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New Developments in Tissue Engineering of Microvascular Prostheses

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1. Introduction

Clinical needs to have a ready-to-use small-diameter vascular prostheses are very remarkable and cover different fields of surgery: plastic and reconstructive surgery (microvascular transfer of free flaps), heart surgery (treatment of ischemic heart diseases), vascular surgery (distal revascularization of lower limbs), neurosurgery (substitution of intracranial arteries), paediatric vascular surgery. In particular, there is a substantial need for tissue-engineered, living, autologous replacement materials with the potential for growth in paediatric applications and for substitute small diameter vessel that up to now are defined the "holy grail" of vascular biology. Completely bio-resorbable vascular prostheses with the capacity for induce regeneration and growth of a new vascular segment may overcome the limitations of contemporary artificial prostheses that are nonviable, artificial, or allogenic materials lacking the capacity of growth, repair, and remodelling. These intrinsic properties limit their long-term function, posing the substantial burden of graft failure and related re-operations, particularly on paediatric patient population. Moreover, these synthetic materials are not suitable for the reconstruction of the coronary, carotid, or femoral arteries as well as other small diameter vessels (< 6 mm). Autologous native vessels, i.e., the saphenous vein and mammary artery, are the most currently used material for small-diameter arterial replacement. Immune acceptance is a major advantage offered by this technique of "ready to use" conduits. However, the availability of suitable native replacements is limited when multiple conduits are required, especially in patients with diffuse vascular disease. The need for a prosthetic graft that performs as a small diameter conduit has led investigators to pursue many avenues in vascular biology (Figure 1). There are four main approaches currently being investigated, all of which satisfy an apparent prerequisite to biocompatibility of a small-diameter graft-that no permanent synthetic materials are used. One approach is acellular, based on implanting decellularized tissues treated to enhance biocompatibility, strength, and cell adhesion/invasion leading to cellularization with host cells. The other three approaches involve implantation of constructs possessing some degree of cellularity. The most recent of these is based on the concept of self-assembly, wherein cells are cultured on tissue culture plastic in medium inducing high ECM synthesis. This leads to sheets of neotissue that are subsequently processed into multilayer tubular form. The other two approaches rely on a polymeric scaffold. One is

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Fig. 1. The ideal micro-vascular prostheses should orchestrate the sequential regeneration of different vascular components: the intimal layer to avoid acute thrombosis, the smooth muscle vascular cell layer to respond to mechanical stimuli and finally the adventitial layer to obtain a long-term vascular patency.

based on forming a tube of a synthetic biodegradable polymer and then seeding the cells (which would not survive the conditions of polymer synthesis), relying on active cell invasion or an applied force to achieve cellularity. The other is based on a tube of a biopolymer, typically a reconstituted type I collagen gel, formed with and compacted by tissue cells, where an appropriately applied mechanical constraint to the compaction yields circumferential alignment of fibrils and cells characteristic of the arterial media. It is this last feature that is most attractive about a biopolymer-based tissue-engineered artery. This follows from two axioms, (i) that native artery function, particularly mechanical function, depends on structure (particularly alignment of the smooth muscle cells and collagen fibers in the medial layer) as much as it depends on composition, and (ii) that the tissueengineered artery should serve as a functional remodelling template, so that while providing function during the remodelling, the artificial tissue also provides a template for the alignment of the remodelled tissue. To some extent, all these approaches rely on the ability of cells (transplanted or host) to adhere to and migrate within the construct, and to remodel its composition and/or structure. This last point is key, as remodelling confers biocompatibility, in principle, by virtue of complete resorption of the initial scaffold. Of course, the initial scaffold must be replaced by functional cell-derived ECM on the same time scale. Remodelling also determines the ultimate mechanical, transport, and biological properties.

The aim of this chapter is to investigate all the materials commonly studied to create small diameter vascular prostheses. In particular, absorbable biomaterials are also reviewed in

depth, for better understanding of the properties of the biomaterials used until now in vascular engineering. A brief analysis of their two main features is necessary: surface properties (haemocompatibility/endothelialisation) and mechanical properties (tensile force/compliance).

2. Material surface

Thrombosis of vascular substitutes is the main mechanism of obliteration and subsequent failure of most vascular conduits. Various methods have been recorded to avoid this phenomenon, such as coatings with antithrombotic drugs, e.g., heparin, hirudin, aspirin, or tissue factor pathway inhibitor (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). There have been attempts to emulate the endothelial cellular surface which, coated with heparan sulphate proteoglycan, produces a negative surface charge which helps to prevent platelet adherence. Some prostheses are therefore coated internally with heparan sulphate, which is quickly degraded, and some materials with an electronegative surface have been created, with uncertain results (Guidoin R et al 1993).

So far, many researchers have described seeding endothelial cells in conduits. A recent study reported a patency rate of 90% in 27 months for ePTFE prostheses used in coronary bypass, after additional incubation with endothelial cells which allowed them to adhere to the material (Laube HR et al 2000). The major limitation of this method is the need for cell cultures and withdrawal of tissue from the patient, and in any case it remains a two-step procedure. A tubular structure of ePTFE is also left in place, with the risk of later infection. Constructs composed entirely of cells (Tissue Engineered Blood Vessels: TEBV) have been devised to overcome these complications (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). Although the method promises amazing results, it is time-consuming and very expensive.

To avoid the cost of cell cultures, many researchers have tried to improve endothelial coverage of prostheses by coating them with endothelial-friendly compounds with good haemocompatibility. For example, e-PTFE prostheses have been coated with perlecan (Zenni GC et al 1993) and endothelial-specific adhesion proteins such as fibrin–gelatin (Kumar TR et al 2002) and hirudin (Salacinski H et al 2002). Fibronectin coating seems to be a successful method, apart from loss of lining at high flow rates. This is why a functional ligand for fibronectin was used, with covalent binding of short peptide sequences (Arg-Gly-Asp, RGD) to improve cell adhesion (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

Instead of coating prostheses with the above substances, another possibility would be to use absorbable, already biocompatible biomaterials, to make entire prostheses (Kannan RY et al 2005).

In spite of all these experiments, endothelialisation in various types of vascular prostheses has been shown in animals but never satisfactorily in humans. The type of material is not the only essential point in favouring endothelialisation. In order to clarify this, we need to go back a little and recall the physiopathology of endothelialisation, which today takes place in three main ways:

- Trans-anastomatic endothelialisation;
- Transmural endothelialisation;
- Endothelialisation due to 'fall-out' of circulating pluripotent cells.

Therefore, trying to enhance endothelialisation means acting on each of these three modalites of cell growth.

Trans-anastomotic endothelialisation (TAE) appears to be very difficult in humans. Early studies on synthetic prostheses report that they cannot be longer than 0.5 cm, even after prolonged implantation. In spite of a long period of observation, internal endothelialisation has not been observed in humans, except in sites of anastomosis (Berger K et al 1972). Several factors have been observed to influence this, such as species, senescence, anatomic dimensions of the vessel, and prosthetic materials (Zilla P et al 2004), but even in animals TAE is limited (Zhang Z et al 2004).

Study of endothelial cells, both human and canine, compared in vitro, suggest that human cells have a greater potential for migration but a lower capacity for adhesion, which may explain the lack of re-endothelialisation in vivo, when blood flow may obstruct cell adhesion (Dixit P et al 2001).

Instead, the transmural pathway seems to enhance rapid endothelialisation, according to recent studies on materials with sufficient porosity. Pore size takes on importance in these studies, since the prosthesis must be sufficiently large to allow cell growth, but not too large to cause loss of intercellular adhesion (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). Materials with differently sized pores inside and outside the conduit have even been experimented, in order to obtain an ecocompatible surface internally and a colonisable one externally (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

Pore size also alters the haemocompatibility of biomaterials, as well as their compliance and degradation time. An optimal pore size for vascular engineering has been hypothesised, ranging from 30 to 50 microns. It appears that smaller pores would not allow growth of endothelial cells, and larger ones would cause excessive leakage of blood (Matsuda T et al 1996). Pores in the walls of prosthetic materials can also avoid intimal hyperplasia. It has been hypothesised that a thrombus initially deposited on the walls of the prothesis later organises itself into muscle-like tissue, which then gives rise to intimal hyperplasia. The precocious growth of endothelial tissue would avoid thrombosis and thus the consequent cascade of events leading to intimal hyperplasia (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). Increased pore size causes increased radial compliance of the material. Several studies have shown that vascular implants with fibers organised in a circular fashion do not cause dilation (Mooney DJ et al 1996; Kim BS et al 1996). This is local, since cells undergo mechanical stress and are thus conditioned in their spatial orientation.

"Fall-out healing" leads to the formation of endothelial islands, with no connection with the formation of trans-anastomotic or transmural tissue. This is a late phenomenon, not of great importance in materials such as Dacron and e-PTFE grafts, and thus it does not play a central role in present-day prostheses (Zilla PD et al 2007), but it appears to be the mechanism for repairing small vascular lesions (Roberts NM et al 2005). However, recent studies show how this mechanism may be enhanced, by attracting EPC cells to participate (Avci-Adali MG et al 2010).

3. Mechanical properties of biomaterials

Before describing the mechanical properties of vascular replacements, we must digress to describe those of vessels. Arteries are mechanically anisotropic, i.e., their elasticity and resistance (maximum pressure tolerated before bursting) varies according to the direction along which they are measured. The capacity for distension of the arterial wall is generally called compliance (radial elasticity), or the difference in diameter obtained by varying

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pressures inside the artery itself ($\Delta D/DP$) X 100). The two main structural proteins, collagen and elastin, confer several properties, enabling arteries to distend more at low pressure and become less elastic at high ones. Both properties are extremely important, since inadequate resistance may lead to rupture, and absence of elasticity may disturb flow, leading to thrombosis. In detail, the velocity of propagation (v) of pressure waves depends on the elasticity of vessel walls (E), their thickness (s) and diameter (D), and on the density (P) of blood, as described by the Moens-Korteweg equation, v= Es/ P D. When a section of artery and a replacement conduit have different radial elastic properties, two consequences may ensue. Discontinuity in the speed of propagation of pressure waves leads to turbulence in the area between the natural and artificial vessels which, in turn, leads to local overpressure, the cause of new aneurisms. Apart from the different degree of distension of arterial sections (original artery/prosthesis), this may put greater stress on sutures.

Different compliance has been associated with intimal hyperplasia in an arterial replacement and the artery itself (Stewart SF et al 1992).

Synthetic tissues are far less elastic than arteries, but absorbable materials which could be replaced by the normal vasal extracellular matrix are believed to avoid this problem. Resorption does very often trigger a response similar to that of a foreign body, which leads to the formation of scar tissue which may deprive the original construct of its elasticity (Zilla PD et al 2007).

From the viewpoint of experimental models, elastic components are more or less the same in various species, in spite of changing sizes. Wolinsky and Glagov (1967) have shown that, whereas the total circumferential tension in the vascular wall of the aorta increases 26 times from mouse to pig, defined by Laplace's law as T=PR, tension per lamellar unit (elastic lamina) is similar, being about 1/ 3Nm-1 in various animal aortas, but unfortunately the above study does not compare peripheral vessels. Arterial elasticity causes a reduction in the pressure gradient generated during cardiac systole/distole (Wolinsky et al 1967; Shadwick RE 1999). In peripheral vessels, flow is continuous, thanks to the reservoir role played by the large vessels during cardiac systole. In humans, the ratio between flow gradient and mean flow falls from a value of 6 near the aortic arch to 2 distal to the femoral artery (Wolinsky et al 1967; Shadwick RE 1999). There is therefore a difference between the elasticity of vessels in relation to their size and position within the circulatory system which may influence the design of ideal replacements for them.

According to the above, and in view of the complexity of the cardiocirculatory system and interactions with blood flow, some components are essential for neovessels if they are to guarantee sufficient resistance and compliance and, at the same time, avoid thrombosis and intimal hyperplasia (Mitchell SL et al 2003). At the present time, always in terms of totally absorbable products, there are many approaches involving heterologous tissues, synthetic polymers, biopolymers and totally engineered products (Isenberg BC et al 2006). Every experiment leads to different conclusions, and associations between materials to exploit desired properties while avoiding problems have also been proposed. For example, PGA is a generally stiff product and is frequently associated with other substances to achieve the necessary elasticity (Shinoka T et al 2008).

4. Materials

Many research groups all over the world have approached the problem of developing the ideal prosthesis in a variety of ways. As in all tissue engineering fields, research ranges round

the triad Scaffold/Cells/Growth Factor (Figure 2). Scaffolds are ideal biomaterials for conduits, and cells can be seeded and cultivated on them, after preconditioning with various growth factors. Very many materials have been used to make scaffolds, generally subdivided into four main categories: biological materials (allografts, xenografts, and derived products), natural proteins, and both permanent and absorbable synthetic polymers.



TEBV (Total Engineered Blood Vessels)

Although this review covers biomaterials used as scaffolds in vascular tissue engineering, mention must be made of the methods for reconstructing small diameter vessels which do not use scaffolds. These mass-produced cell laminae are called TEBV (total engineered blood vessels). They have proven mechanical properties comparable with those of the saphenous vein until 8 months after implant. Their anatomic integration is excellent, showing the formation of vasa vasorum histologically in context and the presence of viable endothelium. Results have been so promising that the first studies for clinical application are under way (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). However, TEBV can only be produced in the laboratory over a total period of about 3 months, which does not make them suitable for urgent procedures; they are also extremely expensive. Of the studies examined,

only 4 dealt with this method. One significant publication was a multi-centre cohort study illustrating the results of implanting TEBV in access routes in patients under dialysis. Although the cohort was composed of high-risk patients, primary patency of 76% was reported in the first 3 months (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

Biological materials

The first attempt at vascular replacement was made in 1908 with an allograft in dog, which earned its inventor the Nobel Prize for medicine (Carrel et al 1906). Since then, biological materials have been tested in various species (xeno- and allotransplants) and after various types of preparatory procedures (decellularisation to reduce the immune response, derivation of material for homogenisation, cryoconservation). These biomaterials are widely available and they are of course excellent substrates for cell adhesion. In addition, the processing method can retain all their advantageous mechanical properties (tensile strength, elasticity) (Schmidt CE et al 2000). As the main disadvantages are possible residual antigenicity and infection after implant (Chlupac JE et al 2009), techniques for their decellularisation and sterilisation have been refined. Articles on TEBV published in the last 5 years (biological materials) mainly deal with materials already nautually present as tubular structures in the body (arteries, veins, urethers) and submitted to decellularisation. They are often studied as allo- or xenografts, and enriched with cells (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996), bFGF (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996), heparin [42] and VEGF (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996) to improve patency in the long term. Of special interest for the physiopathology of tissue healing after implant is one study reporting trends after implants of decellularised porcine arteries in rat, concluding that the initial inflammation due to integration in tissues does not interfere with long-term modelling (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). One in vitro study examines the creation of a biotube produced by reaction to a foreign body (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). Other studies examine the use of SIS (Small Intestinal Submucosa), already amply employed in clinical management of wounds. These studies show the good mechanical properties of this biomaterial (Hinds MT et al 2006), but also the poor long-term patency of conduits (Pavcnik D et al 2009).

Permanent materials

To replace large diameter vessels, synthetic materials are now routinely used in clinical practice, but synthetic polymers such as e-PTFE and Dacron have not given good results in small diameter vessels (<6 mm). In view of the enormous number of works on the subject, the problems of permanent synthetic polymers are clearly difficult to overcome. Many works report variable results in terms of long-term vasal patency, with consequent infection of the operation site (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). During experimental studies, variable patency turned out to be considerable. These materials have been implanted in humans, but do not develop an endothelialised surface (Mooney DJ et al 1996; Kim BS et al 1996), thus causing platelet adhesion and the development of a fibrin layer which may lead to thrombosis. Later failure may also be due to thrombosis after stenotic occlusion of the vessel consequent upon the development of endothelial hyperplasia. Several methods have been applied in the past to reduce thrombosis after surgery (e.g., anti-thrombotic drugs in their surface or surface ligands (Kidane AG et al 2004). Results were better, although clinically satisfactory criteria were not achieved. In the articles examined – that is, those published in the last 5 years – the main non-degradable

biomaterials were PTFE and polyurethanes. A total of 20 articles on PTFE were found, 16 of which described coating surfaces to avoid thrombosis or tissue hyperplasia. This approach is normally followed by endothelialisation of prostheses, and 8 articles described techniques for this (cell cultures). One review (Bordenave L et al 2005) illustrates how this procedure, in time, has moved from one-stage to two-stage techniques although, in spite of discouraging results, not much space was devoted to clinical practice, mainly because of its three most serious limitations: the impossibility of executing these techniques in emergencies; the need for prior withdrawal of cells; and the need for a GLP laboratory to treat human cells.

The remaining approaches for surface coating do not involve cell cultures, and may be interesting as regards future applications of these products in combination with biodegradable materials. Fibrin has been proposed as a coating: results have been either discouraging (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996) or encouraging as regards anti-adhesive action in platelets and perlecan (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996); one article reported an elastin-like recombining protein [84, 85, 88]. Single peptides like RGD, cyclic RGD (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996) have also been proposed, but only in vitro studies are available (Walpoth BH et al 2007; Lord MS et al 2009; Jordan SW et al 2007; Tang C et al 2009; Larsen CC et al 2007).

Polyurethane materials

Polyurethanes are polymers composed of chains of organic units joined by urethane bonds, formed by polymerisation, generally with reagents like monomers containing al least 2 hydroxyl groups (diol), dioisokyanate, and a chain extender. They react to form linear copolymers, segments consisting of alternating stiff and soft segments. The soft segments are derived from polyols such as polyester and the hard ones from isocyanate and chain extenders (e.g., lactic acid/ethylenglycol) (Atala et al 2008). These polymers are biocompatible and highly versatile, since their tensile strength and radial compliance vary according to segment composition (Tiwari et al 2003), stiff segments being responsble for tensile strength and soft segments for elasticity (Kannan et al 2005). Originally produced as permanent biomaterials, they do deteriorate in vivo, due to oxidation and enzymatic and cell-mediated degradation, with the result that their biostability is under revision (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). Oxidation of PU is initiated by oxidase, free radicals and enzymes. The phenomenon of environmental stress cracking is also due to oxidation, a process in which the surface of the biomaterial is coated with proteins which recall adhesion by macrophages, which release oxidising factors (Stokes KR et al 1995). These discoveries were made in the early 1990s and led to the classification of PU as a new category of absorbable materials (Santerre JP et al 2005).

The differing composition of PU segments may lead to products with various degrees of biostability. As regards dioisokyanates/, the aromatic forms are more stable than the aliphatic ones (Fromstein JDW et al 2006; Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996), although the former were abandoned after it was noted that they release toxic substances with carcinogenic effects (hepatocarcinoma) in laboratory animals (Gunatillake et al 2003). PU have been combined with highly crystalline segments such as polycarbonates and silicon oligomers to inrease their stability (Atala et al 2008). Degradable polyesters such as PLA, PGA and PCL have also been associated as weak segments. Carbonate PU have shown good resistance to hydrolysis and oxidative stress (Salacinski HJ et al 2002). In one in vivo study, such prostheses were implanted in the aorto-iliac segment of dog, where they remained viable for more than 36 months (Mooney DJ et al 1996; Kim BS et al 1998; Wake

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MC et al 1996). PU have excellent radial compliance, but are structurally weaker than the others, being more rapidly degraded due to their typical ester bonds (Guan J et al 2002). As well as biodegradability, PU have shown good biocompatibility in in vivo tissue studies (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

The development of small diameter vascular prostheses in the last 5 years revealed a total of 22 articles on polyurethanes, 14 in vitro, 4 on production of material and and its medical properties, and only 4 in vivo. The cellular compatibility of several PU (associated with other substances) has also been studied according to method of preparation, e.g., the use of porous structures.

Electrospinning has been applied to other materials in the field of vascular engineering (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996), and produces small diameter fibers with good tensile strength on the final material. However, this method is difficult to apply when large pores are required. In theory, the immersion-leaching technique (Pan S et al 2005) should not create interconnected pores and thus not be favourable to cell growth, whereas phase inversion (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996) may fail in both interconnections and pore size. One in vivo study examined the PU produced by induction of thermal phase separation, but the resulting biomaterial had poor tensile strength and the overall results in terms of implant viability, presence of aneurisms and vessel functionality were mediocre (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

As regards chemical composition, PU has been combined with silk fibroin, showing better histocompatiblity of pure PU after implant in rat muscular tissue (Wang W et al 2010). Many experiments have also been made on the mixed-composition PU PDMS (polydimethylsiloxane), a silicon-based polymer (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). In this case, PDMS not only increased biostability but also increased haemocompatibility and immunocompatibility (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996). In in vivo studies (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996) show encouraging long-term viability: in one (Khorasani MT et al 2006), a PEUU/PDMS polymer was created with the spray phase inversion technique in a tubular form with two-phase porosity: a highly porous internal wall (mean interfibrillar distance 40 microns) and an only slightly porous outer one (mean interfibrillar distance 30 microns). It showed good re-endothelialisation 24 months after implant, with remodelling of the vessel parallel with digestion of the material, without aneurismatic dilation or calcification. However, the sample size was very small, and the prostheses showed uniform dilation over time.Another in vivo study in this series used poly(ester urethane)urea (PEUU) combined with a thrombogenic polymer not similar to a phospholipid, poly(2-methacryloyloxyethyl phosphorylcholine-co-methacryloyloxyethyl butylurethane) (PMBU), to create a fibrillar scaffold by electrospinning, with good tensile strength and compliance. In addition, the association with PMBU made the PU less prone to platelet deposition and hypertrophy of muscle cells. The in vivo patency of 1.3-mm conduits implanted in rat aorta after 8 weeks varied from 40% for pure PU to 67% for PU PMBU (Hong Y et al 2009).

Bioresorbable materials

In the last five years, most research groups have concentrated on testing absorbable/ biomaterials to achieve the ideal vascular conduit. As already mentioned, the materials in question may be synthetic or biopolymers already constituting the extracellular matrix.

The most common absorbable biomaterials are polyesters. This category contains $poly(\alpha - hydroxylester poly(L-lactic acid)$ (PLLA), poly(-glycolic acid) (PGA), polylactone

polyorthoesters (POE) and polycarbonates. When these materials are implanted in living tissues, their polymeric structure is subject to hydrolysis and the resulting products, such as lactic and glycolic acids, are metabolized. Their safety and biocompatibility are now established (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

It is generally difficult to examine the use of these biomaterials separately, as they are all linked in the field of vascular tissue engineering. The first to be examined was polyglcolic acid, an asborbable polyester, which has shown good biocompatibility and is chosen for many applications (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

Polyglycolic acid was the first biodegradable polymer to be used in vascular engineering [84, 85, 88]. It was tested in combination with cultivated bovine muscle cells and then preconditioned in a pulsatile flow bioreactor. After 8 weeks, this construct revealed collagen and had good mechanical properties. Its tensile strength is comparable to that of a vein. However, it also begins to lose its mechanical resistance within 4 weeks of implant and is completely degraded at 6 months. Degradation speed can be controlled by associating it with other polymers such as poly-L-lactic acid (PLLA), polyhydroxyalkanoate (PHA), polycaprolactonecopolylactic acid, and polyethylene glycol (Mooney DJ et al 1996; Kim BS et al 1998; Wake MC et al 1996).

However, the mechanical resistance of these products does not reach the desired levels – an anticipated outcome, as PGA was originally in the form of a non-woven fabric, and thus does not have measurable tensile strength (Sodian R et al 2000). Most studies have therefore concentrated on preconditioning methods to increase resistance, e.g., use of pulsatile flow bireactors, alternative techniques of cell culture, and administration of various growth factors (Niklason LE et al 1997).

Another substantial problem with PGA is its stiffness, which does not confer the elastic properties typical of arterial tissues (Sodian R et al 2000). In this case too, the use of copolymers has improved results. A fibrillar scaffold based on polyglycolic or polylactic acid coated with a 50:50 L-lactate or L-caprolactone (PCLA/PGA or PCLA/PLA) copolymer has been specifically tested for vascular repair, resulting in compliance closer to that of the original vessel with better surgical handling (Watanabe M et al 2001).

One study showed how PGA-based matrices have greater cellularity and production of proteins of extracellular matrices based on PHAV and P4HB. The authors explained this phenomenon as due to the higher porosity of PGA (> 90%), yielding a contact surface greater than that of cells (Sodian R et al 2000).

To support the remodeling process in vivo, a biomaterial that functions only as a temporary absorbable guide, similar to an in vivo "Artery-Bioregeneration Assist Tube" (ABAT), which can promote the sequential and complete regeneration of vascular structures at the implantation site, entirely made of Hyaluronic Acid was used in different in vivo experimental model (Lepidi et al 2006; Pandis L et al 2010; Zavan B et al).

5. Conclusion

Critical reading of researches in the field of microvascular tissue engineering gave the general impression of progress in the search for an ideal replacement for small diameter vessels.

Most studies indicate the use of absorbable biomaterials, in view of their good integration, with the hope of developing autogenous vessels to replace prostheses. However, not one of these products has yet been approved for clinical experimentation, unlike TEBV and products of biological origin. Degradability is one of the characteristics which tend to

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Fig. 3. Regenerative medicine is based on "intelligent" biomaterials able.

dissuade surgeons at the crucial moment of implant. In addition, other variants have been added, making the subject a spiny one. For example, on one hand, the porosity or fibrillar form of these materials not only alters their biostability but also their mechanical characteristics, which are today believed to be essential for implant success. On the other hand, the variability of clinical applications, which may differentiate the desired characteristics of each type of material, requires reflection.

There are many gaps in the examined articles. The first problem, already examined by many authors, is variability in animal models, which hinders direct comparison of results. Homogeneous studies on mechanical studies are also lacking, since so many of them focus on tensile strength, and neglect compliance, which is an essential feature of vessels.

An effective model of an artificial vessel is very far from being achieved, and its development must take into account the context in which it could be applied. Experimental models have already been super-ceded, if we think that the application of a bio-absorbable prosthesis means that cells must be able to reconstruct a new artery and that, in clinical practice, this must be achieved in already damaged arteries.

In elective vascular surgery (e.g., arterial insufficiency in the lower limbs), cellularised replacements are possible, tailored to suit single patients according to their tissue biopsy. However, the procedures are time-consuming and very expensive, requiring dedicated laboratories able to guarantee sterility and suitability for in vivo re-implantation of cell cultures.

As regards urgent procedures, such as revascularisation of all types, the cell culture step should be avoided. The ideal choice would be ready-to-use materials (Figure 3).

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These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentials of different synthetic and engineered biomaterials. Contributions were not selected based on a direct market or clinical interest, than on results coming from very fundamental studies which have been mainly gathered for this book. This fact will also allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The book collects 22 chapters related to recent researches on new materials, particularly dealing with their potential and different applications in biomedicine and clinics: from tissue engineering to polymeric scaffolds, from bone mimetic products to prostheses, up to strategies to manage their interaction with living cells.

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