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Tissue engineering of small diameter cardiovascular grafts

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ABSTRACT

To design a biocompatible, nonthrombogenic, compliant, infection resistant and technically facile small diameter vascular graft is a surmounting feat to achieve. A whole host of biological grafts and synthetic prostheses are available. Saphenous vein and internal mammary artery grafts were initially used for coronary artery bypass grafting (CABG). Clinical efficiency of synthetic, allogenic or xenogenic graft is limited by thrombosis, rejection, chronic infection and poor mechanical properties. Commonly used synthetic grafts like ePTFE and Dacron (PET) are limited by thrombogenicity, neointimal hyperplasia and low patency rate. New surface processing techniques such as glutaraldehyde fixation, treatment with polyepoxy compounds and dye mediated photo oxidation improves the patency rates and reduces immunogenicity but has its own disadvantages like increased tissue calcification and cytotoxicity. Tissue engineering offers an alternative, in which endothelial cells are seeded on either decellularized arteries or polymeric nanofibrous scaffolds which are prepared by non woven electro spinning technology. This technique improves graft patency. The surface of the polymer scaffold could be modified using peptides like Arginine-Glycine-Asparate (RGD), heparin or treated with recombinant type VIII collagen for better endothelial cell (EC) attachment and retention. Advancing scaffold technology in collaboration with biological, chemical and materials science engineering will lead to the design of ideal cardiovascular graft. This article reviews the current strategies that are employed clinically to treat blocked blood vessels and the importance of tissue engineering of small diameter arteries.

KEYWORDS

Tissue engineering;
Vascular graft;
Synthetic polymers;
Electrospinning.

INTRODUCTION

Cardiovascular disease affects millions of people worldwide and accounts for 30% mortality worldwide[1]. Cardiovascular disease is a broad all encompassing term with two main components-disease of heart (cardio) and disease of blood vessel (vascular). TABLE 1 lists some common types of disease of both the categories[2].

The major risk factors which promote these diseases are hypertension, high blood cholesterol, diabetes, smoking and heredity[3-8]. Both natural and syn-

thetic biomaterials are currently used for the reconstruction of the blood vessels. Natural vessels can be further categorized as autograft, allograft and xenografts[9]. Vascular surgery was first defined as area of interest for massive research with the initial publication of Alexis Carrel's work in 1902[10]. Lexer in 1907 performed the first free autogenous vein graft by replacing an axillary artery defect with greater saphenous vein from the same patient[11,12]. Cryopreserved allograft exhibit excellent mechanical properties but their use is limited by high occlusion rates[13,14]. Xenogenic prostheses like glutaraldehyde treated Bovine pericardial and porcine carotid arterial graft have good homeostatic characteristics but display chronic infections and calcification effects[15]. The main limitations of harvesting native vessel are multiple surgical procedures at the cost of patient which causes longer healing time and donor site morbidity. Synthetic polymers like polytetrafluoroethylene (PTFE), polyethyleneterephthalate (Dacron) and polyurethane are widely used for vascular surgery[16]. The first synthetic graft was reported in 1952 when Voorhees described his initial work in dogs using tubes of Vinyon N cloth to bridge arterial grafts[17]. Autologous small diameter grafts have been harvested from the saphenous vein and used mainly in coronary artery bypass grafting (CABG)[18]. Large diameter vascular graft is mainly used for aortic/iliac artery reconstructions with Dacron and ePTFE dominating the market[19]. The main drawbacks of synthetic grafts are poor patency, high thrombogenicity and neointimal hyperplasia which results in the failure of the prostheses[20].

Tissue engineering is the development of biological substitute and strategies for regeneration that can be used to replace, enhance, repair and regenerate tissue/organ function[21]. Tissue offers a viable alternative to engineer an ideal small diameter vascular graft[22-24].

Natural biomaterial as cardiovascular graft

Natural vessels can be broadly classified as autografts, allografts and xenografts. A tissue surgically removed from one area of person's own body and transplanted in another site on the same person is called autograft. Autologous vessels such as greater saphenous vein, internal mammary artery or radial artery are appropriate for coronary artery bypass grafting procedures[25]. Lantz et al. grafted dog jejunal submucossa as an autologous vascular graft and found that after 28 days the tissue was covered by a layer of endothelium and resembled the native vessel. On long term follow up there were no evidences of graft failure and infection[26]. These results were further confirmed by the work of Huynh et al. who used acellular submucossal graft enriched with bovine collagen[27]. Small intestinal submucosa is a cell free collagen matrix derived from porcine small intestine and is used as a scaffold for vascular reconstruction. It was demonstrated that they have the ability to be remodel into host tissue[28,29].

TABLE 1: Cardiovascular diseases

Disease of heart	Disease of vessel
Coronary heart disease	Arteriosclerosis and atherosclerosis
Coronary artery disease	High blood pressure
Cardiomyopathy	Stroke
Valvular heart disease	Aneurysm
Pericardial disease	Peripheral arterial disease and claudication
Congenital heart disease	Vasculitis
Heart failure	Venous thrombosis

Allografts are tissues that are obtained from another person or from cadavers. Since they are obtained from cadavers, they are available in plenty, and eliminate secondary surgery and the associated pain and cost[30-31]. However, they are limited by the possibility of immunological rejection and disease transmission[32]. Cryopreserved allografts are important component in the repair of many congenital heart defects, particularly to enlarge luminal diameter of obstructed outflow tracts and large vessel stenosis[13-14]. Knosalla et al. examined the efficacy of allograft in dogs that had been implanted with *S.epidermidis* infected aortic prosthetic grafts and concluded that cryopreserved aortic allograft were more resistant to reinfection than are synthetic grafts after *in situ* replacement[33]. Rowe et al. compared cryo preserved allografts and collagen impregnated Dacron grafts and found that the latter had more patency[34]. Furthermore Lehalle et al. demonstrated allograft related complications of degeneration and rupture[35]. Xenografts are tissues removed from an animal donor and transplanted to a human. Xenogeneic organs used as scaffold include decellularised porcine, common carotid arteries, iliac arteries and bovine ureter[36-40]. The main advantage of these graft material is that they can be preserved as off shelf material and are in abundant in supply.

All these grafts require lot of physical and chemical pretreatment to control the chemical and enzymatic

Review

degradation, preserve the native tissue and most importantly kill the pathogens to avoid disease transmission. Lots of tissues processing techniques are carried out in this respect and the most common method is to crosslink the scaffold using glutaraldehyde or formaldehyde[41-43]. Glutaraldehyde is a main cross linking agent which has five carbon bifunctional aldehyde that reacts with lysyl residues in proteins and forms interchain cross links which stabilizes tissue against degradation. Bovine pericardial grafts are treated with glutaraldehyde to yield good homeostatic result. However, this treatment is cytotoxic and leads to chronic calcifications. Further it was demonstrated that calcification is the principal cause of failure for tissue-derived cardiac valves replacement pre-treated with glutaraldehyde[15]. Glutaraldehyde incorporation and cross links introduced upon pretreatment creates gaps or void spaces in the bioprosthetic tissue which serve as nucleation sites for calcification. It was analyzed that local cytotoxicity of glutaraldehyde cross linked bioprostheses is due to unstable glutaraldehyde polymers that persist in the interstices of cross linked tissues[44]. Another promising cross linking agent with fewer side effects is polyepoxy compound which reacts with amide, carboxy, phenol and alcohol groups to form intrahelical collagen cross links[45-46]. Ekada *et al.* compared the cytotoxic effects of glutaraldehyde and polyepoxy cross-linked materials and found that all the diepoxy compounds investigated in the study exhibited lower cytotoxicity than formaldehyde, glutaraldehyde, and a water-soluble carbodiimide[47]. They provide good resistance to calcification, thrombosis and aneurysm apart from stabilizing the tissue against degradation. Another approach is to cross link the graft material catalyzed by dye mediated photo oxidation[48]. In the presence of some photo sensitizers like methylene blue certain amino acids such as tryptophan and histidine are oxidized. With respect to photo oxidation of histidine, it has been reported that aspartic acid and urea were the final products of this reaction, and a detailed mechanism for the oxidation reaction has been described by Ukita et al.[49]. Studies have shown that dye-mediated photo oxidation can be used to stabilize intact collagen based tissues such as bovine or sheep pericardium and small-diameter arteries for these applications, photo-oxidation serves as a catalytic process that induces modification and crosslink formation within the existing matrix components, resulting in a more natural material[48]. Photo-oxidized bovine pericardial tissue (Photo Fix TM) is non immunogenic and biocompatible material suitable for vascular repair[50]. Native vessels are indeed the first choices for vascular repair but have their own limitations of aneurysm and need a long term follow-up[51].

Synthetic biomaterials as cardiovascular grafts

The most commonly used synthetic grafts are polyethyleneterephthalate (PET), polytetrafluoro ethylene (PTFE) and polyurethanes[52]. The ideal properties of synthetic vascular grafts are chemical stability, biocompatibility, hemocompatibility, appropriate mechanical property and porosity[53]. The following sections deals with the different synthetic biomaterials that are currently used as cardiovascular grafts.

Polyethyleneterephthalate (PET) or dacron

The synthetic polymer polyester terephthalate (PET), also known as Dacron, is biocompatible, resilient, flexible, durable, and resistant to biodegradation and sterilization. Dacron grafts have good strength, endurance and higher compatibility with human host[52]. Different types of Dacron (woven, knitted and crimped) have been fabricated with velour construction (Bard®, De Bakey®) on their inner and outer surfaces to enhance tissue incorporation. In order to reduce the blood loss, knitted grafts have been pre-clotted prior to insertion[54]. The pre-clotting procedure is less frequently used in woven grafts because of much smaller pores produced in this technique. Dacron grafts coated with proteins (collagen/albumin) and antibiotics have recently been manufactured to reduce the blood loss and infection respectively[55]. However Dacron grafts suffer from thrombogenicity and poor patency[56,57].

Expanded PTFE (ePTFE)

Polytetrafluoroethylene is a resin which was invented by Sumitomo electrics in 1963[58]. PTFE provides a smooth surface which is less thrombogenic and requires externally support to avoid kinking[59]. Microporous expanded polytetrafluoroethylene grafts were introduced in 1970s[60]. Sawyer et al. invented ePTFE which is a synthetic polymer of carbon and fluorine produced by mechanical stretching[61]. An implantable microporous ePTFE tubular vascular graft exhibits long term patency, superior radial tensile strength and sufficient porosity to

promote cell endothelialization, tissue ingrowths and healing. Moreover, they retard aneurysm formation, have excellent heat & chemical resistance and are highly impermeable to blood[62]. Successful animal studies were performed by Soyer et al. and Campbell et al.[18,19]. Hanson et al. improved the performance of ePTFE grafts by modifying the physical properties of the graft surface[63]. Neointimal hyperplasia within ePTFE grafts is significantly reduced by the local application of nonporous silicon polymer coating or by peptide fluorocarbon-surfactant polymer coating[64,65]. Recently Chaikof et al. has used a recombinant elastin-mimetic triblock protein polymer with an inverse transition temperature (approximately 20^0C) and seeded it on small-diameter ePTFE vascular graft. The results demonstrate that elastin-mimetic triblock protein polymer can be used as nonthrombogenic coating or as a component of a tissue-engineered composite. ePTFE graft is preferred than Dacron for venous reconstruction in the absence of autologous vein[66].

Polyurethanes

Polyurethanes (PU) were first introduced as grafts in the early 1950s[67]. The advantages of polyurethanes include a very smooth non-thrombogenic inner surface, a thin-walled graft with compliance and improved handling characteristics[68-69]. The efficacy PU grafts can be improved by providing carbon coating, heparin grafting and endothelial cell seeding. However, the existing PU grafts have not been successful for use as smaller diameter vessels[70].

Many strategies such as seeding endothelial cells onto the synthetic surface have been employed made to improve the function and patency of polymeric grafts so that they became less thrombogenic[71]. Dacron and ePTFE grafts have been pre-seeded with autologous endothelial cells to avoid immunological reactions[72-74]. Since the adhesion of endothelial cells on the surface of Dacron and ePTFE was poor, the graft surface properties were altered by either using a recombinant fibronectin-like adhesion factor or by ammonia plasma treatment[75,76]. Modifying the surface of the grafts did not show any improvement in the patency. However, there is a huge clinical demand for an alternative supply of vessels to replace diseased arteries[76].

Tissue engineering of blood vessels

Tissue engineering merges the fields of cell biology, engineering, materials science and surgery using scaffolds in the presence or absence of living cells to maintain, restore or improve the function of damaged tissues and organs[77,78]. Due to the limitations of the current small diameter arteries, it is required to develop an artificial construct that would mimic the native coronary artery. Considerable success has been achieved with such constructs for the replacement of larger vessels, whereas small-diameter arteries with a diameter less than 6 mm have not been successful. Hence a tissue engineered blood vessel (TEBV) that can replace an autologous vein or artery with appropriate performance after implantation and at affordable cost is desirable[79]. The basic TEBV requirements to mimic the functional characteristics of a living blood vessel are nonthrombogenicity, non-immunogenicity, exhibits vasoactivity and possess mechanical properties matching those of the native vessel[80].

Basic understanding of the physiological characteristics of blood vessels is required to engineer a suitable cardiovascular graft. An artery consists of three distinct regions, all of them containing specialized cells and the extra cellular matrix (ECM). Anatomically, from the outside of the vessel to the inside, these regions are called the tunica adventitia, tunica media and tunica intima. Furthermore, the tunica intima is lined with a specialized, single layer of endothelial cells[79].

The tunica adventitia, the outermost layer of a blood vessel, consists of mainly fibro-elastic connective tissue and ECM, supplying most of the mechanical strength and structural integrity to the vessel[81].

The tunica media contains mainly smooth muscle cells and elastin fibers and the smooth muscle cells are highly abundant and make up the bulk of the vessel wall thickness. The elastin provides the visco-elastic property to the vessel and the media rests on an internal elastic lamina for structural support thereby separating it from the innermost layer, the tunica intima[82].

The tunica intima or intima is usually the thinnest structural layer present in a vessel and is made of a single layer of endothelial cells (ECs) mounted on a basement membrane. The surface of the EC layer expresses glycoproteins, together called the glycocalyx, which prohibits blood cells and plasma proteins to migrate into the vessel wall under normal conditions; hence it is a charged barrier[82].

Review

Scaffold fabrication

Tissue engineering scaffolds are three-dimensional structures that provide a site for cells to attach, proliferate, differentiate and secrete an extra-cellular matrix, eventually leading to tissue formation[83]. In addition to optimizing scaffold structure to ensure that such desired cellular activities occur, it is also possible to guide cells into forming a neo-tissue of predetermined three-dimensional shape and size[84]. Basically tissue-engineered scaffolds should mimic the function of extra cellular matrix. ECM is composed mainly of fibers such as collagen, elastin and largely amorphous interfibrillary matrix such as proteoglycans, non collagenous cell binding adhesive glycoprotein solutes and water[85]. The main function of ECM is to support cell anchorage, oriented cell adhesion, proliferation and differentiation[62]. Recent advances in biomedical nanotechnology and electrospinning techniques enable the production of ultra fine solid and continuous fibers of biodegradable polymers[86]. They can be electrospun to form aligned or random nanofibres depending on the application and nanofibers have diameter in the range of 1-1000nm. In the nanodimension, surface to volume ratio is large providing a higher surface area which benefits cell adhesion and proliferation. These nanofibrous mats obtained via electrospinning mimics extracellular matrix in characteristics and function if functionalized suitably[87].

Electrospinning

Electrospinning is a cost effective and elegant method to produce aligned or random nanofibers depending upon the application[88-92]. When a polymer solution or melt is exposed to high voltage, the drop formed at the end of the capillary overcomes the surface tension and forms a Taylor cone which further splits into fine jets of the fibers. As the fibers travels towards the grounded target the solvent evaporates forming a series of interconnected web of fine fibers[93].

Electrospun nanofibrous mat for blood vessel engineering

Ramakrishna et al. demonstrated the preparation of poly (L-lactide-co-ε-caprolactone)[P (LLA-CL)] biodegradable structure via electrospinning method[94]. Further the[P (LLA-CL)] copolymer surface was modified with collagen coating which enhances spreading, viability and attachment of human coronary artery endothelial cells (HCAVEs)[95]. Surface modification of electrospun polyethylene terephthalate (PET) nanofibers with gelatin and polymethacrylic acid were also studied[96]. Vacanti et al. reported that electrospun nanofibrous polycaprolactone (PCL) meshes show good adherence and proliferation of rat cardiomyo cytes[97]. Poly (ethylene glycol)-poly (D, L-lactide) electrospun fine-textured scaffolds were constructed for heart tissue regeneration by Xong et al.[98]. Richard et al. fabricated polycaprolactone-polyurethane (PCL-PCU) composite scaffold for vascular repair and carried out human vascular endothelial cells adherence and proliferation studies[99]. The results demonstrated that the luminal PCL surface of the scaffold supports the formation of stable functional EC monolayers and the overall scaffold is good for vascular tissue engineering.

Vascular applications of tissue engineering

The earliest application of tissue engineering to blood vessel replacement was the endothelial cell (EC)-seeding on synthetic grafts to resist thrombogenicity[100]. For the past three decades various approaches such as EC-seeded synthetic grafts, collagen-based blood vessel analogs, self-assembled blood vessels and decellularized tissue approaches to blood vessel grafting have been studied[79]. Initially to increase the patency of synthetic grafts (ePTFE and Dacron) endothelialization was carried out[76]. Bhattacharya et al. showed the result of enhanced endothelialization and micro vessel formation in polyester grafts which were seeded with CD34+ bone marrow cells[101]. Many modifications of the synthetic vascular grafts were carried out to enhance their patency. Plasmids encoding for human vascular endothelial growth factor (pNGVL3-VEGF) were administered to improve early endothelialization[102]. Polyurethane vascular prosthesis was incorporated with polyethylene glycol and YIGSR peptide to promote endothelialization[103]. Synthetic peptide Arg-Glu-Asp-Val was immobilized on polymer surface and enhanced endothelialization was observed[104]. RGD and heparin were covalently bonded onto Myolink® to improve the cell retention on graft surface[64]. Recently it was demonstrated that vascular endothelial growth factor (VEGF) gene plasmid carried by polytetrafluoroethylene (PTFE) vascular graft could transfect endothelial cells and promote their growth[105].

In the area of biodegradable synthetic polymer-

based constructs lot of polymer combinations or co-polymers are tried to produce an ideal scaffold for cardiovascular tissue engineering. Fabrication of the vascular substitute by seeding bovine endothelial cells on poly (D, L-lactide-co-glycolide) scaffold was carried out[106]. Collagen coated poly (L-lactide-co-ε-caprolactone) nanofiber mesh was seeded with human coronary artery endothelial cells (HCAEC) for making suitable vascular substitute[107]. Enhanced spreading was observed on all above matrices. Recently scaffolds made of polycaprolactone and polyurethane was seeded with endothelial cells and were evaluated for blood vessel engineering. Attached cells demonstrated abundant release of von Willebrand factor, nitric oxide and ICAM-I and exhibited response to lipopolysaccharide[99].

First tissue engineered blood vessel was produced by Weinberg and Bell in 1986 and this provided the foundation for cardiovascular tissue engineering[24]. It gives us the insight to develop a completely biological, living and autologous blood vessel produced *in vitro* for small vessel reconstructions, also having the advantages of good mechanical strength without the use of a synthetic scaffold. Bell *et al.* used animal collagen gels and cultured bovine endothelial cells (ECs), smooth muscle cells and fibroblasts. Other reinforced methods were developed and tested due to the poor mechanical performance observed in first case. A very innovative approach was taken by Huereux et al. in the year 1998 to tissue engineer a blood vessel based exclusively on the use of cultured human cells and without synthetic or exogenous biomaterials[108]. Sheets were produced with human vascular smooth muscle cells cultured with ascorbic acid and placed on a tubular support to produce the media. Similar sheet over the media provided the adventitia and ECs were seeded in the lumen. Campbell et al. carried forward these approaches by growing a vascular graft in recipient's own peritoneal cavity. After two weeks of implantation, layers of myofibroblasts, collagen matrix and single layer of mesothilium had covered the silastic tubing which was inserted in the cavity. Tube of living tissue was everted and was found suitable for designing a small diameter vessel[109].

Research is being carried out in the field of allografts and xenografts for tissue engineering purpose. Haverich et al. designed a graft by seeding human cells in decellularized porcine matrix[110]. Mechanical and compliance properties of the scaffold were studied and it were found to be durable, compliant and to match the mechanical properties of the native small diameter blood vessels. Endothelial cells were cultured on acellularized rat arteries and the mechanical properties of the matrix were comparable to native vessels presenting an alternating method for reconstruction of small vessels[31]. Cryopreserved allograft veins were taken and precoated with autologous serum for small caliber grafts application[13,14]. Biomechanical properties of decellularized porcine common carotid arteries were reported by Roy et al.[111]. The results demonstrated that the process of decellularization yields vessels that can withstand inflation pressures but have the disadvantages of geometrical and compliance mismatch also compliance mismatch may promote graft-artery intimal hyperplasia by altering the suture line stresses. Recently a tissue engineered graft was constructed by self derived cells and heterogeneous acellular matrix. Experiments were performed on thoracic aorta of adult sheep and endothelial cells were derived from two weeks piglet and the results showed no thrombosis and calcification was observed[112].

In another novel approach bacterial cellulose derived from the bacteria *Acetobacter xyllinium* was used as a scaffold for TEBV[113]. These scaffolds supported the growth of blood vessels with no signs of inflammation. In more recent findings scientists have designed the first TEBV suitable for autologous small diameter arterial revascularization in adult patients[114]. A novel method named sheet based tissue engineering was used to assemble TEBV, where fibroblasts were cultured to produce a cohesive sheet comprising of living cells and a well organized endogenous matrix. Also this model eliminates the need of smooth muscle cells which is reported to be associated with decreased burst pressure in human model[115]. Thus, cardiovascular tissue engineering provides a viable platform for designing small diameter vascular conduit for appropriate site application[116].

Summary

Small diameter cardiovascular graft can be modeled from natural vessels like allograft and xenografts but their uses are limited by infections and immunogenicity. Surface modification using cross linking techniques results in thrombogenicity and calcification. Synthetic grafts like ePTFE and Dacron are most suitable for large vessel reconstructions and have their own disadvan-

Review

tages of kinking, thrombogenicity, infections, poor compliance mismatch and patency. Tissue engineering seems to be the best possible solution for designing a small diameter vascular graft with decellularized allograft, xenografts and polymeric nanofibers as suitable matrices. Also electrospinning process has been recognized as an ideal technique to fabricate polymeric nanofibrous scaffolds for vascular tissue engineering. Furthermore, by using electrospinning and tissue engineering process one can develop mechanically strong cardiovascular graft with enhanced patency.

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Review

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