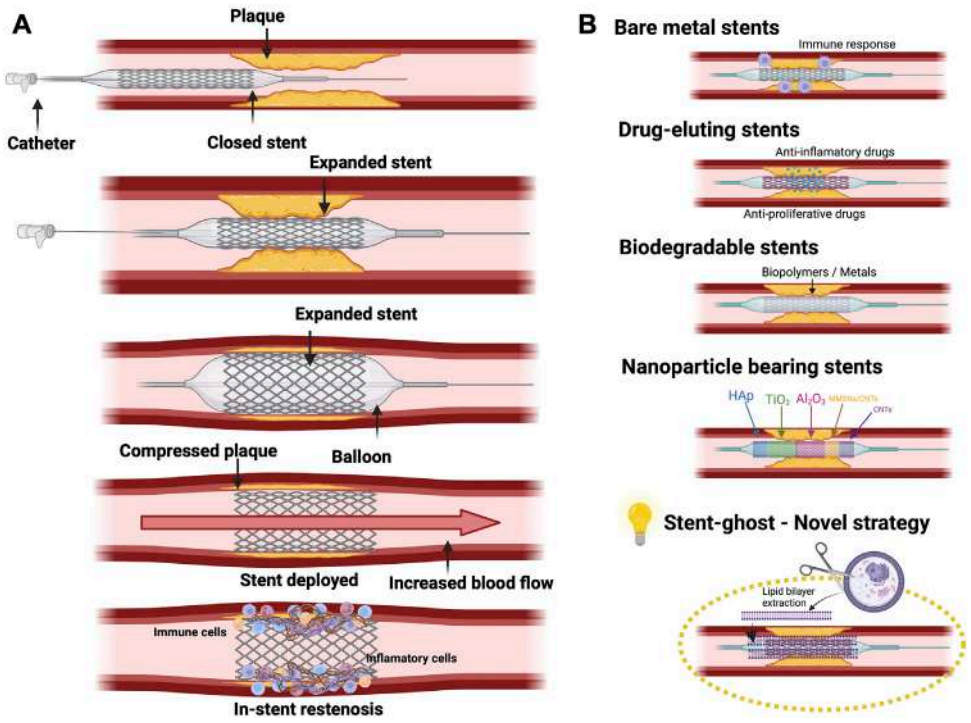


FIGURE 2
(A) Representative stent implantation procedure, including implantation, expansion and lastly, in stent-restenosis formation. **(B)** Representation of different Stents used in clinic: Bare metal stents; Drug-eluting stents; Biodegradable stents; nanoparticle bearing stents. The use of a "Stent ghost" which is a stent coated with cell membranes could represent an innovative therapy to escape the immune surveillance.

regards to targeting lesion revascularization and the acquired results depending on the patient type and device used (Brener et al., 2013; Sakurai et al., 2013). Apart from the mTOR pathway inhibition, other strategies have been considered to avoid restenosis. For instance, potassium channels blockade, which are necessary for SMC activation (Lasch et al., 2020). In fact, it has been shown that the inhibition of voltage-gated K + 1.3 channels could reduce restenosis by targeting vascular SMC (Bobi et al., 2020; Ye et al., 2020).

Anti-inflammatory drugs are used to inhibit the inflammatory response derived from stent implantation, reducing one of the main events that promote neointimal overgrowth. The promising use of Dexamethasone has been proved in preclinical models and in clinical studies (Radke et al., 2004; Braga et al., 2019). For instance in porcine damaged coronary arteries to study drug delivery to the tissue, with effectivity being seen in the first 28 days (Lincoff et al., 1997), and in canine femoral arteries that showed a significant decrease in the neointimal hyperplasia (Strecker et al., 1998). In other clinical studies, the dexamethasone-eluting stents have shown benefits compared to BMS, demonstrating a significant reduction of major adverse cardiac events at 12 months and in the restenosis/neointimal proliferation rates at 6 months (Lincoff et al., 1997; Strecker et al., 1998; Pesarini et al., 2009; Park et al., 2013). It is important to note that 10% of the patients treated with DES continue to suffer from restenosis and angina. Further, a delay in the healing of the injured vessel and very late thrombosis after DES implantation have been described (van Beusekom et al., 2007; Taniwaki et al., 2016). Therefore, there is still a necessity to develop less harmful stents/drug association to reduce their adverse effects (Wang et al., 2019).

Dual drug-eluting stents (Dual-DES) combine the beneficial effects of various compounds, while reducing individual dosage and associated adverse effects (Zhang et al., 2017; Cha et al., 2020). For instance, the combined effect of sirolimus (antiproliferative) and triflusal (antithrombotic) eluting stents, have been proven, *in vitro* and *in vivo,* to efficiently deliver both drugs, each in its appropriate dose, and to gain greater reduction of restenosis compared to the single drug-eluting stents (Huang et al., 2010). Other example are prednisolone plus sirolimus Dual-DES, in which, prednisolone enhances the effect of sirolimus, achieving a greater reduction of SMC proliferation, restenosis, fibrin expression and inflammation as well as an enhanced re-endothelialization (Lee et al., 2016). Furthermore, Byrne et al. (2009) have proved that Dual-DES (rapamycin and probucol) in patients with CAD showed a higher reduction of restenosis than simple stents.

Bioabsorbable/biodegradable stents (BS) are another new generation of stents that try to reduce injury by naturally dissolving or being absorbed by the body (Erne et al., 2006). These types of stents can be composed of metals, such as magnesium (Mg) or zinc (Zn), or biodegradable polymers such as poli-D,L-lactic acid (PDLLA), poli-L-glycolic acid (PLGA) or poly(L-lactide–co-ε-caprolactone) (PLCL) that can be reabsorbed in the body after some time (Jinnouchi et al., 2019; Beshchasna et al., 2020). BS present an advantage over other types of stents because they eradicate the factor of a foreign material remaining in the body

permanently which is essential in avoiding immune reactions and having to remove the stent later on if necessary (Borhani et al., 2018). Obviously, BS have to be made by biocompatible and non-toxic materials (Joner et al., 2018; Zhang et al., 2021a). In this respect, Mg and its alloys have been used in the development of the first metallic BS because Mg is highly biocompatible and also presents low thrombogenicity. In addition, in an aggressive chloride environment like the human body, its degradation is fast (Gu et al., 2009). Other metal alloys containing iron (Fe) or Zn have also been among the pioneer metals used to manufacture BS (McKavanagh et al., 2018; Bowen et al., 2019). In fact, Bowen et al. (2013) described that Zn and its alloys had a slower degradation rate in comparison to Mg and Fe. Combining this with its good biocompatibility and mechanical properties, Zn and its alloys have been shown to be a safer choice to avoid issues associated with Mg and Fe BS since Mg has a faster corrosion rate than Zn and the corrosion of Fe produces non-bioresorbable iron oxides.

The second generation of BS was composed of biodegradable polymers that generate more innocuous products during their degradation than metallic oxides. One of the most frequently used biodegradable polymers is PLLA due to its high biocompatibility (Xiao et al., 2015). In a period of 12–18 months, PLLA is metabolized into Carbon dioxide and water *via* the Krebs cycle with no toxic products resulting from the degradation. The first globally reported fully degradable stent was the PLLA-based BS by Tamai et al., 2000. Down the road, there have been more advances in bioabsorbable polymeric stents in terms of their capability in drug deliverance and the way they are manufactured. Other polymers like PLGA, polyhydroxycarboxylic acids (PHCA), poly(3-hydroxybutyrate) (P3HB) (Williams et al., 2013) and PLCL are under study to assess their biocompatibility and functionality. Further examples of PLLA stents are Tissue Gen, ARTDIVA and Elixir, whose mechanical characteristics have been extensively studied (Charpentier et al., 2015; Zhao et al., 2016; Wang et al., 2022). In fact, it was shown in the study about everolimus eluting PLLA stent Absorb-BVS-System that the mechanical strength of the stent rapidly deteriorated after the first 3 months following implantation (Bil and Gil, 2016). There have also been other polymers like poly(vinylidene fluoride)-hexafluoropropylene (PVDF-HFP) that have been used with second or third generation DES (Waksman, 2017; McKavanagh et al., 2018). Sirolimus and salicylic acid have also been combined with a poly-anyhydride ester to create a BS that has been found to have both anti-inflammatory and anti-proliferative characteristics (Charpentier et al., 2015). Although, there is a number of polymers being used for medical purposes with properties that make them suitable for stent manufacturing, there are still some important issues to resolve, for example, the generation of toxic products, poor mechanical properties and an unsatisfactory degradation rate (McKavanagh et al., 2018). Taking all this into account, BS still do not present enough radial strength and stiffness in comparison with BMS and this can lead to fatigue and fracturing issues after the stent is implanted. In regards to this, research has been carried out to improve the mechanical properties of BS and it has been shown that plasticizing is an effective solution (75). The sterilization techniques used on the stent must also be considered to avoid affecting the molecular weight and crystallinity of the material (Weir et al., 2004).

Bioresorbable drug eluting vascular scaffolds (BVS) are made up of polymers that will disappear after drug elution (Weir et al., 2004). Interestingly, several randomized trials have shown promising results when comparing BVS with traditional DES (Ellis et al., 2015; Nogic et al., 2018). Although the advantage of BVS is the transitivity of the scaffold, while presenting a longer period of time drug delivery, their efficacy and safety over time have not yet been proven due to limited data.

Nevertheless, it is important to remark that bioabsorbable stents have not represented a significant improvement clinically despite their initial promising results. To date, only two bioabsorbable stents are commercially available, Magnesium Magmaris (Biotronik) and polylactic Absorb (Abott) (Mridha et al., 2019). In addition, a recent randomized meta-analysis study comparing the mid- and long-term clinical outcomes of both durable polymer drug-eluting stents (DP-DES) and bioabsorbable polymer drug-eluting stents (BP-DES) revealed no statistically significant differences in cardiac mortality, stent thrombosis, target lesion revascularization, target vessel failure or reinfarction rates (Mridha et al., 2019).

In fact, a conducted trial to compare BVS *versus* metallic evorilimus-eluting stents (EES) did not produce conclusive results, since no significant differences were found between patients from either group in terms of cause of death after 1 year (Tamburino et al., 2016).

The first BVS to be put on the market and to be clinically used was the Absorb BVS (Abbott Vascular, Santa Clara, CA, United States) but even it had limitations in regards to scaffold recoil and thrombosis (Seo et al., 2020). A longer randomized study that tested the differences between BVS and DES was carried out in over 500 patients and there was a noted decrease in angina recurrence and deterioration in patients after 12 months (Caiazzo et al., 2015). None the less, after 3 years, there was in-stent loss and nitrate induced vasomotion observed. The original Absorb BVS had a strut thickness of 150 μm and this has been attributed to being the cause of the adverse reactions observed (Caiazzo et al., 2015). Thus, this led to the development of second and third generation BVS to tackle the issues of the stent's strut thickness. Therefore, a next-generation of scaffolds with smaller strut thickness were developed, for example, Biolute BRS with a strut thickness of 108 μm and MeRes and DESsolve, both with a strut thickness of 100 μm (Seo et al., 2020). The expected advantages from this new generation of BVS would be lessening the flow disturbances and in the long run, reducing platelet activation and thus the scaffold's thrombogenicity. Besides the strut thickness of the BVS, another promising research area is reducing the reabsorption time of the scaffold. In this regard, it has been demonstrated that DESsolve scaffolds are bio-absorbed and biodegraded within 1–2 years (Seo et al., 2020).

All this does not come without limitations and as mentioned before, this is a field that is promising but one that requires further research. Kozuma et al., further prove this with a 5 years follow up study they carried out to assess the long-term results of using BVS in comparison to DES that showed comparable results in regards to patient and device based outcomes (Serruys et al., 2015).

In short, there is still a need of more studies to prove the benefits of BVS in comparison to DES since studies carried out in the past 10 years showed BVS´s adverse effects and non-significant improvement in terms of patient´s outcomes in comparison to other types of stents (Serruys et al., 2015; Kozuma et al., 2020).

Other strategies to improve stent hemocompatibility and reduce restenosis are based on the implementation of nanotechnology (Zhao et al., 2016; Botelho et al., 2021; Georgakarakos et al., 2021). The use of nanoparticles (NPs) to inhibit restenosis has been reviewed (Karimi et al., 2016). In short, different types of NPs have been tested, such as liposomes, phospholipid-based micelles; polymeric nanoparticles; hydrogel nanospheres and magnetic nanoparticles leading to reduction of inflammation and angiogenesis (Zhao et al., 2016; Hu et al., 2020).

Interestingly, similar to stents, the clearance of systemically inoculated nanoparticles by immune surveillance also represents a major barrier in the context of nanotherapy which has been extensively assessed (Hu et al., 2020). Thus, from a new perspective, the use of therapeutic nanoparticles, for instance directed against tumour cells, could be compared with the implantation of a stent. In both cases, foreign elements are recognized by the immune system and they cause rejection. Therefore, the advances related to the use of nanoparticles in other diseases can be applied to the field of stent engineering. In this regard, several strategies have been designed in order to avoid immune reaction, for example, the classical nanoconstructs surface grafting with polyethylene glycol (PEG) polymers to reduce their recognition by the reticuloendothelial immune system (Senti et al., 2022). Nevertheless, the authors also highlighted the short-term perspectives of this approach due to the generation of anti-PEG antibodies by the host adaptive immune response. Furthermore, the functionalization of nanoplatforms with CD47, a self-recognition molecule, with the aim of avoiding or reducing their clearance by the innate immune system has also been shown (Pham et al., 2021).

On the other hand, significant advances in the last few years have led to the trend of cell membrane-coated nanoformulations with the ability to biomimic and efficiently evade their removal by phagocytosis (Cai et al., 2022). According to these data, it seems reasonable to suggest the translation of nanotherapy-associated advances, regarding to immune evasion, to the development of new generation stents with reduced restenosis induction and improved biocompatibility. For instance, it may be interesting to look into the development of a CD47-homologous peptide-coated stent to increase its biocompatibility (Inamdar et al., 2020) or even cell membrane-coated stents. To this end, several cell membranes could be used, highlighting autologous endothelial cell-derived membranes in order to fabricate a kind of stent which may simulate a continuity of the host endothelial barrier. Obviously, this is a mere theoretical suggestion which should be experimentally assessed. In this respect, the importance of the cell-derived membrane orientation during the coating process should be remarked upon since it has been reported that the right-side-out orientation is the one which provides the appreciated immune-evasive properties (Bu et al., 2021).

3 Tissue-engineered vascular grafts

Currently, the leading, long-term therapeutic strategy to treat severe, but not extremely urgent, obstructions in small-diameter vessels (lower than 6 mm), highlighting coronary arteries, consists of performing bypass grafting using autologous vasculature like the saphenous vein, radial arteries or internal thoracic artery (Sánchez et al., 2018). Nevertheless, bypass grafting involving autologous vessels may not be suitable in many cases due to a range of circumstances (i.e., inappropriate graft size, previous diseases, unfavourable operation history or the multiple nature of the vascular occlusion) (Cai et al., 2021; Georgakarakos et al., 2021). Additionally, other inconveniences should be highlighted, such as the vein graft failure due to stenosis (Botelho et al., 2021). In a particular example, the internal mammary artery is commonly used for bypassing the left anterior descending artery in patients with CAD. In this regard, a clinical trial study revealed that the failure of this procedure had a statistical frequency near 10% and it was associated with some risk factors including vessel stenosis or the presence of additional bypass grafts in the diagonal branch (Harskamp et al., 2016). Indeed, the study established a link between the higher incidence of acute clinical events and an increased rate of repeat revascularization (probably derived from the traumatization of the body because of the external manipulation of autologous vessels) (Harskamp et al., 2016).

Large diameter synthetic grafts that are available on the market usually exhibit an acceptable long-term patency rates, however, small diameter synthetic vascular grafts present limited clinical application, in regards to poor mechanical properties and the appearance of infections, induction of intimal hyperplasia or thrombosis (Weekes et al., 2022). In addition, there is no commercially available synthetic vascular graft for small diameter blood vessels. As an alternative, the replacement of the damaged vasculature by the development of tissue-engineered vascular grafts (TEVGs) which can be generally classified into scaffold-based TEVGs or self-assembled scaffold-free TEVGs has been proposed (Chen et al., 2021).

For instance, an alternative to constructed scaffolds is the use of natural scaffolds which already have all the properties that are needed, hence the use of decellularized vessels. The decellularization strategy relies on obtaining an acellular biological scaffold by removing donor tissue-resident cellular populations in order to minimize adverse host immune rejection, while simultaneously preserving the extracellular matrix and tri-dimensional structure. However, in order to have a practical clinical application as a biological scaffold, the decellularized vessels must be previously re-cellularized, preferably, with the patient´s own cells (Lin et al., 2018). As an example, the decellularization of porcine carotid arteries with the aim of obtaining biological vascular scaffolds feasible to be used as vascular grafts has been described (López-Ruiz et al., 2017; Cai et al., 2021; Heng et al., 2021). Nevertheless, small-diameter TEVGs based on allogenic or xenogenic decellularized scaffolds still have several limitations to be addressed before translation to clinical practice. For instance, the induction of host immune response against the vascular graft, inappropriate physical properties of the graft compared with native vessels which usually promotes the generation of aneurysms, graft-related thrombosis or graft-associated infections (Lin et al., 2018). In addition, developing an appropriate decellularization protocol still remains a matter of debate considering it should effectively remove not only host cells but also immunogenic antigens like α-Gal (Jin et al., 2022).

The development of techniques such as electrospinning and 3D printing have facilitated the fabrication of scaffolds that mimic

natural vessels' structures (Weekes et al., 2022). To date, *in vitro* and *in vivo* experiments have shown encouraging outcomes (Fazal et al., 2021; Rickel et al., 2021). Regarding the use of synthetic scaffolds, the development of a thermoplastic polyurethane synthetic vascular graft, using 3D printing technology, with improved mechanical and biocompatibility features compared with commercially available, non-biodegradable polytetrafluoroethylene grafts has been shown. In this study, the vascular graft was implanted into a rat abdominal aorta model using a patch technique. Despite it not being a full graft technique, the synthetic graft was shown to reduce calcification and thrombus formation in comparison to the standard polytetrafluoroethylene graft 30 days post-operation (Sohn et al., 2021). However, biofabrication techniques, including bioprinting, still have some insurmountable limitations. Microfabrication technologies need to better mimic native vascular anatomy to minimize dimensional disparities at the anastomosis site and TEVG (Henry et al., 2017; Fazal et al., 2021). Along with this, further research with large animals is needed, since only a few studies have been performed (Fang et al., 2021). To date, most of the studies have been conducted on rats and rabbits. Moreover, there is a need for longer *in vivo* studies (>12 months) since the patency rate is still considerably lower than in autologous grafts (Horakova et al., 2018).

In fact, self-assembled, scaffold-free vascular grafting is a novel strategy that takes advantage of recent advances in bioreactors and bioprinting technologies. In this line, Itoh and colleagues patented a procedure to create small-diameter, scaffold-free TEVGs by combining 3D bioprinting and bioreactor-based culturing approaches. First, tubular structures composed of human umbilical endothelial cells, human aortic smooth muscle cells and human dermal fibroblasts were generated using a 3D bioprinter. Then, these tubular structures were cultured in a perfusion system (bioreactor) and matured before their implantation in a rat model (Itoh et al., 2015). Another alternative to constructed scaffolds is the use of natural scaffolds which already have all the properties that are needed, hence the use of decellularized vessels. The decellularization strategy relies on obtaining an acellular biological scaffold by removing donor tissue-resident cellular populations in order to minimize adverse host immune rejection, while simultaneously preserving the extracellular matrix and tri-dimensional structure. However, in order to have a practical clinical application as a biological scaffold, the decellularized vessels must be previously re-cellularized, preferably, with the patient´s own cells (Lin et al., 2018). As an example, the decellularization of porcine carotid arteries with the aim of obtaining biological vascular scaffolds feasible to be used as vascular grafts has been described (López-Ruiz et al., 2017; Cai et al., 2021; Heng et al., 2021). Nevertheless, small-diameter TEVGs based on allogenic or xenogenic decellularized scaffolds still have several limitations to be addressed before translation to clinical practice. For instance, the induction of a host immune response against the vascular graft, inappropriate physical properties of the graft compared with native vessels which usually promotes the generation of aneurysms, graft-related thrombosis or graft-associated infections (Lin et al., 2018). In addition, developing an appropriate decellularization protocol still remains a matter of debate considering it should effectively remove not only host cells but also immunogenic antigens like α-Gal (Jin et al., 2022).

The development of a TEVG is still a challenge due to anatomical complexity. Additionally, the mechanical and biological functions of TEVGs need to be improved for TEVGs to make their way to the clinical use (Konig et al., 2010). One of the main causes of vascular graft failure is the lack of a confluent endothelium due to the thrombogenicity of the graft (Joseph et al., 2022; Weekes et al., 2022). Acellular and decellularized TEVGs are commonly affected by thrombosis and thereby early failure (Owens et al., 2015). A variety of studies have focused on the issue of vascular regrowth after TEVG implantation due to its high complexity. To reduce the thrombogenicity of the TEVG, multiple solutions have been explored including the combination of synthetic and natural materials, the incorporation of anticoagulant molecules (e.g., heparin), or the functionalization with several drugs and factors (e.g., VEGF) to promote endothelialization (Rickel et al., 2021). Recently, a new magnetic approach to accelerate cell retention and improve cellular density which consists of the use of a magnetic hydrogel based on bacterial cellulose to target vascular cells has been proposed (Arias et al., 2018).

Nevertheless, the key to long-term thrombosis prevention is the formation of both the ECs and the SMCs layers. Both layers are critical to prevent intimal hyperplasia and for the function of native vessels (Ju et al., 2017). Moreover, the properties of the polymers must be carefully managed to avoid excessive migration and proliferation of cells which can lead to intimal or neointimal hyperplasia and stenosis, which typically reduces the patency of the vessel graft (Leal et al., 2021).

Given the importance of developing cellularized- TEVGs, different cell sources "to dress" the grafts have been proposed. In this respect, autologous cells are the ideal candidate to minimize host immune reactions against the vascular grafts (Chen et al., 2021). However, using autologous cells, such as, endothelial and smooth muscle cells, still present several hurdles like limited harvesting potential and the inability to reconstitute neo-tissues due to poor *ex vivo* differentiation and expansion rates. To overcome these limitations, the use of human skeletal myoblasts has been proposed as an alternative cell source, taking advantage of their high *in vitro* proliferative capacity (Saito et al., 2021). Another approach could rely on the generation of autologous induced pluripotent stem cells from peripheral blood-derived mononuclear cells and inducing their differentiation towards endothelial and smooth muscle cells to develop TEVGs. This approach can´t be translated clinically but it could be useful as a model of developing pathologic vessels with patient-specific cells to test novel therapeutic agents (Generali et al., 2019).

Interestingly, another alternative to generate highly compatible autologous TEVGs consists of the subcutaneous implantation of tubular mandrels in the own host that will receive the future TEVG. This strategy takes advantage of the host immune reaction against the foreign body (tubular mandrel) around which a fibrotic capsule will be generated. After the maturation of the construct, the formed fibrotic conduit is extracted and used as a TEVG and this is very advantageous because of the short process duration (approximately 4 weeks) and the mechanical strength expressed by the resulting matrix *in vivo* (Qiu et al., 2021).

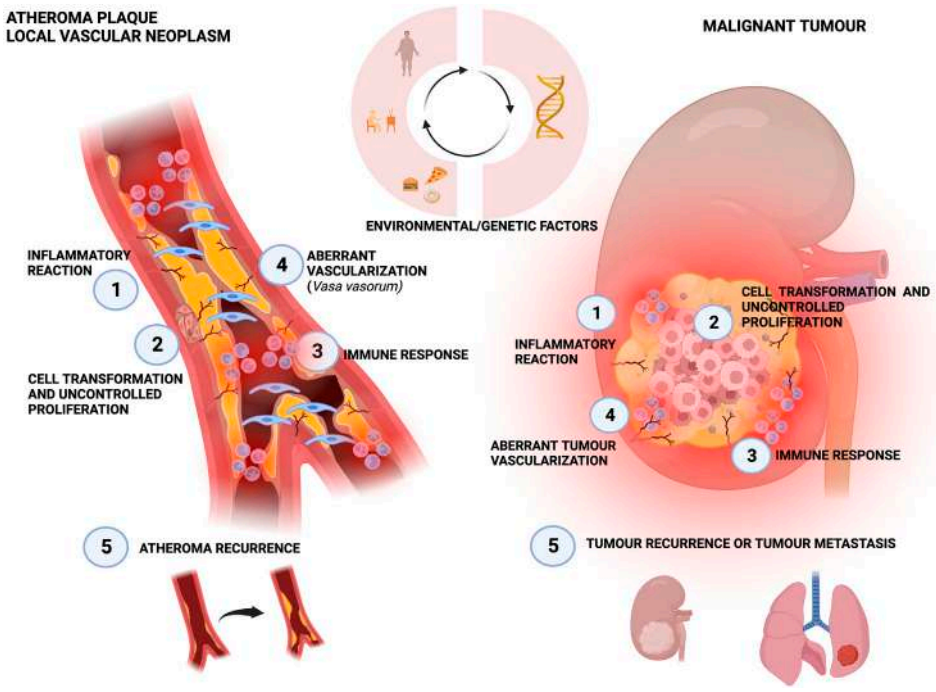


FIGURE 3
Similarities between atherosclerotic plaque and local neoplasia. The three circular arrows show that both diseases can be influenced by these same risk factors. Both processes are initiated by an inflammatory reaction (Roth et al., 2017), that leads to cell transformation and uncontrolled proliferation (Kubota et al., 2017), which in turn triggers the immune response (Tappia and Blewett, 2020) and an aberrant vascularization (Libby, 2021a). Finally, recurrence is frequently present for both atheroma and cancer (Herrington et al., 2016).

Nevertheless, the clinical translation potential of fibrotic capsule-based TEVGs may be deterred as it represents an invasive procedure highly dependent on either the host physiological characteristics (gender, age or pre-existing diseases like diabetes), the implant location or the implant features (i.e., chemical composition or topography). Additionally, it also requires an extensive period of time for its incubation (several weeks) (Geelhoed et al., 2017), so this therapeutic strategy remains mainly in the pre-clinical field. In fact, efforts are being made to shorten TEVGs production time to under 2 weeks in order to make them suitable for translation to clinical practice (Von Bornstädt et al., 2018). Considering the high recurrence frequency of atherosclerosis, one strategy to reduce the time-limiting factor of TEVGs in the case of disease-recurrent patients could be their initiation at the time of the first therapeutic intervention, immediately after the recurrence of the vascular obstruction, as a preventive measure. Moreover, a second surgical intervention may increase post-operatory complications and significantly reduce patient quality of life (Bianco et al., 2021). Considering the period between the application of the by-pass and noticeable reactions to it in the patient as a crucial clinical determinant, it seems reasonable to remark upon the importance of understanding the biological nature of atherosclerotic plaque formation. Such knowledge may provide us with relevant cues to develop specific drugs with the potential to promote atherosclerotic plaque reabsorption or, at least, mitigate its progression in order to gain time before obtaining an appropriate TEVG.

4 Inflammation leads to atheroma formation and tumour cell mass initiation

Traditional concepts in which a low-density lipoprotein is the sole cause of atherosclerosis are lately being questioned. The idea that atherosclerosis is a chronic inflammatory disease, with inflammation playing a central role in each stage of the atherosclerotic plaque life cycle has been gaining interest over the past few years (Libby, 2021b). In fact, the use of inflammatory biomarkers has proven to be able to predict the risk of cardiovascular disease before any signals of the appearance of symptoms (Ridker et al., 2018). Similarly, aberrant tumour cell growth is also supported by a background of chronic inflammation which represents a main risk factor for oncogenesis (Liu et al., 2021; Neufert et al., 2021). In this respect, although atherosclerosis and cancer are two different diseases with distinct clinical management, and cancer is commonly associated with genetic mutations whereas atherosclerosis is rather related to environmental causative factors, there are still some similitudes that are relevant to remark (Figure 3). In fact, an interesting point of view relies on considering atheroma formation as a kind of local malignancy rising from the vascular wall (Fasehee et al., 2019), thus being a “wound that never heals” (Garcia-Sabaté et al., 2020). The start point of atheroma formation involves the activation of endothelial cells from a quiescent-like phenotype towards a proliferative one (da Luz et al., 2018) and interestingly, several stimuli can trigger this event through a pro-inflammatory signalling cascade, including LDL accumulation in the

subendothelial space (Guo et al., 2021), blood flow disturbance at arterial curvatures or branch points (Chien et al., 2021).

Thus, the relevance of NFkβ pro-inflammatory axis on a molecular level in the activation of endothelial cells to generate atherosclerotic lesions in response to changes in blood flow hemodynamics has been noted (Singh et al., 2021). Remarkably, a link between single-nucleotide genetic alterations and inflammation can be established by focusing on DNA-based deaminases like APOBEC3s family members or AID which induce the transition from Cytosine to Thymine and are enhanced by pro-inflammatory signals (Petljak et al., 2019; Zong et al., 2019). Considering these facts and the documented association between DNA-based deaminases and carcinogenesis (Nishikori et al., 2021), it may be tempting to suggest the existence of an important role of deaminases in atherosclerosis.

Furthermore, the activated endothelium can undergo a process known as endothelial-to-mesenchymal transition (endMT) by which endothelial cells can acquire mesenchymal-like features partially losing their endothelial markers. In fact, the endMT process confers a remarkable phenotypic plasticity since it has been shown that endothelial cells can become smooth muscle-like cells and fibroblast-like cells during atherosclerosis (Zhao et al., 2021). On a molecular level, the importance of the TGFβ/SMAD signalling pathway along with the Wnt2 axis in the progression of endMT, playing a key role in the increase of endothelial cells migration, invasion and neointimal formation has been reported (Zhang et al., 2021b). Moreover, it may be reasonable to propose that the activation of TGFβ and the endMT process may be early events in atherosclerosis since non-laminar blood flow and DNA methylation changes have been associated with the promotion of the endMT process and Wnt/β-catenin signaling pathway in endothelial cells (Björck et al., 2018). Interestingly, the endMT process can be considered as the homologue of epithelial-to-mesenchymal transition (EMT) by which almost all types of tumours exhibit a more aggressive phenotype.

The uncontrolled proliferation of vascular SMC which migrate from the media to the intima vascular layer and contribute to neointimal hyperplasia and arterial remodelling during atherosclerotic plaque progression (An et al., 2022) could resemble oncogenesis. Specifically, vascular SMC undergo a phenotypic switch from a quiescent and contractile phenotype towards a proliferative and migratory one with enhanced capacity to synthesize extracellular matrix components like collagen. Notably, the key role of miRNAs in such a dedifferentiation process by regulating intimal thickening has been reported, with the miR-146b-3p/PIK3CG axis as a representative example (Zhuang et al., 2021). Of note, the central role of transcription factor KLF4 in this phenotypic change triggered by several stimuli including the exposure of contractile vascular SMC to oxidized LDL has been highlighted (Wang et al., 2021). Parallelly, the phenotypic switching undergone by cancerous cells, from a well-differentiated phenotype towards a cancer stem-like one, which could also be termed as a "dedifferentiation process" has been widely described, with crucial roles during tumour progression of enhancing cellular aggressiveness and metastasis (Sandiford et al., 2021). Supporting the homology between vascular SMC and malignant cells, the clonal expansion of vascular SMC which have the potential to originate the majority of the cell types within atherosclerotic lesions has been confirmed (Wang et al., 2020). Interestingly, this fact may resemble the ability of cancer stem cells to generate all cancer subpopulations within a tumour mass. Indeed, the existence of a kind of "atherosclerotic stem cell" with an SMC lineage has already been suggested (Direnzo et al., 2017) which reflects a homologous potential to cancer stem cells.

In the same line, it has been revealed that vascular SMC phenotypic plasticity is far more complex than described above since SMC could even acquire distinct cellular fates, according to some single-cell analysis-based studies, such as myofibroblast-like (Wirka et al., 2019) or macrophage-like phenotypes (Brandt et al., 2022). More importantly, the recruitment of circulating innate immune cells, including monocytes/macrophages, into the intimal vascular layer in an early stage of atheroma biology has been confirmed. Specifically, the activation of endothelium triggered, for instance, by blood flow disturbances can lead to the expression of adhesion molecules, like ICAM1, in the surface of activated endothelial cells which promotes leucocytes extravasation (Murphy et al., 2018). Therefore, these data may suggest the relevance of both fibroblast-like and macrophage-like cells in atherosclerotic occlusions. Similarly, the generation of a complex tumour microenvironment around solid tumour masses, with cancer-associated fibroblasts (CAFs) and tumour-associated macrophages (TAMs) playing significant roles in the support of cancer progression has been widely observed (Tang et al., 2022). Focusing on plaque-associated macrophages (PAMs), it is relevant to note the distinct polarization they can acquire within the plaque, with pro-inflammatory, M1-like PAMs being correlated with atherosclerosis progression and plaque instability/rupture whereas anti-inflammatory/regenerative, M2-like PAMs are correlated with disease regression, plaque stability and better prognosis (Garcia-Sabaté et al., 2020). An equal bimodal polarization can also be identified regarding to TAMs but, intriguingly, with opposite roles since M1 TAMs have been associated with an anti-cancer behaviour while M2 TAMs have been classified as pro-tumorigenic cells (Hernández-Camarero et al., 2021). In any case, macrophage-like cell specific polarization may be a factor of key importance in both disorders. Remarkably, M1-like polarization in atherosclerotic plaque triggered by vascular SMC secretome has been shown to exhibit a diminished potential to recognize and remove opsonized diseased/unwanted cells, like proliferative vascular SMC (Wang et al., 2020). In other words, pro-atherosclerotic vascular SMC can modify immune cells like PAMs in order to escape immune surveillance, in a similar fashion to cancerous cells.

Next, it may be relevant to highlight that the intima-media thickening mentioned earlier, along with the accumulation of pro-inflammatory factors and chemokines in the adventitia vascular layer, could induce oxygen deficiency which may trigger abnormal vasa vasorum neoangiogenesis within atherosclerotic lesions (Li et al., 2021). On the other hand, solid malignancies have also been characterized by strongly enhanced angiogenesis within the tumour mass. Indeed, it is thought that uncontrolled cellular proliferation and growth lead to local lack of oxygen and nutrients which may trigger the formation of abnormal intra-tumour vasculature characterized, for instance, by deficient pericyte coverage (Xu et al., 2021).

Traditionally, the main strategy to prevent/manage atherosclerosis relies on controlling its risk factors, i.e., blood LDL levels, hypertension or life habits like smoking. Although, atherosclerotic occlusion can be removed by atherectomy, a common recurrence of the disease within

2 years after the surgical intervention in up to 50% of patients has been noted (Bath et al., 2021). Interestingly, this fact may be similar to the well-known recurrence of malignant growth after the surgical removal of tumour mass. Thus, it is key to consider atherosclerotic plaque formation as a continuous process which may progress over time, so any effective therapeutic strategy may be a long-term treatment rather than a punctual procedure (Botts et al., 2021).

Considering the implication of the chronic inflammatory response in atherosclerosis, the administration of anti-inflammatory drugs like Omentin-1 has been proposed (Lin et al., 2021).

Recent clinical trials have shown that targeting inflammation can reduce cardiovascular events. The "Canakinumab Anti-inflammatory Thrombosis Outcomes Study" (CANTOS) which was a randomized double-blind trial, targeting the interleukin-1β innate immunity pathway with the monoclonal antibody canakinumab concluded that the antiinflammatory therapy led to a significantly lower rate of recurrent cardiovascular events than the placebo, independent of lipid-level lowering (Ridker et al., 2017a). Although patients treated with canakinumab showed greater incidence of infections, one positive event was the reported highly significant reduction in incident and fatal lung cancer (Ridker et al., 2017b).

In addition, two trials using the natural anti-inflammatory factor Colchicine at different dosages have been conducted. The "Colchicine Cardiovascular Outcomes Trial" (COLCOT) also showed improvements in reducing recurrent cardiovascular events after the development of acute coronary syndromes, but the incidence of pneumonia increased in the treated group. In the second trial, "Low Dose Colchicine 2" (LoDoCo2), the diminution in Colchicine administration also reported a reduction in recurrent events, similar to COLCOT, supporting the role of inflammatory pathways in the pathogenesis of atherosclerosis (Tardif et al., 2019; Nidorf et al., 2020).

Following the rationale of relating atheroma biology with clinical approaches, it might also be tempting to specifically target activated endothelial cells in order to reverse the endMT process (Zhang et al., 2021b; Huang et al., 2021), or specifically focus on inhibiting the dedifferentiation of vascular SMC by promoting the recovery of their contractile phenotype (Shi et al., 2021). Another potential therapeutic strategy could be the enhancing of efferocytosis, the removal of unwanted/pathogenic plaque-associated cells by own phagocytes, in order to induce plaque regression. In this regard, the neutralization of CD47-expressing M1-like PAMs has been proposed with the aim of improving their sensibility to opsonized vascular SMC within atherosclerotic lesions (Wang et al., 2020). Furthermore, the modulation of PAMs phenotype could also be an interesting immunotherapy-based idea. In fact, enhancing M2 PAM polarization and increasing M2/M1 PAM ratio within atherosclerotic occlusion may improve clinical outcomes and plaque regression (Zhang et al., 2021c).

Finally, it has been noted that the abnormal vasa vasorum neo-angiogenesis within atherosclerotic plaque usually leads to the establishment of weak vessels prone to rupture and induce intra-plaque haemorrhages and instability. Thus, the inhibition of vasa vasorum neo-angiogenesis and/or the promotion of the correct maturation of newly formed intra-plaque vasculature could be an interesting therapeutic approach with the aim of reinforcing plaque stability (Li et al., 2021).

5 Conclusion

Novel stents have been developed and they have revolutionized interventional cardiology, however, restenosis and immune responses against the stent are still two of the main hurdles in this therapeutic approach. Although improvements have been made in stent design, such as the use of bioabsorbable materials combined with drug delivery, their capacity to maintain tissue function over prolonged periods of time needs to be improved.

Precise and accurate technological advances like electrospinning and bioprinting have allowed the fabrication of new generations of vascular scaffolds. Furthermore, the use of descellularized vessels or *in situ* vessel creation by subcutaneous implantation of tubular mandrels are novel approaches that are being tested in animal models. In addition, advances in strategies to cover stents with therapeutically designed nanoparticles, can be applied in the development of stent-coating to avoid immune rejection. For instance, the use of a CD47-homologous peptide-coated stent or even cell membrane-coated stents in order to fabricate "stent-ghosts" may open a door for new strategies to increase stent biocompatibility and provide the appreciated immune-evasive properties.

Finally, understanding inflammation as the driving force that pushes plaque formation could help to improve common CVD therapies. In addition, our growing knowledge of the biology of atherosclerosis could help to develop novel strategies based on anti-proliferative therapies, cell reprogramming approaches or immunomodulatory treatments to improve prevention and treatment of vascular related diseases.

Author contributions

The review was designed by MP, EL-R, and SB. SB and PH-C wrote the manuscript and MP and EL-R revised and edited the manuscript thoroughly. MP, EL-R, JM, GM-T and RV-M revised the manuscript critically. All authors contributed to the manuscript and approved the submitted version.

Funding

PH-C is supported by an FPU grant from the Ministry of Education, Culture and Sport. This work has been partially funded by the University of Jaen, Acción I apoyo a la investigación (BIO-349) and by Modeling Nature (MNat), Project number QUAL21-11.

Acknowledgments

All figures were created with BioRender.com with the help of María Belén Toledo.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

An, W., Luong, L. A., Bowden, N. P., Yang, M., Wu, W., Zhou, X., et al. (2022). Cezanne is a critical regulator of pathological arterial remodelling by targeting β-catenin signalling. *Cardiovasc Res.* 118 (2), 638–653. doi:10.1093/cvr/cvab056

Arias, S. L., Shetty, A., Devorkin, J., and Allain, J. P. (2018). Magnetic targeting of smooth muscle cells *in vitro* using a magnetic bacterial cellulose to improve cell retention in tissue-engineering vascular grafts. *Acta Biomater.* 1 (77), 172–181. doi:10.1016/j.actbio.2018.07.013

Bath, J., Lawrence, P. F., Neal, D., Zhao, Y., Smith, J. B., Beck, A. W., et al. (2021). Endovascular interventions for claudication do not meet minimum standards for the Society for Vascular Surgery efficacy guidelines. *J. Vasc. Surg.* 73 (5), 1693–1700.e3. doi:10.1016/j.jvs.2020.10.067

Beshchasna, N., Saqib, M., Kraskiewicz, H., Wasyluk, Ł., Kuzmin, O., Duta, O. C., et al. (2020). Recent advances in manufacturing innovative stents. *Pharmaceutics* 12 (4), 349. doi:10.3390/pharmaceutics12040349

Bianco, V., Kilic, A., Gleason, T. G., Aranda-Michel, E., Habertheuer, A., Humar, R., et al. (2021). Long-term outcomes after reoperative coronary artery bypass grafting. *Ann. Thorac. Surg.* 111 (1), 150–158. doi:10.1016/j.athoracsur.2020.04.092

Bil, J., and Gil, R. J. (2016). Bioresorbable vascular scaffolds-what does the future bring? *J. Thorac. Dis.* 8 (8), E741–E745. doi:10.21037/jtd.2016.05.80

Björck, H. M., Du, L., Pulignani, S., Paloschi, V., Lundströmer, K., Kostina, A. S., et al. (2018). Altered DNA methylation indicates an oscillatory flow mediated epithelial-to-mesenchymal transition signature in ascending aorta of patients with bicuspid aortic valve. *Sci. Rep.* 8 (1), 1–15. doi:10.1038/s41598-018-20642-4

Bobi, J., Garabito, M., Solanes, Nú., Cidad, P., Ramos-Pérez, V., Ponce, A., et al. (2020). Kv1.3 blockade inhibits proliferation of vascular smooth muscle cells *in vitro* and intimal hyperplasia *in vivo*. *Transl. Res.* 224, 40–54. doi:10.1016/j.trsl.2020.06.002

Bønaa, K. H., Mannsverk, J., Wiseth, R., Aaberge, L., Myreng, Y., Nygård, O., et al. (2016). Drug-eluting or bare-metal stents for coronary artery disease. *N. Engl. J. Med.* 375 (13), 1242–1252. doi:10.1056/NEJMoa1607991

Borhani, S., Hassanajili, S., Ahmadi Tafti, S. H., and Rabbani, S. (2018). Cardiovascular stents: Overview, evolution, and next generation. *Prog. Biomater.* 7 (3), 175–205. doi:10.1007/s40204-018-0097-y

Botelho, F. E., Cacione, D. G., Leite, J. O., and Baptista-Silva, J. C. (2021). Endoluminal interventions versus surgical interventions for stenosis in vein grafts following infrainguinal bypass. *Cochrane Database Syst. Rev.* 4 (4), CD013702. doi:10.1002/14651858.CD013702.pub2

Botts, S. R., Fish, J. E., and Howe, K. L. (2021). Dysfunctional vascular endothelium as a driver of atherosclerosis: Emerging insights into pathogenesis and treatment. *Front. Pharmacol.* 1, 1–18. doi:10.3389/fphar.2021.787541

Bowen, P. K., Drelich, J., and Goldman, J. (2013). Zinc exhibits ideal physiological corrosion behavior for bioabsorbable stents. *Adv. Mater* 25 (18), 2577–2582. doi:10.1002/adma.201300226

Bowen, P. K., Shearier, E. R., Zhao, S., Guillory, R. J 2nd., Zhao, F., Goldman, J., et al. (2019). Biodegradable metals for cardiovascular stents: From clinical concerns to recent Zn-alloys. *Adv. Healthc. Mater* 5 (10), 1121–1140. doi:10.1002/adhm.201501019

Braga, S. F., Neves, J. R., Ferreira, J., Carrilho, C., Simões, J. C., Mesquita, A., et al. (2019). Neointimal hyperplasia. *Rev Port Cir cardio-toracica e Vasc orgao da Soc Port Cir Cardio-Toracica e Vasc.* 26 (3), 213–217.

Brandt, K., Burger, F., Baptista, D., Roth, A., FernandesDa Silva, R., Montecucco, F., et al. (2022). Single-cell analysis uncovers osteoblast factor Growth Differentiation Factor 10 as mediator of vascular smooth muscle cell phenotypic modulation associated with plaque rupture in human carotid artery disease. *Int. J. Mol. Sci.* 23 (3), 1796. doi:10.3390/ijms23031796

Brener, S. J., Kereiakes, D. J., Simonton, C. A., Rizvi, A., Newman, W., Mastali, K., et al. (2013). Everolimus-eluting stents in patients undergoing percutaneous coronary intervention: Final 3-year results of the clinical evaluation of the XIENCE V everolimus eluting coronary stent system in the treatment of subjects with de Novo native coronary artery lesions trial. *Am. Heart J.* 166 (6), 1035–1042. doi:10.1016/j.ahj.2013.08.030

Brugaletta, S., Gomez-Lara, J., Ortega-Paz, L., Jimenez-Diaz, V., Jimenez, M., Jiménez-Quevedo, P., et al. (2021). 10-Year follow-up of patients with everolimus-eluting versus bare-metal stents after ST-segment elevation myocardial infarction. *J. Am. Coll. Cardiol.* 77 (9), 1165–1178. doi:10.1016/j.jacc.2020.12.059

Bu, Y., Zhang, X., Zhu, A., Li, L., Xie, X., and Wang, S. (2021). Inside-out oriented cell membrane biomimetic magnetic nanoparticles for high-performance drug lead discovery. *Anal. Chem.* 93 (22), 7898–7907. doi:10.1021/acs.analchem.1c00567

Buccheri, D., Piraino, D., Andolina, G., and Cortese, B. (2016). Understanding and managing in-stent restenosis: A review of clinical data, from pathogenesis to treatment. *J. Thorac. Dis.* 8 (10), E1150–E1162. doi:10.21037/jtd.2016.10.93

Byrne, R. A., Mehilli, J., Iijima, R., Schulz, S., Pache, J., Seyfarth, M., et al. (2009). A polymer-free dual drug-eluting stent in patients with coronary artery disease: A randomized trial vs. polymer-based drug-eluting stents. *Eur. Heart J.* 30 (8), 923–931. doi:10.1093/eurheartj/ehp044

Cai, J. X., Liu, J. H., Wu, J. Y., Li, Y. J., Qiu, X. H., Xu, W. J., et al. (2022). Hybrid cell membrane-functionalized biomimetic nanoparticles for targeted therapy of osteosarcoma. *Int. J. Nanomedicine* 17, 837–854. doi:10.2147/IJN.S346685

Cai, Z., Gu, Y., Xiao, Y., Wang, C., and Wang, Z. (2021). Porcine carotid arteries decellularized with a suitable concentration combination of Triton X-100 and sodium dodecyl sulfate for tissue engineering vascular grafts. *Cell Tissue Bank.* 22 (2), 277–286. doi:10.1007/s10561-020-09876-7

Caiazzo, G., Kilic, I. D., Fabris, E., Serdoz, R., Mattesini, A., Foin, N., et al. (2015). Absorb bioresorbable vascular scaffold: What have we learned after 5 years of clinical experience? *Int. J. Cardiol.* 201, 129–136. doi:10.1016/j.ijcard.2015.07.101

Carrabba, M., and Madeddu, P. (2018). Current strategies for the manufacture of small size tissue engineering vascular grafts. *Front. Bioeng. Biotechnol.* 6, 41. doi:10.3389/fbioe.2018.00041

Cha, J. J., Kim, G. C., Hur, S. H., Bae, J. H., Choi, J. W., Jin, D. K., et al. (2020). Efficacy and safety of dual-drug-eluting stents for de Novo coronary lesions in South Korea-the effect trial. *J. Clin. Med.* 10 (1), 69. doi:10.3390/jcm10010069

Charpentier, E., Barna, A., Guillevin, L., and Juliard, J. M. (2015). Fully bioresorbable drug-eluting coronary scaffolds: A review. *Arch. Cardiovasc Dis.* 108 (6–7), 385–397. doi:10.1016/j.acvd.2015.03.009

Chen, S. G., Ugwu, F., Li, W. C., Caplice, N. M., Petcu, E., Yip, S. P., et al. (2021). Vascular tissue engineering: Advanced techniques and gene editing in stem cells for graft generation. *Tissue Eng.* 27 (1), 14–28. doi:10.1089/ten.TEB.2019.0264

Chien, C. S., Li, J. Y. S., Chien, Y., Wang, M. L., Yarmishyn, A. A., Tsai, P. H., et al. (2021). METTL3-dependent N6-methyladenosine RNA modification mediates the atherogenic inflammatory cascades in vascular endothelium. *Proc. Natl. Acad. Sci. U S A* 118 (7), 1–12. doi:10.1073/pnas.2025070118

Cornelissen, A., and Vogt, F. J. (2019). The effects of stenting on coronary endothelium from a molecular biological view: Time for improvement? *J. Cell Mol. Med.* 23 (1), 39–46. doi:10.1111/jcmm.13936

Crea, F., and Libby, P. (2017). Acute coronary Syndromes.The way forward from mechanisms to precision treatment. *Circulation* 136 (12), 1155–1166. doi:10.1161/CIRCULATIONAHA.117.029870

da Luz, P. L., Chagas, A. C. P., Dourado, P. M. M., and Laurindo, F. R. M. (2018). "Endothelium in atherosclerosis: Plaque formation and its complications," in *Endothelium and cardiovascular diseases: Vascular biology and clinical syndromes* (Netherlands: Elsevier).

Dai, X., Wiernek, S., Evans, J. P., and Runge, M. S. (2016). Genetics of coronary artery disease and myocardial infarction. *World J. Cardiol.* 26, 1–23. doi:10.4330/wjc.v8.i1.1

Denktas, A. E. (2010). Primary percutaneous coronary intervention in ST-segment elevation myocardial infarction is more effective than fibrinolysis at reducing the composite outcome of death or reinfarction after 8 years. *Evid. Based Med.* 15 (5), 145–146. doi:10.1136/ebm1108

Direnzo, D., Owens, G. K., and Leeper, N. J. (2017). Attack of the clones" commonalities between cancer and atherosclerosis. *Circ. Res.* 120 (4), 624–626. doi:10.1161/CIRCRESAHA.116.310091

Drozd, J., Wójcik, J., Małek, R., Korona, B., Zapolski, T., Wysokiński, A., et al. (2010). Acute coronary syndrome - a frequent clinical manifestation of bare metal in-stent restenosis. *Kardiol. Pol.* 68 (6), 637–645.

Ellis, S. G., Kereiakes, D. J., Metzger, D. C., Caputo, R. P., Rizik, D. G., Teirstein, P. S., et al. ABSORB III Investigators. (2015). Everolimus-eluting bioresorbable scaffolds for coronary artery disease. *N. Engl. J. Med.* 373, 1905–1915. doi:10.1056/NEJMoa1509038

Erne, P., Schier, M., and Resink, T. J. (2006). The road to bioabsorbable stents: Reaching clinical reality? *Cardiovasc Interv. Radiol.* 29 (1), 11–16. doi:10.1007/s00270-004-0341-9

Fang, S., Ellman, D. G., and Andersen, D. C. (2021). Review: Tissue engineering of small- diameter vascular grafts and their *in vivo* evaluation in large animals and humans. *Cells* 10 (3), 713. doi:10.3390/cells10030713

Fasehee, H., Fakhraee, M., Davoudi, S., Vali, H., and Faghihi, S. (2019). Cancer biomarkers in atherosclerotic plaque: Evidenced from structural and proteomic analyses. *Biochem Biophys. Res. Commun.* 509 (3), 687–693. doi:10.1016/j.bbrc.2018.12.160

Fazal, F., Raghav, S., Callanan, A., Koutsos, V., and Radacsi, N. (2021). Recent advancements in the bioprinting of vascular grafts. *Biofabrication* 28 (3), 032003. doi:10.1088/1758-5090/ac0963

Gaba, P., Gersh, B. J., Ali, Z. A., Moses, J. W., and Stone, G. W. (2021). Complete versus incomplete coronary revascularization: Definitions, assessment and outcomes. *Nat. Rev. Cardiol.* 18, 155–168. doi:10.1038/s41569-020-00457-5

Garcia-Sabaté, A., Mohamed, W. K. E., Sapudom, J., Alatoom, A., Al Safadi, L., and Teo, J. C. M. (2020). Biomimetic 3D models for investigating the role of monocytes and macrophages in atherosclerosis. *Bioengineering* 7 (3), 113–120. doi:10.3390/bioengineering7030113

Geelhoed, W. J., Moroni, L., and Rotmans, J. I. (2017). Utilizing the foreign body response to grow tissue engineered blood vessels *in vivo*. *J. Cardiovasc Transl. Res.* 10 (2), 167–179. doi:10.1007/s12265-017-9731-7

Generali, M., Casanova, E. A., Kehl, D., Wanner, D., Hoerstrup, S. P., Cinelli, P., et al. (2019). Autologous endothelialized small-caliber vascular grafts engineered from blood-derived induced pluripotent stem cells. *Acta Biomater.* 97, 333–343. doi:10.1016/j.actbio.2019.07.032

Georgakarakos, E., Tasopoulou, K., Koutsoumpelis, A., and Argyriou, C. (2021). The "pull, cast, and fix" technique for bypass in the midpopliteal (P2) arterial segment in chronic femoropopliteal occlusions. *Case Rep. Ann. Vasc. Surg.* 71, 523–527. doi:10.1016/j.avsg.2020.08.153

Grabow, N., Schlun, M., Sternberg, K., Hakansson, N., Kramer, S., and Schmitz, K. P. (2005). Mechanical properties of laser cut poly(L-lactide) micro-specimens: Implications for stent design, manufacture, and sterilization. *J. Biomech. Eng.* 127 (1), 25–31. doi:10.1115/1.1835349

Gu, X., Zheng, Y., Cheng, Y., Zhong, S., and Xi, T. (2009). *In vitro* corrosion and biocompatibility of binary magnesium alloys. *Biomaterials* 30 (4), 484–498. doi:10.1016/j.biomaterials.2008.10.021

Guo, J., Li, J., Zhang, J., Guo, X., Liu, H., Li, P., et al. (2021). LncRNA PVT1 knockdown alleviated ox-LDL-induced vascular endothelial cell injury and atherosclerosis by miR-153-3p/GRB2 axis via ERK/p38 pathway. *Nutr. Metab. Cardiovasc Dis.* 31 (12), 3508–3521. doi:10.1016/j.numecd.2021.08.031

Harskamp, R. E., Alexander, J. H., Ferguson, T. B., Jr., Hager, R., Mack, M. J., Englum, B., et al. (2016). Frequency and predictors of internal mammary artery graft failure and subsequent clinical outcomes: Insights from the project of *ex-vivo* vein graft engineering via transfection (PREVENT) IV trial. *Circulation* 133 (2), 131–138. doi:10.1161/CIRCULATIONAHA.115.015549

Heng, J. W., Yazid, M. D., Abdul Rahman, M. R., and Sulaiman, N. (2021). Coatings in decellularized vascular scaffolds for the establishment of a functional endothelium: A scoping review of vascular graft refinement. *Front. Cardiovasc Med.* 29 (8), 677588. doi:10.3389/fcvm.2021.677588

Henry, J. J. D., Yu, J., Wang, A., Zhang, Y., Li, X. S., Guex, A. G., et al. (2017). A compliant and biomimetic three-layered vascular graft for small blood vessels. *Biofabrication* 9, 025010. doi:10.1088/1758-5090/aa6bae

Hernández-Camarero, P., López-Ruiz, E., Marchal, J. A., and Perán, M. (2021). Cancer: A mirrored room between tumor bulk and tumor microenvironment. *J. Exp. Clin. Cancer Res.* 40 (1), 217. doi:10.1186/s13046-021-02022-5

Herrington, W., Lacey, B., Sherliker, P., Armitage and, J., and Lewington, S. (2016). Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ. Res.* 118 (4), 535–546. doi:10.1161/CIRCRESAHA.115.307611

Horakova, J., Mikes, P., Lukas, D., Saman, A., Jencova, V., Klapstova, A., et al. (2018). Electrospun vascular grafts fabricated from poly(L-lactide-co-ε-caprolactone) used as a bypass for the rabbit carotid artery. *Biomed. Mater.* 13, 065009. doi:10.1088/1748-605X/aade9d

Hsich, E. M. (2016). Matching the market for heart transplantation. *Circ. Heart Fail* 9 (4), e002679. doi:10.1161/CIRCHEARTFAILURE.115.002679

Hu, B., Boakye-Yiadom, K. O., Yu, W., Yuan, Z. W., Ho, W., Xu, X., et al. (2020). Nanomedicine approaches for advanced diagnosis and treatment of atherosclerosis and related ischemic diseases. *Adv. Healthc. Mater* 9 (16), e2000336. doi:10.1002/adhm.202000336

Huang, Q., Gan, Y., Yu, Z., Wu, H., and Zhong, Z. (2021). Endothelial to mesenchymal transition: An insight in atherosclerosis. *Front. Cardiovasc Med.* 8, 734550–734559. doi:10.3389/fcvm.2021.734550

Huang, Y., Venkatraman, S. S., Boey, F. Y. C., Lahti, E. M., Umashankar, P. R., Mohanty, M., et al. (2010). *In vitro* and *in vivo* performance of a dual drug-eluting stent (DDES). *Biomaterials* 31 (15), 4382–4391. doi:10.1016/j.biomaterials.2010.01.147

Inamdar, V. V., Fitzpatrick, E. G., Alferiev, I. S., Levy, R. J., Stachelek, S. J., and Fishbein, I. (2020). Mitigation of blood borne cell attachment to metal implants through CD47-derived peptide immobilization. J. Vis. Exp. *JoVE.* 166, 1–15. doi:10.3791/61545

Iqbal, J., Gunn, J., and Serruys, P. W. (2013). Coronary stents: Historical development, current status and future directions. *Br. Med. Bull.* 106, 193–211. doi:10.1093/bmb/ldt009

Itoh, M., Nakayama, K., Noguchi, R., Kamohara, K., Furukawa, K., Uchihashi, K., et al. (2015). Scaffold-free tubular tissues created by a bio-3D printer undergo remodeling and endothelialization when implanted in rat aortae. *PLoS One* 10 (9), 1–15. doi:10.1371/journal.pone.0136681

Iwata, H., Osborn, E. A., Ughi, G. J., Murakami, K., Goettsch, C., Hutcheson, J. D., et al. (2021). Highly selective pparα (Peroxisome proliferator-activated receptor α) agonist pemafibrate inhibits stent inflammation and restenosis assessed by multimodality molecular-microstructural imaging. *J. Am. Heart Assoc.* 10 (20), e020834. doi:10.1161/JAHA.121.020834

Jin, X., Kang, R., Deng, R., Zhao, X., Wang, Z., Rong, W., et al. (2022). Fabrication and characterization of an acellular annulus fibrosus scaffold with aligned porous construct for tissue engineering. *J. Biomater. Appl.* 36 (6), 985–995. doi:10.1177/08853282211041956

Jinnouchi, H., Torii, S., Sakamoto, A., Kolodgie, F. D., Virmani, R., Finn, A. V., et al. (2019). Fully bioresorbable vascular scaffolds: Lessons learned and future directions. *Nat. Rev. Cardiol.* 16 (5), 286–304. doi:10.1038/s41569-018-0124-7

Joner, M., Ruppelt, P., Zumstein, P., Lapointe-Corriveau, C., Leclerc, G., Bulin, A., et al. (2018). Preclinical evaluation of degradation kinetics and elemental mapping of first- and second-generation bioresorbable magnesium scaffolds. *EuroIntervention J Eur Collab Work Gr Interv Cardiol Eur Soc Cardiol* 14 (9), e1040–e1048. doi:10.4244/EIJ-D-17-00708

Joseph, J., Domenico Bruno, V., Sulaiman, N., Ward, A., Johnson, T. W., Baby, H. M., et al. (2022). A novel small diameter nanotextile arterial graft is associated with surgical feasibility and safety and increased transmural endothelial ingrowth in pig. *J. Nanobiotechnology* 20 (1), 71. doi:10.1186/s12951-022-01268-1

Ju, Y. M., Ahn, H., Arenas-Herrera, J., Kim, C., Abolbashari, M., Atala, A., et al. (2017). Electrospun vascular scaffold for cellularized small diameter blood vessels: A preclinical large animal study. *Acta Biomater.* 59, 58. doi:10.1016/j.actbio.2017.06.027

Karacsonyi, J., and Brilakis, E. S. (2017). Bypass graft failure: Changing what we can, accepting what we cannot change, and developing the science to know the difference. *Circ.* Cardiovasc. Interv. *United States* 10, e005212. doi:10.1161/CIRCINTERVENTIONS.117.005212

Karimi, M., Zare, H., Nik, A. B., Yazdani, N., Hamrang, M., Mohamed, E., et al. (2016). Nanotechnology in diagnosis and treatment of coronary artery disease. *Nanomedicine (Lond)* 11 (5), 513–530. doi:10.2217/nnm.16.3

Keulards, D. C. J., Karamasis, G. V., Alsanjari, O., Demandt, J. P. A., Van't Veer, M., Zelis, J. M., et al. (2020). Recovery of absolute coronary blood flow and microvascular resistance after chronic total occlusion percutaneous coronary intervention: An exploratory study. J. Am. Heart Assoc. 9 (9), e015669. doi:10.1161/JAHA.119.015669

King, S. B., and ii, I. (2021). How does angioplasty work. *Cardiovasc. revascularization Med.* 24, 89–90. doi:10.1016/j.carrev.2020.12.008

Konig, G., Mcallister, T. N., Dusserre, N., Garrido, S. A., Iyican, C., Marini, A., et al. (2010). Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. *Biomaterials* 30, 1542–1550. doi:10.1016/j.biomaterials.2008.11.011

Kozuma, K., Tanabe, K., Hamazaki, Y., Okamura, T., Ando, J., Ikari, Y., et al. (2020). ABSORB Japan investigators long-term outcomes of Absorb bioresorbable vascular scaffold vs. Everolimus-eluting metallic stent - a randomized comparison through 5 Years in Japan. *Circ. J.* 84, 733–741. doi:10.1253/circj.CJ-19-1184

Kubota, Y., Evenson, K. R., Maclehose, R. F., Roetker, N. S., Joshu, C. E., et al. (2017). Physical activity and lifetime risk of cardiovascular disease and cancer. *Med. Sci. Sports Exerc* 49 (8), 1599–1605. doi:10.1249/MSS.0000000000001274

Lasch, M., Caballero Martinez, A., Kumaraswami, K., Ishikawa-Ankerhold, H., Meister, S., Deindl, E., et al. (2020). Contribution of the potassium channels K(V) 1.3 and K(Ca)3.1 to smooth muscle cell proliferation in growing collateral arteries. *Cells* 9 (4), 913. doi:10.3390/cells9040913

Leal, B. B. J., Wakabayashi, N., Oyama, K., Kamiya, H., Braghirolli, D. I., and Pranke, P. (2021). Vascular tissue engineering: Polymers and methodologies for small caliber vascular grafts. *Front. Cardiovasc Med.* 7, 592361. doi:10.3389/fcvm.2020.592361

Lee, S. Y., Bae, I. H., Sung Park, D., Jang, E. J., Shim, J. W., Lim, K. S., et al. (2016). Prednisolone- and sirolimus-eluting stent: Anti-inflammatory approach for inhibiting in-stent restenosis. *J. Biomater. Appl.* 31 (1), 36–44. doi:10.1177/0885328216630498

Li, M., Qi, Z., Zhang, J., Zhu, K., and Wang, Y. (2021). Effect and mechanism of Si-Miao-Yong-An on vasa vasorum remodeling in ApoE−/− mice with atherosclerosis vulnerable plague. *Front. Pharmacol.* 12, 634611–634612. doi:10.3389/fphar.2021.634611

Libby, P. (2021). Inflammation during the life cycle of the atherosclerotic plaque. *Cardiovasc Res.* 117 (13), 2525–2536. doi:10.1093/cvr/cvab303

Libby, P. (2021). The changing landscape of atherosclerosis. *Nature* 592 (7855), 524–533. doi:10.1038/s41586-021-03392-8

Lin, C. H., Hsia, K., Ma, H., Lee, H., and Lu, and J. H. (2018). *In vivo* performance of decellularized vascular grafts: A review article. *Int. J. Mol. Sci.* 19 (7), 2101. doi:10.3390/ijms19072101

Lin, S., Li, X., Zhang, J., and Zhang, Y. (2021). Omentin-1: Protective impact on ischemic stroke via ameliorating atherosclerosis. *Clin. Chim. Acta* 517, 31–40. doi:10.1016/j.cca.2021.02.004

Lincoff, A. M., Furst, J. G., Ellis, S. G., Tuch, R. J., and Topol, E. J. (1997). Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J. Am. Coll. Cardiol.* 29 (4), 808–816. doi:10.1016/s0735-1097(96)00584-0

Liu, C. H., Chen, Z., Chen, K., Liao, F. T., Chung, C-E., Liu, X., et al. (2021). Lipopolysaccharide-Mediated chronic inflammation promotes tobacco carcinogen-induced lung cancer and determines the efficacy of immunotherapy. *Cancer Res.* 81 (1), 144–157. doi:10.1158/0008-5472.CAN-20-1994

López-Ruiz, E., Venkateswaran, S., Perán, M., Jiménez, G., Pernagallo, S., Díaz-Mochón, J. J., et al. (2017). Poly(ethylmethacrylate-co-diethylaminoethyl acrylate) coating improves endothelial re-population, bio-mechanical and anti-thrombogenic properties of decellularized carotid arteries for blood vessel replacement. *Sci. Rep.* 7 (1), 407. doi:10.1038/s41598-017-00294-6

MacRae, J. M., Dipchand, C., Oliver, M., Moist, L., Lok, C., Clark, E., et al. (2016). Arteriovenous access failure, stenosis, and thrombosis. *Can. J. kidney Heal Dis.* 3, 2054358116669126. doi:10.1177/2054358116669126

Manubolu, V. S., and Budoff, M. J. (2022). Achieving coronary plaque regression: A decades-long battle against coronary artery disease. *Expert Rev. Cardiovasc Ther.* 20 (4), 291–305. doi:10.1080/14779072.2022.2069559

Martín-Timón, I., Sevillano-Collantes, C., Segura-Galindo, A., and Del Cañizo-Gómez, F. J. (2014). Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World J. Diabetes* 5 (4), 444–470. doi:10.4239/wjd.v5.i4.444

Marx, S. O., Totary-Jain, H., and Marks, A. R. (2011). Vascular smooth muscle cell proliferation in restenosis. *Circ. Cardiovasc Interv.* 4 (1), 104–111. doi:10.1161/CIRCINTERVENTIONS.110.957332

McKavanagh, P., Zawadowski, G., Ahmed, N., and Kutryk, M. (2018). The evolution of coronary stents. *Expert Rev. Cardiovasc Ther.* 16 (3), 219–228. doi:10.1080/14779072.2018.1435274

Melly, L., Torregrossa, G., Lee, T., Jansens, J. L., and Puskas, J. D. (2018). Fifty years of coronary artery bypass grafting. *J. Thorac. Dis.* 10 (3), 1960–1967. doi:10.21037/jtd.2018.02.43

Mridha, N., Subhaharan, D., Niranjan, S., Rashid, M. K., Psaltis, P., and Singh, K. (2019). A meta-analysis of randomized controlled trials to compare long-term clinical outcomes of bioabsorbable polymer and durable polymer drug-eluting stents. *Eur. Heart J. Qual. Care Clin. Outcomes* 1-5 (2), 105–113. doi:10.1093/ehjqcco/qcy036

Murphy, P. A., Butty, V. L., Boutz, P. L., Begum, S., Kimble, A. L., Sharp, P. A., et al. (2018). Alternative RNA splicing in the endothelium mediated in part by rbfox2 regulates the arterial response to low flow. *Elife* 7, 1–21. doi:10.7554/eLife.29494

Naegeli, K. M., Kural, M. H., Li, Y., Wang, J., Hugentobler, E. A., Niklason, L. E., et al. (2022). Bioengineering human tissues and the future of vascular replacement. *Circ. Res.* 131 (1), 109–126. doi:10.1161/CIRCRESAHA.121.319984

Neufert, C., Heichler, C., Brabletz, T., Scheibe, K., Boonsanay, V., Greten, F. R., et al. (2021). Inducible mouse models of colon cancer for the analysis of sporadic and inflammation-driven tumor progression and lymph node metastasis. *Nat. Protoc.* 16 (1), 61–85. doi:10.1038/s41596-020-00412-1

Nidorf, S. M., Fiolet, A. T. L., Mosterd, A., Eikelboom, J. W., Schut, A., Opstal, T. S. J., et al. (2020). Colchicine in patients with chronic coronary disease. *N. Engl. J. Med.* 383 (19), 1838–1847. doi:10.1056/NEJMoa2021372

Nishikori, A., Nishimura, Y., Shibata, R., Ohshima, K. I., Gion, Y., Ikeda, T., et al. (2021). Upregulated expression of activation-induced cytidine deaminase in ocular adnexal marginal zone lymphoma with IgG4-positive cells. *Int. J. Mol. Sci.* 22 (8), 4083. doi:10.3390/ijms22084083

Nogic, J., McCormick, L. M., Francis, R., Nerlekar, N., Jaworski, C., West, N. E. J., et al. (2018). Novel bioabsorbable polymer and polymer-free metallic drug-eluting stents. *J. Cardiol.* 71 (5), 435–443. doi:10.1016/j.jjcc.2017.12.007

Owens, C. D., Gasper, W. J., Rahman, A. S., and Conte, M. S. (2015). Vein graft failure. *J. Vasc. Surg.* 61 (1), 203–216. doi:10.1016/j.jvs.2013.08.019

Palmerini, T., Serruys, P., Kappetein, A. P., Genereux, P., Riva, D. D., Reggiani, L. B., et al. (2017). Clinical outcomes with percutaneous coronary revascularization vs coronary artery bypass grafting surgery in patients with unprotected left main coronary artery disease: A meta-analysis of 6 randomized trials and 4,686 patients. *Am. Heart J.* 190, 54–63. doi:10.1016/j.ahj.2017.05.005

Park, Y. M., Han, S. H., Lee, K., Suh, S. Y., Oh, P. C., Chung, W. J., et al. (2013). Dexamethasone-eluting stents had sustained favorable ischemic driven target lesion revascularization rates over 5 years: A randomized controlled prospective study. *Int. J. Cardiol.* 165, 359–362. doi:10.1016/j.ijcard.2012.08.033

Pesarini, G., Ferrero, V., Tomai, F., Paloscia, L., De Cesare, N., Tamburino, C., et al. (2009). Steroid-eluting stents in patients with acute coronary syndromes. Angiographic results of DESIRE: Dexamethasone-Eluting Stent Italian REgistry. *J. Invasive Cardiol.* 21 (3), 86–91.

Petljak, M., Alexandrov, L. B., Brammeld, J. S., Price, S., Wedge, D. C., Grossmann, S., et al. (2019). Characterizing mutational signatures in human cancer cell lines reveals episodic APOBEC mutagenesis. *Cell* 176 (6), 1282–1294. doi:10.1016/j.cell.2019.02.012

Pham, L. M., Poudel, K., Phung, C. D., Nguyen, T. T., Pandit, M., Nguyen, H. T., et al. (2021). Preparation and evaluation of dabrafenib-loaded, CD47-conjugated human serum albumin-based nanoconstructs for chemoimmunomodulation. *Colloids Surfaces B Biointerfaces* 208, 112093. doi:10.1016/j.colsurfb.2021.112093

Qiu, X., Li-Ping Lee, B., Wong, S. Y., Ding, X., Xu, K., Zhao, W., et al. (2021). Cellular remodeling of fibrotic conduit as vascular graft. *Biomaterials* 268, 120565. doi:10.1016/j.biomaterials.2020.120565

Radke, P. W., Weber, C., Kaiser, A., Schober, A., and Hoffmann, R. (2004). Dexamethasone and restenosis after coronary stent implantation: New indication for an old drug? *Curr. Pharm. Des.* 10 (4), 349–355. doi:10.2174/1381612043453324

Rickel, A. P., Deng, X., Engebretson, D., and Hong, Z. (2021). Electrospun nanofiber scaffold for vascular tissue engineering. *Mater Sci. Eng. C Mater Biol. Appl.* 129, 112373. doi:10.1016/j.msec.2021.112373

Ridker, P. M., Everett, B. M., Thuren, T., MacFadyen, J. G., Chang, W. H., Ballantyne, C., et al. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* 377 (12), 1119–1131. doi:10.1056/NEJMoa1707914

Ridker, P. M., Koenig, W., Kastelein, J. J., Mach, F., and Lüscher, T. F. (2018). Has the time finally come to measure hsCRP universally in primary and secondary cardiovascular prevention? *Eur. Heart J.* 39 (46), 4109–4111. doi:10.1093/eurheartj/ehy723

Ridker, P. M., MacFadyen, J. G., Thuren, T., Everett, B. M., Libby, P., Glynn, R. J., et al. (2017). Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: Exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet* 390 (10105), 1833–1842. doi:10.1016/S0140-6736(17)32247-X

Roth, G. A., Johnson, C., Abajobir, A., Abd-Allah, F., Abera, S. F., Abyu, G., et al. (2017). Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J. Am. Coll. Cardiol.* 70 (1), 1–25. doi:10.1016/j.jacc.2017.04.052

Saito, J., Yokoyama, U., Nakamura, T., Kanaya, T., Ueno, T., Naito, Y., et al. (2021). Scaffold-free tissue-engineered arterial grafts derived from human skeletal myoblasts. *Artif. Organs* 45 (8), 919–932. doi:10.1111/aor.13930

Sajadian, M., Alizadeh, L., Ganjifard, M., Mardani, A., Ansari, M. A., and Falsoleiman, H. (2018). Factors affecting in-stent restenosis in patients undergoing percutaneous coronary angioplasty. *Galen. Med. J.* 7, e961. doi:10.22086/gmj.v0i0.961

Sakurai, R., Inajima, T., Kaneda, H., Nagai, R., and Hashimoto, H. (2013). Sirolimus-eluting stents reduce long-term mortality compared with bare metal stents in ST-segment elevation myocardial infarction: A meta-analysis of randomized controlled trials. *Int. J. Cardiol.* 167 (1), 162–167. doi:10.1016/j.ijcard.2011.12.054

Sánchez, P. F., Brey, E. M., and Briceño, J. C. (2018). Endothelialization mechanisms in vascular grafts. *J. Tissue Eng. Regen. Med.* 12 (11), 2164–2178. doi:10.1002/term.2747

Sandiford, O. A., Donnelly, R. J., El-Far, M. H., Burgmeyer, L. M., Sinha, G., Pamarthi, S., et al. (2021). Mesenchymal stem cell–secreted extracellular vesicles instruct stepwise dedifferentiation of breast cancer cells into dormancy at the bone marrow perivascular region. *Cancer Res.* 81 (6), 1567–1582. doi:10.1158/0008-5472.CAN-20-2434

Sekuli, c. M., Zacharias, M., and Medalion, B. (2019). Ischemic cardiomyopathy and heart failure. *Circ. Heart Fail* 12 (6), e006006. doi:10.1161/CIRCHEARTFAILURE.119.006006

Senti, M. E., de Jongh, C. A., Dijkxhoorn, K., Verhoef, J. J. F., Szebeni, J., Storm, G., et al. (2022). Anti-PEG antibodies compromise the integrity of PEGylated lipid-based nanoparticles via complement. *J. Control. Release* 341, 475–486. doi:10.1016/j.jconrel.2021.11.042

Seo, J., Ahn, J. M., Hong, S. J., Kang, D. Y., Hong, S. J., Her, A. Y., et al. (2020). Bioresorbable vascular scaffolds versus drug-eluting stents for diffuse long coronary narrowings. *Am. J. Cardiol.* 125 (11), 1624–1630. doi:10.1016/j.amjcard.2020.02.031

Serruys, P. W., Chevalier, B., Dudek, D., Cequier, A., Carrié, D., Iniguez, A., et al. (2015). A bioresorbable everolimus-eluting scaffold versus a metallic everolimus-eluting stent for ischaemic heart disease caused by de-novo native coronary artery lesions (ABSORB II): An interim 1-year analysis of clinical and procedural secondary outcomes from a randomised controlled trial. *Lancet* 385 (9962), 43–54. doi:10.1016/S0140-6736(14)61455-0

Sharifkazemi, M., Zafar, H., Sharif, R., Wyns, W., and Sharif, F. (2022). Do bioresorbable vascular scaffold stents reduce very late stent thrombosis? A systematic review. SN Compr. Clin. *Med.* 4, 64. doi:10.1007/s42399-022-01147-8

Shi, X., Zhang, Y., Han, J., Peng, W., Fang, Z., Qin, Y., et al. (2021). Tryptanthrin regulates vascular smooth muscle cell phenotypic switching in atherosclerosis by AMP-activated protein kinase/acetyl-CoA carboxylase signaling pathway. *J. Cardiovasc Pharmacol.* 77 (5), 642–649. doi:10.1097/FJC.0000000000001008

Shlofmitz, E., Iantorno, M., and Waksman, R. (2019). Restenosis of drug-eluting stents: A new classification system based on disease mechanism to guide treatment and state-of-the-art review. *Circ. Cardiovasc Interv.* 12 (8), e007023. doi:10.1161/CIRCINTERVENTIONS.118.007023

Sigwart, U., Puel, J., Mirkovitch, V., Joffre, F., and Kappenberger, L. (1987). Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. *N. Engl. J. Med.* 316 (12), 701–706. doi:10.1056/NEJM198703193161201

Singh, B., Kosuru, R., Lakshmikanthan, S., Sorci-Thomas, M. G., Zhang, D. X., Sparapani, R., et al. (2021). Endothelial Rap1 (Ras-Association proximate 1) restricts inflammatory signaling to protect from the progression of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 1, 638–650. doi:10.1161/ATVBAHA.120.315401

Sohn, S. H., Kim, T. H., Kim, T. S., Min, T. J., Lee, J. H., Yoo, S. M., et al. (2021). Evaluation of 3d templated synthetic vascular graft compared with standard graft in a rat model: Potential use as an artificial vascular graft in cardiovascular disease. *Mater. (Basel)* 14 (5), 1239. doi:10.3390/ma14051239

Strecker, E. P., Gabelmann, A., Boos, I., Lucas, C., Xu, Z., Haberstroh, J., et al. (1998). Effect on intimal hyperplasia of dexamethasone released from coated metal stents compared with non-coated stents in canine femoral arteries. *Cardiovasc Interv. Radiol.* 21 (6), 487–496. doi:10.1007/s002709900309

Tamai, H., Igaki, K., Kyo, E., Kosuga, K., Kawashima, A., Matsui, S., et al. (2000). Initial and 6-month results of biodegradable poly-l-lactic acid coronary stents in humans. *Circulation* 102 (4), 399–404. doi:10.1161/01.cir.102.4.399

Tamburino, C., Capranzano, P., Gori, T., Latib, A., Lesiak, M., Nef, H., et al. (2016). 1-Year outcomes of everolimus-eluting bioresorbable scaffolds versus everolimus-eluting stents: A propensity-matched comparison of the GHOST-EU and XIENCE V USA registries. *JACC Cardiovasc Interv.* 9 (5), 440–449. doi:10.1016/j.jcin.2015.10.042

Tang, P. C. T., Chung, J. Y. F., Xue, V. W., Xiao, J., Meng, X. M., Huang, X. R., et al. (2022). Smad3 promotes cancer-associated fibroblasts generation via macrophage-myofibroblast transition. *Transit. Adv. Sci.* 9 (1), 1–14. doi:10.1002/advs.202101235

Taniwaki, M., Radu, M. D., Zaugg, S., Amabile, N., Garcia-Garcia, H. M., Yamaji, K., et al. (2016). Mechanisms of very late drug-eluting stent thrombosis assessed by optical coherence tomography. *Circulation* 133 (7), 650–660. doi:10.1161/CIRCULATIONAHA.115.019071

Tapia-Vieyra, J. V., Delgado-Coello, B., and Mas-Oliva, J. (2017). Atherosclerosis and cancer; A resemblance with far-reaching implications. *Arch. Med. Res.* 48 (1), 12–26. doi:10.1016/j.arcmed.2017.03.005

Tappia, P. S., and Blewett, H. (2020). Nutrition and cardiovascular health. *Int. J. Mol. Sci.* 21, 2284. doi:10.3390/ijms21072284

Tardif, J. C., Kouz, S., Waters, D. D., Bertrand, O. F., Diaz, R., Maggioni, A. P., et al. (2019). Efficacy and safety of low-dose colchicine after myocardial infarction. *N. Engl. J. Med.* 381 (26), 2497–2505. doi:10.1056/NEJMoa1912388

Tibaut, M., Mekis, D., and Petrovic, D. (2017). Pathophysiology of myocardial infarction and acute management strategies. *Cardiovasc Hematol. Agents Med. Chem.* 14 (3), 150–159. doi:10.2174/1871525714666161216100553

van Beusekom, H. M. M., Saia, F., Zindler, J. D., Lemos, P. A., Swager-Ten Hoor, S. L., van Leeuwen, M. A. H., et al. (2007). Drug-eluting stents show delayed healing: Paclitaxel more pronounced than sirolimus. *Eur. Heart J.* 28 (8), 974–979. doi:10.1093/eurheartj/ehm064

Von Bornstädt, D., Wang, H., Paulsen, M. J., Goldstone, A. B., Eskandari, A., Thakore, A., et al. (2018). Rapid self-assembly of bioengineered cardiovascular bypass grafts from scaffold-stabilized, tubular bilevel cell sheets. *Circulation* 138 (19), 2130–2144. doi:10.1161/CIRCULATIONAHA.118.035231

Waksman, R. (2017). A new generation of drug-eluting stents: Indications and outcomes of bioresorbable vascular scaffolds. *Cleve Clin. J. Med.* 84, e20–e24. doi:10.3949/ccjm.84.s4.05

Wang, X., Chen, X., Sun, W., Tian, T., Zhou, S., Zhang, Z., et al. (2019). Very late stent thrombosis in drug-eluting stents new observations and clinical implications. *Cardiol. Rev.* 27 (6), 279–285. doi:10.1097/CRD.0000000000000283

Wang, Y., Li, G., Yang, L., Luo, R., and Guo, G. (2022). Development of innovative biomaterials and devices for the treatment of cardiovascular diseases. *Adv. Mater* 34, e2201971. doi:10.1002/adma.202201971

Wang, Y., Nanda, V., Direnzo, D., Ye, J., Xiao, S., Kojima, Y., et al. (2020). Clonally expanding smooth muscle cells promote atherosclerosis by escaping efferocytosis and activating the complement cascade. *Proc. Natl. Acad. Sci. U S A* 117 (27), 15818–15826. doi:10.1073/pnas.2006348117

Wang, Z. Y., Cheng, J., Liu, B., Xie, F., Li, C. L., Qiao, W., et al. (2021). Protein deglycase DJ-1 deficiency induces phenotypic switching in vascular smooth muscle cells and exacerbates atherosclerotic plaque instability. *J. Cell Mol. Med.* 25 (6), 2816–2827. doi:10.1111/jcmm.16311

Weekes, A., Bartnikowski, N., Pinto, N., Jenkins, J., Meinert, C., and Klein, T. J. (2022). Biofabrication of small diameter tissue-engineered vascular grafts. *Acta Biomater.* 15 (138), 92–111. doi:10.1016/j.actbio.2021.11.012

Weir, N. A., Buchanan, F. J., Orr, J. F., Farrar, D. F., and Boyd, A. (2004). Processing, annealing and sterilisation of poly-L-lactide. *Biomaterials* 25 (18), 3939–3949. doi:10.1016/j.biomaterials.2003.10.076

Williams, S. F., Rizk, S., and Martin, D. P. (2013). Poly-4-hydroxybutyrate (P4HB): A new generation of resorbable medical devices for tissue repair and regeneration. *Biomed. Tech. Berl.* 58 (5), 439–452. doi:10.1515/bmt-2013-0009

Wirka, R. C., Wagh, D., Paik, D. T., Pjanic, M., Nguyen, T., Miller, C. L., et al. (2019). Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis. *Nat. Med.* 25 (8), 1280–1289. doi:10.1038/s41591-019-0512-5

Xiao, J., Feng, G., Kang, G., Lan, Z., Liao, T., Kislauskis, E., et al. (2015). 6-Month follow-up of a novel biodegradable drug-eluting stent composed of poly-L-lactic acid and amorphous calcium phosphate nanoparticles in porcine coronary artery. *J. Biomed. Nanotechnol.* 11 (10), 1819–1825. doi:10.1166/jbn.2015.2102

Xie, Q., Huang, J., Zhu, K., and Chen, Q. (2021). Percutaneous coronary intervention versus coronary artery bypass grafting in patients with coronary heart disease and type 2 diabetes mellitus: Cumulative meta-analysis. *Clin. Cardiol.* 44 (7), 899–906. doi:10.1002/clc.23613

Xu, Z., Guo, C., Ye, Q., Shi, Y., Sun, Y., Zhang, J., et al. (2021). Endothelial deletion of SHP2 suppresses tumor angiogenesis and promotes vascular normalization. *Nat. Commun.* 12 (1), 6310–6315. doi:10.1038/s41467-021-26697-8

Ye, M., Guo, X., Wang, H., Wang, Y., Qian, X., Deng, H., et al. (2020). Mutual regulation between β-TRCP mediated REST protein degradation and Kv1.3 expression controls vascular smooth muscle cell phenotype switch. *Atherosclerosis* 313, 102–110. doi:10.1016/j.atherosclerosis.2020.08.018

Zhang, B., Zheng, B., Wang, X., Shi, Q., Jia, J., Huo, Y., et al. (2017). Polymer-free dual drug-eluting stents evaluated in a porcine model. *BMC Cardiovasc Disord.* 17 (1), 222. doi:10.1186/s12872-017-0654-7

Zhang, J., Rojas, S., Singh, S., Musich, P. R., Gutierrez, M., Yao, Z., et al. (2021). Wnt2 contributes to the development of atherosclerosis. *Front. Cardiovasc Med.* 8, 751720–751729. doi:10.3389/fcvm.2021.751720

Zhang, Q., He, J., Xu, F., Huang, X., Wang, Y., Zhang, W., et al. (2021). Supramolecular copolymer modified statin-loaded discoidal rHDLs for atherosclerotic anti-inflammatory therapy by cholesterol efflux and M2 macrophage polarization. *Biomater. Sci.* 9 (18), 6153–6168. doi:10.1039/d1bm00610j

Zhang, Z. Q., Yang, Y. X., Li, J. A., Zeng, R. C., and Guan, S. K. (2021). Advances in coatings on magnesium alloys for cardiovascular stents - a review. *Bioact. Mater* 6 (12), 4729–4757. doi:10.1016/j.bioactmat.2021.04.044

Zhao, G., Lu, H., Liu, Y., Zhao, Y., Zhu, T., Garcia-Barrio, M. T., et al. (2021). Single-cell transcriptomics reveals endothelial plasticity during diabetic atherogenesis. *Front. Cell Dev. Biol.* 9, 1–14. doi:10.3389/fcell.2021.689469

Zhao, K., Li, D., Shi, C., Ma, X., Rong, G., Kang, H., et al. (2016). Biodegradable polymeric nanoparticles as the delivery carrier for drug. *Curr. Drug Deliv.* 13 (4), 494–499. doi:10.2174/1567201813041605210046O9

Zhuang, X., Gao, F., Shi, L., Liu, W., Wang, W., He, X., et al. (2021). MicroRNA-146b-3p regulates the dysfunction of vascular smooth muscle cells via repressing phosphoinositide-3 kinase catalytic subunit gamma. *Bioengineered* 12 (1), 2627–2638. doi:10.1080/21655979.2021.1937904

Zong, C., Kimura, Y., Kinoshita, K., Takasu, S., Zhang, X., Sakurai, T., et al. (2019). Exposure to 1,2-dichloropropane upregulates the expression of activation-induced cytidine deaminase (AID) in human cholangiocytes Co-cultured with macrophages. *Toxicol. Sci.* 168 (1), 137–148. doi:10.1093/toxsci/kfy280