Drug releasing systems in cardiovascular tissue engineering

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Abstract

Heart disease and atherosclerosis are the leading causes of morbidity and mortality worldwide. The lack of suitable autologous grafts has produced a need for artificial grafts; however, current artificial grafts carry significant limitations, including thrombosis, infection, limited durability and the inability to grow. Tissue engineering of blood vessels, cardiovascular structures and whole organs is a promising approach for creating replacement tissues to repair congenital defects and/or diseased tissues. In an attempt to surmount the shortcomings of artificial grafts, tissue-engineered cardiovascular graft (TECVG), constructs obtained using cultured autologous vascular cells seeded onto a synthetic biodegradable polymer scaffold, have been developed. Autologous TECVGs have the potential advantages of growth, durability, resistance to infection, and freedom from problems of rejection, thrombogenicity and donor scarcity. Moreover, polymers engrafted with growth factors, cytokines, drugs have been developed allowing drug-releasing systems capable of focused and localized delivery of molecules depending on the environmental requirements and the milieu in which the scaffold is placed. A broad range of applications for compound-releasing, tissue-engineered grafts have been suggested ranging from drug delivery to gene therapy. This review will describe advances in the development of drug-delivery systems for cardiovascular applications focusing on the manufacturing techniques and on the compounds delivered by these systems to date.

Keywords: tissue engineering • cardiovascular graft • drug delivery • growth factors

Introduction

Heart disease and atherosclerosis still represent the leading causes of morbidity and mortality worldwide. The lack of suitable autologous grafts has produced a need for artificial grafts but the patency of these grafts is limited in comparison to natural materials. Tissue engineering, of blood vessels, cardiovascular structures and whole organs holds promises as a new approach for creating replacement tissues to repair congenital defects and/or diseased tissues. Current artificial grafts carry significant limitations, including thrombosis, infection, limited durability and inability to grow. In an attempt to surmount these shortcomings, creation of tissue-engineered cardiovascular vascular grafts (TECVG), constructs by using cultured autologous vascular wall cells seeded onto a synthetic biodegradable polymer scaffold, have been developed [1, 2]. Autologous TECVGs have the potential advantages of growth, durability, resistance to infection, and freedom from problems of rejection, thrombogenicity and donor scarcity. Both pre-clinical and clinical studies have demonstrated the feasibility of constructing functional large-diameter (6 mm) vascular grafts from autologous vascular cells seeded onto a biodegradable tubular matrix [3–5].
Until now several types of polymers have been proposed to obtain vascular grafts and poly(lactic-co-glycolic acid) polymers (PLGA) and poly-L-lactic acid (PLLA) represent the most attractive avenue for this purpose as their advantageous mechanical properties, plasticity and biocompatibility [6]. These materials are biodegradable, with the aim of in vivo vascular cell remodelling to enable the final functional conduit to perform the structural role as well. To this extent, the degradation process with further possibility of remodelling is to be pursued especially to avoid aneurysmal degeneration and rupture, as it does confer adequate strength to the conduit. There is a good piece of evidence underlining biodegradable porous polymer scaffolds as an attractive alternative to develop temporal templates for tissue regeneration, prosthetic devices and stents.

Many different techniques have been developed for the construction of vascular scaffolds including solvent casting/particulate leaching, phase separation, emulsion freeze drying, fibre extrusion and fabric forming processing, gas foaming and lately the electro-spinning technique [7].

Moreover, polymers engrafted with growth factors, cytokines and drugs have been developed [8–10], thus obtaining drug-releasing systems capable of focused and localized delivery of molecules depending on the environment requirements and the actual milieu in which the scaffold is placed. A broad range of applications for electrospun fibres have been suggested [11, 12] ranging from drug delivery [13–17] to gene therapy [18].

However, in many cases of tissue-engineering applications, cell differentiation towards a specific phenotype is highly desirable. In situ local delivery of various drugs and protein growth factors within tissue-forming cell/scaffold constructs has been investigated [19, 20]. Local sustained delivery of various growth factors such as epidermal growth factor [21], transforming growth factor (TGF) [22], vascular endothelial growth factor (VEGF) [23, 24] and bone morphogenetic proteins (BMPs) [25] within the biodegradable scaffolds has been used to stimulate the differentiation of seeded cells to desirable specific organized tissues. To this extent, because of their biochemical properties, it is possible to produce growth factor microspheres to encapsulate within polymeric biomaterials or direct incorporate into the void space of the scaffolds to achieve sustained release [20].

In tissue engineering of blood vessels and cardiovascular structures, several autologous cell types have been used to seed the constructs in particular endothelial cells obtained from great saphenous vein even if this raise a good number of issues concerning cells amount and availability [26]. Recently, bone marrow stem cells were seeded into macroporous biodegradable polymer scaffolds and implanted to regenerate or heal injured tissues [27]. Previously, a 3D collagen type I matrix has been seeded with human umbilical cord blood mononuclear cells and grafted onto infarcted ventricles in an ischemic heart murine model with notable improvement in the left ventricular function and myocardial remodelling [28]. Recently, a similar approach has been successfully used in a clinical trial (MAGNUM Trial), with a bone marrow cells seeded collagen sponge applied on top of the scarred area aiming at regenerating myocardial cells and restoring the extracellular matrix (ECM) function which is deeply altered following myocardial infarction [29, 30].

This review will describe the actual advances in development of drug-delivery systems (DDSs) for cardiovascular applications focusing on the manufacturing techniques and on the molecules delivered by these systems until now.

**Drug delivery systems in cardiovascular applications – what are the aims/targets?**

In the cardiovascular research field, biosynthetic and biodegradable materials have been largely investigated. A wide range of natural materials ranging from collagen [31–33], fibrin [34], hyaluronic acid [35] to approaches involving tissue decellularization [36, 37] have been evaluated (Table 1). With the aim to improve the long- and short-term management of the tissue-engineering vascular graft following its implantation, a variety of local DDSs have been developed.

In the early post-implantation phase, the local environment undergoes a number of dramatic changes that are considered to be at the root of the loss of in vivo performance of biomedical devices. Implanted devices have been shown to typically trigger a cascade of immunogenic reactions in response to the implantation-related trauma that results in acute inflammation with dense infiltration of inflammation-mediating cells at the device–tissue interface [38]. The acute inflammation associated to the presence of a foreign object can eventually lead to complications that can adversely impact or compromise the performance of implantable devices [39]. The maintaining of the inflammatory stimulus provoked by chronically implanted devices turns in the accumulation of dense fibrotic tissue which encapsulates the device with the regression of blood vessel growth, remarking a shift to a chronic state of the inflammation process [40]. In myocardial tissue engineering this process results further in issues concerning mechanical resistance and endothelialization of the inner graft’s surface [41].

Cells survival, proliferation and differentiation require the development of an adequate vascularization especially at the early stage of implantation. Even if the foreign body response to the implanted material is known to induce a certain degree of capillary ingrowth over time, this native angiogenic process is likely to be either too slow or quantitatively inadequate in terms of capillary density, especially considering dimensions and thickness of implanted grafts. Mass transfer limitations and fibrosis associated with the foreign body response can result in non-sufficient nutrient transport for most cells in constructs approximately 1-mm thick and greater [42–44] leading to implant necrosis [45]. Carrier et al. [46, 47] and Radisic et al. [48–50] demonstrated the importance of the oxygen supply in producing thick and resistant new tissue on tissue-engineered cardiac grafts (TECGs). They found that only increasing the oxygen concentration supply to an in vitro TEGC, improved the production of cardiac muscle [47, 51]. Increasing oxygen availability by increasing endothelialization is especially important as most TECGs and myocardial patches are directly applied to an infarcted area in in vivo experimental models to provide ventricular mechanical support.
Table 1 Biomaterial used for cardiovascular applications

| Source        | Biomaterial                                      | Application          |
|---------------|--------------------------------------------------|----------------------|
| Natural       | Matrigel [31, 209, 210]                          | Cardiac              |
|               | Matrigel [32]                                    | Cell differentiation |
|               | Collagen [28, 31, 33]                            | Cardiac              |
|               | Collagen [211]                                   | Vascular             |
|               | Hyaluronic acid [35]                             | Vascular             |
|               | Alginate [61]                                    | Cell differentiation |
|               | Fibrin [34]                                      | Cardiac              |
|               | Fibrin [35]                                      | Vascular             |
|               | Decellularized vessel [37]                       | Vascular             |
|               | Decellularized small intestine [36]               | Vascular             |
|               | submucosa                                        |                      |
| Synthetic     | Poly-L-lactic acid (PLLA) [212]                   | Cell differentiation |
|               | PLLA [213]                                       | Vascular             |
|               | Poly(lactic-co-glycolic acid) (PLGA) [212]        | Cell differentiation |
|               | PLGA [40]                                        | Vascular             |
|               | Polyglycolic acid [214]                          | Vascular             |
|               | poly(epsilon-caprolactone) (PCL) [215]           | Vascular             |
|               | PCL- Poly Urethan [216]                          | Vascular             |
|               | Peptide nanofibres [64, 217]                     | Cardiac              |
|               | Peptide nanofibres [64]                          | Vascular             |
|               | Poly(diol citrates) [218, 219]                   | Vascular             |
|               | Poly(glycerol-sebacate) [220]                    | Cardiac and vascular |
|               | Bioglass [221]                                   | Vascular             |

Thus, stimulation of rapid formation of high-density local vasculature is crucial for tissue regeneration. A highly open porous and sponge-like structure is desirable in order to provide a sufficient cell seeding density and to ease mass transfer rates of nutrients and oxygen within the cell-seeded scaffolds. In this environment, microvessels may sprout from host vasculatures and penetrate into the scaffolds by a natural angiogenesis process. However, this type of neovascularization phenomenon is rare and insufficient to support the whole area of implanted constructs leading to insufficient tissue formation with massive cell death often observed in the early-phase of implantation [52, 53].

Therefore, inducing angiogenesis into the inner pore space of scaffolds is essential. Incorporation of angiogenic growth factors such as basic fibroblast growth factor (bFGF) and VEGF, among others, into scaffolds for controlled release has been shown to promote local angiogenesis [42, 54] (Fig. 1). This effect could be crucial in pathological states as critical limb ischemia. Here the sustained pro-angiogenic stimulus driven by the inappropriate tissue oxygen level could be fostered by the release of specific growth factors leading to a rapid vessel sprouting and collateralization. Moreover, addition of growth factors and other types of molecular signals to the scaffolds could result in the generation of a molecular device providing a leading framework for guided cell differentiation and tissue regeneration (Table 2). In the vascular field, trophic cutaneous lesions related to microvessel diseases as diabetic or venous insufficiency-related ulcers, could benefit from these approaches for skin reconstruction following tissue loss. But this concept becomes even more relevant in cardiac tissue engineering where use of artificial myocardial patches is desirable to both limit geometrical and shape remodelling of the infarcted myocardium and to promote tissue restoration. At the cellular level the sudden suppression of oxygen supply triggers molecular cascades and a series of other dramatic biological events all modulated by intricate molecular networks. The inflammatory state associated with the early-phases of wound healing is characterized by a high regenerative and proliferative capacity of both cardiac and mobilized stem cells. These initial adaptive mechanisms, especially sustained by neurohormonal activation, further lead to a deleterious counterpart represented by fibroblast proliferation and ECM deposition with the aim to compensate tissue loss and prevent ventricular dilation. At this point the proliferative capacity and the actual viability of myocardiocytes are dramatically reduced with the generation of a non-functional scar. To this extent, engineered functionalized myocardium might represent an amenable alternative as it could provide both a mean for mechanical restraint and prevention of ventricular dilation. At the same time it could generate an environment to maintain the proliferative capacity of the cells surrounding the infarcted area providing a molecular pathway to promote cell differentiation (Fig. 2).

Approaches, methodologies and manufacturing techniques

Since a lack of blood supply to transplanted cells at the early stage of implantation retards the survival, proliferation and differentiation of cells, therapeutic angiogenesis via sustained delivery of angiogenic growth factors at a localized region is essential for tissue regeneration [23, 55] Several different techniques have been used for the fabrication of scaffolds for tissue engineering. These include solvent casting/particulate leaching, phase separation, emulsion freeze drying, fibre extrusion and fabric forming processing and gas foaming [7]. Of late, the electrospinning technique is becoming one of the most effective and functional approaches [56] with the possibility to be combined to the newest methodologies of cell seeding [57, 58]. The electrospinning process allows for control over the morphology of the fibres and utilization of a wide variety
of polymers. The small fibre diameters produced by electrospinning have the advantage of a large surface-to-volume ratio, as well as a high permeability and interconnecting pore structure, both of which are desirable in a biological setting (Fig. 3). Further, this material can be functionalized with compounds encouraging cell survival, proliferation and differentiation obtaining a DDS.

An appealing class of delivery vehicles is represented by hydrogels, as they can be introduced into the body with minimally invasive procedures [59] and are often highly biocompatible, owing to their high water content. Alginate is a naturally occurring (linear unbranched) polysaccharide composed of an α-L-guluronic and β-D-mannuronic acid sugar residues. Sodium salts of alginate are water soluble, but in the presence of divalent cations (such as Ca\(^{2+}\)), alginate chains form ionic cross-links, leading to gelation. Owing to the gentle nature of this gelation process, Ca\(^{2+}\)-cross-linked alginate hydrogels have been widely used in several medical applications (dental impressions, drug-delivery devices and as immobilization matrices for cells) [60, 61]. The main issue with these materials concerns the fact that, despite their biocompatibility, these gels undergo a slow and unpredictable degradation in vivo harnessing their utility. Several studies have reported attempts to control alginate gel degradation by partial oxidation of the polymer chains with sodium periodate [62], with the lower levels of oxidation maintaining the gel biocompatibility [63]. An alternative strategy relies on controlling the polymer molecular weight distribution, with the use of a binary molecular weight formulation that allows the incorporation of low molecular weight chains, which more readily disassociate from the gel and can be subsequently excreted from the body [62]. To this extent, growth factor release could be tuned by controlling both diffusion and degradation. Another interesting class of compound amenable for tissue engineering and drug-releasing systems is represented by self-assembling peptides, a class of synthetic, ionic, oligopeptides that spontaneously assemble into gels with an ECM-like microarchitecture when exposed to salt [64].

These peptide scaffolds can be further functionalized through direct solid-phase synthesis extension of short-sequence motifs from two major proteins of the basement membrane, laminin 1 (YIGSR, RYVVLPR) and collagen IV (TAGSCLRKFSTM). It has been shown that these tailor-made scaffolds increased the formation of...
confluent cell monolayers with cobblestone phenotype of human aortic endothelial cells in culture. These functionalized scaffolds can maintain the typical phenotype of endothelial cells which not only preserve low-density lipoprotein uptake activity but also enhance their nitric oxide release capacity, and can promote basement membrane protein deposition as laminin 1 and collagen IV, which are physiological prerequisites for successful culture and expansion of endothelial cells [64]. Moreover, these biomimetic ECM-like materials support capillary morphogenesis and thus may be useful for supporting vascularization [65].

However, for tissue regeneration of complex structures such as cardiovascular components, scaffolds aiming to recapitulate the native histoarchitecture are needed. Again, the necessity to create a specific microenvironment with factors able to promote and orientate cell proliferation requires a careful evaluation and widely spans in the drug-delivery spectrum of interest. Until now several methods to incorporate growth factors into synthetic scaffolds have been developed [42, 55, 66–69]. Growth factors can be adsorbed to the scaffold [70]. Adsorption efficiency is material related and concerns not only the chemical influence but also physical properties of the scaffold itself as the porosity and the nature of the pores. Moreover, it depends on the structure of the growth factors too, which can in turn affect release kinetic. As proteins are generally adsorbed through electrostatic attractions between anionic group and the scaffold surface, growth factors presenting only a few of these reactive groups can display lower adsorption capacities on biomaterials as elegantly showed by Ziegler and colleagues who combined ceramic materials with bFGF and VEGF [70].

It is also possible to blend growth factor containing microspheres into the scaffold [71] without interfering with its macro and microstructure. Microsphere incorporation allows for a fine modulation of the release kinetics by adjusting the molecular weight and composition of the copolymer, eventually avoiding undesirable initial releases bursts.

Recently, Patil and colleagues developed a novel PLGA microsphere/poly(vinyl alcohol) (PVA) hydrogel composite DDS that may be suitable for coating implantable devices for localized delivery of medicinal agents. These composites combine the structural mechanical properties and relative tissue compatibility of physically cross-linked PVA hydrogels with the slow release properties of PLGA microspheres to provide a drug-delivery coating device able to provide a zero-order release profile [40].

Alternatively, a direct mixing growth factor containing protein powder into the scaffold during processing can be performed [24, 53, 72].

Adsorbing growth factor onto the scaffold has the drawback of low loading efficiencies and rapid release together with the degradation of growth factors such as rhVEGF, bone morphogenetic protein 4 (BMP-4) and bFGF during a very short release time [70]. Loading growth factor into microspheres, even if elicits a regulated sustained release, could be harnessed by a loss in bioactivity due to harsh solvents such as methylene. Incorporating growth factors directly into the scaffold can potentially surmount these shortcomings. In particular, the use of a thermoplastic scaffold would be amenable to the mixing of growth factor during polymer processing providing an appropriately mild solvent or low processing temperature. Guan and colleagues have developed a biodegradable, elastomeric poly(ester urethane)urea (PEUU) scaffold capable of releasing bioactive bFGF over a period of several weeks [73].

Nevertheless, more controllable growth factor delivery systems are definitely needed for angiogenesis. Another approach

| Drug/growth factor               | Biomaterial                                                                 |
|----------------------------------|-----------------------------------------------------------------------------|
| Dexamethasone                    | Poly(D,L-lactic-co-glycolic acid) (PLGA) [89]                                |
|                                  | PLGA microspheres/poly(vinyl alcohol) hydrogel composite [40]                |
| VEGF                             | Poly(lactide-co-glycolide) (PLG) [109]                                       |
|                                  | Fibrin [222]                                                                |
|                                  | Collagen [223]                                                              |
|                                  | Gelatine [224]                                                              |
|                                  | Alginate [114]                                                              |
| PDGF-BB                          | Poly(D,L-lactic-co-glycolic acid) (PLGA) [71]                                |
| b-FGF                            | Alginate [69]                                                               |
|                                  | poly(D,L-lactic-co-glycolic acid) (PLGA) [132]                               |
|                                  | Poly(ester urethane)urea [73]                                               |
| TGF-β                            | Fibrin [225]                                                                |
|                                  | Gelatine [226]                                                              |
| Insulin-like growth factor 1     | Gelatine [227]                                                              |
| HGF                              | Self-assembling nanofibres [134]                                            |
| Heparin                          | Gelatine [228]                                                              |
|                                  | Alginate [81]                                                               |
|                                  | Chitosan [82]                                                               |
|                                  | Hyaluronic acid [83]                                                        |
|                                  | Poly(D,L-lactic-co-glycolic acid) (PLGA) [80]                               |
| Heparin                          | Poly(epsilon-caprolactone)(PCL) [9]                                         |
|                                  | Polymer-coated metallic wires [139]                                         |
| Diazoniumdiolates nitric oxide donor | Polyurethane [143]                                 |
|                                  | Polyethylene glycol (PEG) [146]                                             |
| Plasmid DNA encoding platelet-derived growth factor (PDGF) gene | Poly(D,L-lactic-co-glycolic acid) (PLGA) [180] |
Surface-modified PLGA scaffolds have been developed in order to enhance adhesion and function of rat hepatocytes, bovine chondrocytes and bone marrow stem cells by surface immobilization of bioactive molecules such as galactose, cell adhesive peptides and hyaluronic acid [74–77].

The idea at the basis of this strategy concerns the mimicry of the natural ECM with the final aim to improve cell adhesion, proliferation and differentiation. Among the ECM components, heparin has been known to specifically bind various angiogenic growth factors such as VEGF, bFGF and TGF-β [23, 78, 79]. Through specific interactions occurring between both 2-O-sulphate group and N-sulphate group of heparin and lysine and arginine residues of the growth factors, heparin could be reliably considered as playing a depot role in the ECM for the growth factors, enabling them to diffuse out in a sustained manner. Thus addition of heparin on a supporting scaffold could represent a functionalization strategy enabling the scaffold itself to increase its drug-delivery capabilities. Furthermore, it has been shown that the binding of bFGF to heparin augments its stability against thermal denaturation and enzymatic digestion at physiological conditions [80] by inducing a conformational change in the molecule. In relation to both this protective effect and the heparin-specific affinity towards angiogenic growth factors, a good piece of literature has been focused on controlled release of growth factors using heparin-conjugated natural polysaccharides such as alginate, chitosan and hyaluronic acid [81–83]. In these studies, heparin-modified hydrogel matrices loaded with growth factors were intended to be injected into a desired tissue defect site to induce local angiogenesis.

However, it is important to consider that the in vivo release of proteins may result in a more severe inflammatory response as a consequence of the body’s response to the foreign protein, in comparison to that resulting from the implant alone. It can further exacerbate the condition and thus aggravate the adverse effects.

Another approach for scaffold surface modification concerns the addition to the polymer of other bioactive materials that present angiogenic capabilities. To this extent, glasses represent an interesting class of compounds. Since the observation by
Hench and colleagues in 1971 that glasses in the system SiO₂–CaO–NaO–P₂O₅ possess bioactivity, these materials have found use in a wide range of biomedical applications including permanent implants, dentistry, bone reconstruction and tissue-engineering scaffolds [84]. More recently, it has been discovered that certain compositions of bioactive glass stimulate a significant increase in the secretion of angiogenic growth factors from fibroblasts and can induce infiltration of a significantly increased number of blood vessels into synthetic scaffolds [85, 86]. This effect can be incorporated into biodegradable tissue-engineering scaffolds in order to obtain bioactive glass-coated polymer composites able to induce angiogenesis. The ability of bioactive glass-coated polymer scaffolds to stimulate the secretion of angiogenic growth factors and subsequent in-growth of blood vessel into the scaffolds could offer a novel way of ensuring that soft-tissue-engineered constructs become sufficiently vascularized. These composites can be obtained by phase separation, solvent casting/salt leaching and gel foaming adding Bioglass particles to the polymer solution and have been shown to retain the surrounding granulation tissue and a higher number of blood vessels in an in vivo model [87]. Moreover, it has been recently suggested that the pro-angiogenic capacity of this material is related to the soluble dissolution products of Bioglass and the subsequent production of cell-secreted angiogenic factors by stimulated cells [88].

**Drug and molecules released**

**Steroids**

With the aim to reduce the foreign body response following graft implantation and interrupt the vicious circle eventually leading to the failure of the biomedical device, corticosteroids have been used during the scaffolds fabrication. Considering this rationale, Yoon and colleagues developed a sustained releasing dexamethasone biodegradable scaffold showing the possibility to achieve an...
inhibiting effect on smooth muscle cell proliferation [89]. From this point of view, dexamethasone releasing porous and biodegradable scaffolds could be potentially used either as an anti-inflammatory porous prosthetic device or as a temporal anti-inflammatory biodegradable stent for reducing intimal hyperplasia in restenosis. Patil and colleagues developed a PLGA microsphere/PVA hydrogel composite DDS with zero-order release profile that provides a reservoir with a constant amount drug delivery. Dexamethasone loading of this device could be more adequate in the control of inflammatory responses at the tissue/implant interface. Moreover, due to its capability to induce cell differentiation, corticosteroid drug-releasing scaffolds could be usefully employed in tissue engineering of stem cells [90]. Dexamethasone, along with other cytokines, has been used as a stimulating agent for the differentiation of stem cells into bone cells [91, 92]. With this in mind drug loaded scaffolds could be employed to create a specific differentiating microenvironment useful in tissue regeneration. For instance, a scaffold implanted in a bone defect site in vivo could be colonized by bone marrow stem cells recruited and migrated from the local tissue, that might more readily differentiate into bony osteoblast cells due to the locally sustained released dexamethasone.

However, although administration of anti-inflammatory agents such as dexamethasone can minimize implantation-associated inflammation [40, 93, 94], their delivery can inhibit endogenous blood vessel growth. A large number of studies proved that dexamethasone administration can inhibit or down-regulate some of the key factors responsible for the formation of blood vessels such as endogenous VEGF and other angiogenic growth factors [95–98]. A decrease in the available blood circulation surrounding the implant could potentially limit graft functionality and retard healing of the trauma related to the implantation itself. Thus an amenable strategy could be the co-administration of both anti-inflammatory corticosteroids as well as growth factors in a localized environment at low doses so that both agents can function symbiotically without interference [93].

**Growth factors**

Localized delivery of growth factors using implantable DDSs has been successfully used to achieve specific pharmacological effects such as neo-angiogenesis using VEGF [99–102] and bone growth using BMPs [103, 104]. As far as pro-angiogenic growth factor treatment is concerned, considering the limited success in clinical investigations of direct bolus injections of pro-angiogenic growth factors [52, 105], a good piece of literature has been focused on the controlled (i.e. sustained and localized) VEGF delivery from polymeric vehicles. This method has been proven to drive vascularization at a desired tissue site, as indicated by increased microvessel density [106]. Moreover, considering the short half-lives of these molecules in vivo [107], a confined delivery of such potent initiators of angiogenesis to the specific site where intervention is desired would be an attractive alternative in order to compensate the short time tissue exposure, minimizing undesired systemic effects.

Delivery of VEGF has been shown to not only stimulate new blood vessels but also improve circulation in the intended target area [100, 102, 104, 108, 109]. Moreover, combinations of anti-inflammatory agents and growth factors to simultaneously control inflammation and ensure neo-angiogenesis have been reported [93]. This could represent an adequate strategy to circumvent the anti-angiogenic effects of the corticosteroids.

A sustained delivery of VEGF by means of a polymer [85:15 poly(lactide-co-glycolide) (PLG)] has been shown to increase angiogenesis and improve perfusion in an ischemic murine hind limb model [109]. VEGF delivery resulted in a greater capillary density and a more mature vasculature in both the muscle surrounding the implanted area and within the scaffold, suggesting that angiogenic process was regulated by the polymer-derived VEGF. Interestingly, VEGF was not sufficient to induce a detectable significant vessel enlargement suggesting the need for a synergic action of additional factors such as platelet-derived growth factor (PDGF) [20].

PDGF has been demonstrated to promote wound healing through enhancing the formation of granulation tissues, and recombinant human PDGF-BB has been approved by US Food and Drug Administration for use in diabetic foot ulcers [110]. Despite its potential role in tissue regeneration enhancement, clinical application of PDGF encounters some hurdles basically represented by requirement for frequent administrations to maintain sufficient therapeutic concentration. In order to surmount these shortcomings, engineering of biomaterials developed a microsphere-based scaffold release system in which PDGF-BB encapsulated PLGA microspheres were incorporated into a biodegradable PLLA scaffold with well interconnected macroporous and nano-fibrous structures [71]. Moreover, given its protective action on cardiomyocyte [111], local myocardial delivery of PDGF by self-assembling peptide nanofibres at the time of myocardial infarction has been attempted, leading to a sustained improvement in cardiac function without induction of cardiac fibrosis or pulmonary hypertension [112].

Polymeric delivery systems allowing localized and sustained release of therapeutic agents may bypass the current limitations of growth factors delivery [113]. Alginate hydrogels may represent an attractive alternative for localized VEGF delivery [114, 115] and recently an injectable biodegradable alginate hydrogel allowing sustained and localized release of VEGF at a desirable concentration has been developed [116]. This system provided a spatiotemporal factor bioavailability leading to a significant angiogenic response in ischemic hind limbs. The importance of a regulated VEGF-induced angiogenesis is demonstrated by the lack of efficient perfusion when an uncontrolled induction of angiogenesis leads to leaky vessels with tortuous profiles that are readily remodelled and eliminated through regression [117, 118].

It is important to focus on the fact that the biocompatibility of the materials currently used does not necessarily imply in its significance that they are inert and will not exert any collateral effect once in vivo setting, specially when drugs and growth factor are involved in the experimental arena. Indeed, several natural
mechanisms involved in circulatory homeostasis maintenance may also affect engineered vascular networks and, on the other side, the addition of exogenous signals would surely affect this delicate equilibrium. One of these mechanisms concerns blood flow to capillary beds. Vasodilating (e.g. nitric oxide, carbon dioxide) and vasoconstricting (e.g. vasopressin, endothelin) factors originated from the vessel endothelium and the surrounding tissue modulate blood flow in a tissue region according to changes in tissue metabolism and blood pressure [119].

The immune system may also affect the functionality of engineered vessels, as invading immune cells secrete angiogenic growth factors and also release these factors from the ECM [120, 121]. Chen and colleagues demonstrated differences in the angiogenic local response in severe combined immunodeficiency (SCID) and immunocompetent mice (C56BL/6) to a porous PLG construct engineered to provide a sustained delivery of VEGF [122]. The VEGF delivered from this system maintained at least 90% bioactivity and induced sprouting angiogenesis from surrounding host vasculature, which infiltrates the highly porous scaffold, resulting in the rapid formation of neovasculature in vivo [20, 123] both within and around the PLG system. The defined area of the scaffold allowed to differentiate between new tissue within the scaffold (i.e. local effects), and the surrounding host tissue (i.e. regional effects), and thus to distinguish between perfusion changes resulting from growth factor-induced angiogenesis (within the scaffold), and the combined effects of angiogenesis and arteriogenesis in the tissue surrounding the scaffold. This study proved that local angiogenesis may be augmented by the combined effects of angiogenesis initiated by the immune response and delivered growth factors, clearly stressing the idea of the need for a careful evaluation of the ‘host factor’ in tissue engineering of cardiovascular structures. Moreover, dosage of the delivered growth factors has to be considered in order to avoid some potential adverse effects as tumourigenesis.

Along with VEGF, b-FGF has been one of the most studied cytokine as for its angiogenic properties. This heparin-binding growth factor is known to induce the proliferation of endothelial cells and has been studied as a potent mitogen for tissue regeneration, wound healing and angiogenesis [124]. In addition, heparin-bound bFGF improved recognition by cellular receptors and enhanced mitogenic activity. b-FGF has been widely used for vascular applications. Intracoronary delivery with increased collateralization has been shown [125]. Treatment of expanded polytetrafluoroethylene (ePTFE) graft with bFGF in order to increase endothelialization [126, 127] and coating with the peptide sequence Arg-Gly-Asp to improve endothelial cell adhesion through the integrins system, have been performed [128]. However, these attempts encounter hurdles related to the synthetic graft materials which still present issues including high mechanical stiffness, thrombogenicity and the elicitation of foreign-body reactions. Additionally, endothelialization has been shown not to inhibit intimal thickening in vascular prostheses [129]. The use of a naturally occurring ECM materials in the form of a decellularized artery as a substrate for an endothelial cell seeded vascular graft, has been experimented with the aim to maintain similar mechanical properties and structure to a native artery [130]. This system has been further biologically improved by means of covalent link of heparin to the graft to reduce thrombogenicity and to act as a substrate for heparin-binding growth factors. Further coating of the decellularized functionalized artery with b-FGF resulted in an increase in cell retention and proliferation rate of both human microvascular endothelial cells and canine endothelial progenitor cells in vitro and may therefore help expedite the endothelialization [131].

Furthermore, b-FGF has been used in an alginate hydrogel sustained delivery system showing, after a subcutaneous implantation, an increase in the number of blood vessels in the granulation tissue even higher than in a VEGF-based similar model [69]. This system could enable maintenance of a growth factor concentration ranging in the therapeutic dosage for a prolonged time window within a distance from the vehicle that is regulated by the effective amount of factor incorporated in the scaffold [69].

More recently, Guan and colleagues developed a thermoplastic elastomer system to provide a controlled release of bFGF. Biodegradable, PEUU scaffolds in tubular and cylinder forms with the ability to release bioactive bFGF over a 3-week period in vitro have been seeded with murine smooth muscle cells. These scaffolds showed mechanical properties attractive for future application in soft tissue engineering as demonstrated by tensile strength, elongation at break and suture retention studies [73]. The release profile of bFGF from heparin containing PEUU scaffolds was found to be similar to that from PLGA scaffolds and methyldiene malonate polymer. Previously, a bFGF-loaded PLGA scaffold fabricated using supercritical CO2 exhibited 45% initial burst release in 1 day [72, 132]. The release of bFGF from methyldiene malonate polymer films had over 30% initial burst release in the first day [133].

These studies clearly demonstrate the possibility to develop a cell-seeded construct that can stimulate local angiogenesis useful for blood vessel and cardiovascular structures tissue engineering. Additionally, the drug-delivery technology can address issues hampering the effectiveness of stem cell therapy regarding cell engraftment, survival and differentiation engineering the local cellular microenvironment. Davies and colleagues designed self-assembling peptide nanofibers for prolonged delivery of insulin-like growth factor 1, a cardiomyocyte growth and differentiation factor, to the myocardium, using a ‘biotin sandwich’ approach [134]. The ability to control the intramyocardial environment by delivering growth factors to injured myocardium maybe crucial to preventing heart failure as it might sustain organ function and improve the endogenous regenerative response providing a chemoattractant signal to promote stem cell migration.

Heparin

Heparin is widely used for a variety of important cardiovascular conditions including deep venous thrombosis, myocardial infarction and to circumvent thrombotic phenomena arising from bare metal stent implantation in coronary arteries or other vascular
procedures. However, heparin has profound effects on many of
the other events that accompany vascular repair. Heparin
markedly and rapidly inhibits DNA and RNA synthesis in cultured
vascular smooth muscle cells, limits leucocyte adhesion to injured
endothelium, restores endothelial integrity, enhances complement
biology and interacts with vascular growth factors being involved
in their signalling cascade. To this extent, heparin is considered
the best alternative to reduce intimal hyperplasia after vascular
interventions [135–138]. Based on the specific affinity between
heparin and angiogenic growth factors, and its capability to increase
stability against thermal denaturation and enzymatic digestion at
physiological conditions, there have been numerous reports about
controlled release of growth factors using heparin-conjugated natu-
ral polysaccharides such as alginate, chitosan and hyaluronic acid
[81–83]. Recently, heparin has been used in association with
biodegradable scaffolds by means of immobilization onto the sur-
face of PLGA constructs to obtain local and sustained delivery of
bFGF [80]. In this study, heparin was immobilized onto the surface
of PLGA scaffolds by covalent conjugation between carboxylic
groups of heparin and surface-exposed amine groups on the PLGA
scaffolds. The heparin-immobilized scaffolds loaded with bFGF
showed a proliferative effect on human umbilical vein endothelial
cells and when implanted subcutaneously in vivo, they effectively
induced the formation of blood vessels in the vicinity of the implant
site. This study demonstrated that local and sustained delivery of
angiogenic growth factor for tissue regeneration could be achieved
by surface modification of porous scaffolds with heparin.

In consideration of the antithrombogenic properties, coiled tubu-
lar structures, constructed from polymer-coated metallic wires with
the addition of heparin to the surface, have been also developed and
demonstrated to support growth of an endothelial cell layer in vitro.
In this study the constructs have been treated with human plasma
proteins in order to mimic the in vivo conditions, and human
microvascular endothelial cells grew adequately with a further a
moderate enhancing effect in the heparin-coated group [139].

Moreover, heparin exerts an important inhibitory effect on
neointimal hyperplasia. Edelman and colleagues described the
design and development of a novel biodegradable system for the
perivascular delivery of heparin to the blood vessel wall with well-
defined release kinetics. Heparin-encapsulated PLGA micros-
pheres were sequestered in an alginate gel and evaluated for their
ability to inhibit growth of bovine vascular smooth muscle cells in
tissue culture. Moreover functionalized alginate gel sheets were
used to wrap arterial vessel in a murine model of vascular injury
induced by endothelial denudation and in a vein vascular graft
interposition intervention. This system showed ability to both
inhibit smooth muscle cell proliferation in vitro and to reduce
neointimal hyperplasia in in vivo models of vascular disease [140].

Nitric oxide

Nitric oxide plays a pivotal role in vascular homeostasis modulat-
ing the activation and adhesion of leucocytes, limiting platelet
aggregation and thrombus formation, and maintaining the vascu-
lar smooth muscle in a non-proliferative state, thereby preserving
blood vessels from adverse vascular remodelling [141]. Nitric
oxide biological characteristics could therefore constitute an
attractive alternative to ameliorate properties of biomaterials for
cardiovascular applications. Polyethylene terephthalate (Dacron,
Invista, KS, USA) and ePTFE (Teflon, DuPont, VA, USA) grafts
currently used for large-diameter (more than 6 mm) vascular sub-
stitutes, encounter several limitations especially concerning
thrombosis, intimal hyperplasia and thus, failure in small-dia-
meter applications. It is noteworthy that one of the reasons thought
to be at the root of the early failure of saphenous vein grafts is the
relative paucity of nitric oxide production by these conduits as
compared with arterial ones.

To this extent, Jun and colleagues developed a diazeniumdiolate-
modified polyurethane for vascular applications. Diazeniumdiolates
are interesting compounds containing the [N(O)NO]- functional

group [142, 143]. The anionic portions of these compounds sponta-
neously decompose in solution to release nitric oxide, leaving the
amine group as a byproduct [144] with rates of dissociation
depending on several factors, such as structure, temperature and
pH of solution [145].

Using this bioactive material authors described a two phases
releasing kinetic with a rapid burst during the first 48 hrs, followed
by a slower, sustained release over a 2-month period. Along with
these findings enhanced rates of proliferation of endothelial cells
and increased rates of migration and coverage of the synthetic
surface during both phases have been demonstrated. Moreover,
nitric oxide releasing polyurethane, while greatly inhibiting smooth
muscle cell growth, reduced platelets adhesion as revealed by
a mepacrine-based assay. More recently, incorporation of a diazeni-
umdiolate nitric oxide donor into the backbone of a polyurethane
containing both Polyethylene glycol (PEG) and the cell adhesive
YIGSR peptide sequence has been performed in order to provide
a nitric oxide local release during the period of graft endothelial-
ization [146]. PEG has been shown to resist to protein adsorption,
platelet adhesion and bacterial adhesion, and numerous methods
have been developed to integrate PEG into biomaterials, including
polyurethanes, through co-polymerization and surface modifica-
tion [147–152]. The laminin-derived cell adhesive peptide
sequence tyrosine-isoleucine-glycine-serine-arginine (YIGSR)
increases endothelial cell adhesion and migration [153], while the
nitric oxide release improves endothelial cell proliferation during
the period of graft endothelialization. Nitric oxide generating, cell
adhesive polyurethane-PEG copolymer showed the ability to
reduce platelet adhesion and smooth muscle proliferation enhanc-
ing endothelial cells attachment and proliferation. If the addition
of the YIGSR peptide sequence, known to improve cell attachment,
spreading and resistance to shear stress, could give an answer to
potential issues concerning the influence of physical forces of the
circulation in vivo settings, other questions regarding the
response of bone marrow-derived endothelial progenitor cells
need to be evaluated [154].

However, the therapeutic potential of nitric oxide generating
polyurethane remains high and it may be suitable as a candidate
material for small-diameter vascular grafts.
Gene delivery

Until now several attempts have been done to promote regeneration of blood vessels using tissue-engineering approaches with genes. The idea at the basis of this strategy regards the possibility of inducing cell proliferation and differentiation or the secretion of ECM components for tissue regeneration by the transfection of genes encoding growth factors [155]. For these purposes, it is crucial that the development of controlled DDSs which allow a therapeutic gene to be delivered specifically to the target cell in an appropriate sequence and timing [156–158]. In gene therapy two kinds of vectors are basically available, viral and non-viral vectors. Viral vectors, such as adenovirus, retrovirus and adeno-associated virus, have been mainly used because of the high efficiency of gene transfection. Nevertheless, limited size of DNA molecules inserted into viral vectors, possible mutagenesis of cells transfected, high immunogenicity and toxicity represent some of the shortcomings related to this approach. On the other hand, despite their low transfection efficiency, non-viral vectors, such as naked plasmid DNA and plasmid DNA complexed with cationic groups may represent a more feasible alternative as for their safety and the greater molecular size of DNA. Therefore, a lot of literature has been focused on the development of synthetic materials, including cationic liposomes [159–161] and cationic polymers like polylysine [162–165] and polyethyleneimine, molecularly designed to improve delivery of plasmid DNA to the target site at high expression levels [166–174].

On account of the similar negative charge of both cell membranes and plasmid DNA used in transfections, complexion of the genomic material with cationic elements is well recognized to be useful to reduce the plasmid DNA molecular size and to promote electrostatic interactions with the cell membrane resulting in the internalization.

However, the plasmid DNA, when given to cells or injected into the body in the solution form, is readily degraded and inactivated by enzymes or cells and it does not have any inherent ability to accumulate in a certain tissue or organ. Moreover, considering gene pharmacokinetics, a successful gene therapy requires a target-specific sustained expression of the construct [175–179]. The time period of gene expression can be prolonged by the controlled release technology of plasmid DNA. To this extent, DDS technology and methodology are promising to enhance the biological effects of genes for therapeutic purposes. Several studies have been done about the controlled release of plasmid DNA with different biodegradable biomaterials [155, 180–203].

This release technology could enhance the level of gene transfection and promote a prolonged transfection time, which are both important to effectively induce tissue regeneration. Use of biodegradable hydrogels has been widely studied. Modifications of their chemical properties by alkaline or acidic processing have been investigated in order to obtain materials with different isoelectric points to improve interaction with growth factor, plasmid DNA, decoy DNA and small interference RNA for release purposes. The time profile of growth factor and DNA release could be controlled by adjusting hydrogel degradation, which is dependent on the extent of the hydrogel cross-linking [204, 205].

Derivatization with cationic compounds gives gelatine cationic moieties which can polyanionically interact with plasmid DNA of anionic nature. The cationized gelatine could be processed with glutaraldehyde to obtain different degrees of cross-linking. Cross-linking extent is important as it directly affects the hydrogel degradation rate. The release of the DNA molecules ionically complexed with the cationized gelatine is thought to occur during the hydrogel degradation process to generate water-soluble cationized gelatine fragments. The combined release of the plasmid DNA molecules as a complex with degraded gelatine fragments of positive charge is conceived to be at the basis of the ionic interaction with the cell surface of negative charge promoting internalization. Moreover, this complex is thought to prevent DNA enzymatic degradation by Dnase [206–208]. In this approach DNA is biologically stabilized by the incorporation into the hydrogel and the controlled release enhances the local concentration of plasmid DNA around cells to be transfected, consequently increasing the efficiency of gene transfection.

Biomaterial based release of genomic material has been experimented in several fields of medical interest including muscle and bone regeneration. For cardiovascular applications, Mooney et al. have reported that the in vivo sustained release of a plasmid DNA encoding PDGF gene with the carrier matrix of PLGA, enhanced matrix deposition and blood vessel formation [180, 181]. The possibility to create polymer scaffolds capable of controlled release of intact and functional plasmid DNA could represent a mean to enhance and regulate gene transfer within a developing tissue, which will increase their utility in tissue engineering avoiding use of viral vectors.

Conclusion

DDSs represent an attractive option in cardiovascular tissue-engineering applications. Controlled delivery may provide an appropriate alternative capable of releasing localized doses of bioactive molecules over sustained periods of time, while minimizing undesired systemic effects. However, until now the current limitation of TECVG lies in the fact that artificial vessels and cardiovascular structures need to have growth potential, and the inherent ability to remodel and to warranty coagulation homeostasis while maintaining biocompatibility. Scaffold-related limitations and the issues of off-the-shelf availability, and ease of harvest and propagation of autologous cells, are the greatest obstacles to the widespread acceptance of tissue-engineering technology in clinical practice. This gap is where stem cell application can be fully utilized. The use of stem cells could be proposed as an adequate alternative to other autologous primary cell lines because of their easy recruitment in clinical settings (i.e. bone marrow aspiration) and their notable plasticity. The exciting bridge that the stem cell represents could be a breakthrough in
tissue engineering especially if combined with biomaterials that actively guide and provide the correct sequence of signals to allow a harmonic ongoing lineage-specific differentiation of these pluripotent precursor cells.

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