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Nanostructured Materials for Cardiovascular Tissue Engineering

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Substantial progress has been made in the field of cardiovascular tissue engineering with an ever increasing number of clinically viable implants being reported. However, poor cellular integration of constructs remains a major problem. Limitations in our knowledge of cell/substrate interactions and their impact upon cell proliferation, survival and phenotype are proving to be a major hindrance. Advances in nanotechnology have allowed researchers to fabricate scaffolds which mimic the natural cell environment to a greater extent; allowing the elucidation of appropriate physical cues which influence cell behaviour. The ability to manipulate cell/substrate interactions at the micro/nano scale may help to create a viable cellular environment which can integrate effectively with the host tissue. This review summarises the influence of nanotopographical features on cell behaviour and provides details of some popular fabricating techniques to manufacture 3D scaffolds for tissue engineering. Recent examples of the translation of this research into fabricating clinically viable implants for the regeneration of cardiovascular tissues are also provided.

Keywords:

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1. INTRODUCTION

Approximately 1 in 3 American adults are thought to suffer from one or more forms of CVD. In addition to the morbidity and mortality associated with CVD, there is also a significant economic burden, estimated to be over $300 bn in 2009. With the continuing social trend towards obesity, in the United States as well as the rest of the world, this problem is only likely to be exacerbated. CVD are the diseases of the heart and blood vessels. The main cause of death in patients with CVD is often myocardial infarction due to acute ischemia caused by occlusions of the vessels supplying nutrients and oxygen to the heart. The cell death caused by ischemia is irreversible. With the shear paucity of treatment options available to clinicians for treating these degenerative diseases the concept of tissue engineering (TE) emerged.

TE aims to repair or regenerate damaged tissue by culturing cells harvested from the patient or donor onto a suitable material which is then implanted in the patient's body at the appropriate anatomical location.[1] With the cells successfully delivered to the desired location, the hope is that they will integrate with the local tissue with the scaffold gradually degrading. Alternatively, the scaffold is directly implanted into the body, stimulating endogenous cells and the surrounding tissue to mature and proliferate on the template itself, resulting in *in vivo* tissue regeneration. Both tissue engineering paradigms rely heavily on novel biomaterials – which can range from natural macromolecules, synthetic polymers, ceramics, and various combinations of these material types – to tailor the physical, chemical, structural, and biological properties to achieve the desired clinical outcome.

The ability to develop and maintain large masses of viable and functional cells is a complex process. Precise control over cell phenotype, integration, function, proliferation and differentiation potential of the implanted device is a multifaceted challenge. A range of factors have been implicated in controlling cell behaviour which include, but are not limited to, endogenous and exogenous mechanical

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forces, biomaterial surface chemistry and environment, and soluble pharmacological factors.[2–5] Whereas the first generation of biomaterials aimed to merely illicit a minimal immune response and provide functional support; the next generation of materials can be tailored to meet specific needs for individual applications. Biomaterials promote tissue regeneration by providing the physical space-porosity of scaffold-for cells to attach, migrate, proliferate and differentiate. The 3 dimensional (3D) architecture plays a critical role in maintaining the appropriate cell phenotype; it regulates the space available for cells to grow, mass transport via diffusion, mechanical properties of the scaffold and the cell-substrate interactions. The surface morphology or topography is known to significantly affect cellular response on biomaterials and thus tissue formation and function.[6]

In the native tissue, cells are in contact with the extracellular matrix (ECM) which provides cells with biophysical cues which include specific surface chemistry and a 3D topography.[7, 8] Whilst single cells are typically tens of micrometers in diameter, subcellular structures such as cytoskeleton elements, transmembrane proteins and filopodia are on the nanometer scale. Furthermore, the ECM consists of nanostructured grooves, ridges, pits and pores and fibrillar networks composed mostly of collagen and elastin fibres with diameters ranging from 10–300 nm suggesting a regulatory role for these features.[9, 10]

By engineering scaffolds at the micro and nano scale; highly precise reactions, at the cellular and molecular scale, can be stimulated allowing more control over cell function.[11] Scaffolds can be further functionalised with various pro-angiogenic and ECM modifying factors which can be released in a controlled spatio-temporal manner to modify both host and transplanted cell response.[12, 13] The principle paradigm being that the scaffold can contain specific chemical and structural information that can control cell behaviour and tissue formation.

The aim of this review is to briefly summarise the role of nanomaterials in cardiovascular TE. The impact of nano-scale surface topography on cell behaviour will he highlighted. Fabrication techniques which can be used to manufacture TE scaffolds, at the nano-scale, providing greater control over cellular behaviour, will be discussed. Finally, recent examples of the applications of nanomaterial scaffolds in the cardiovascular TE field will be provided particularly focussing on vascular, heart valve and myocardium regeneration. Whilst nanomaterials have found uses elsewhere for drug delivery and controlled release applications these will not be discussed here and have been reviewed extensively elsewhere.[14]

2. NANO-SCALE CONTROL OF CELL BEHAVIOUR

Numerous studies have alluded to the fact that surface topography impacts cell behaviour; however, the exact mechanism remains unclear and is still being actively investigated. Advances in nanotechnology have allowed researches to create structures from the atomic to macromolecular scales in a controlled manner allowing the systematic investigation into cell behaviour. A number of techniques have been utilised ranging from chemical vapour deposition, colloidal lithography, e-beam lithography and photolithography – a thorough review of this literature is beyond the scope of this review but can be found elsewhere.[15–17]

The dimension and type of surface feature is an important parameter in regulating cell adhesion and spreading and subsequently gene expression, proliferation and differentiation. The influence of surface protrusions on primary cardiomyocytes isolated from Sprague-Dawley rats was evaluated using well defined nanopillar array of polyethylene glycol (PEG) hydrogel.[18] Ultravoilet assisted capillary lithography was used to fabricate highly uniform pillars ~150 nm wide and ~400 nm high. Cell adhesion was found to be significantly enhanced on the nanopillars compared to the bare control. Various elements of the cytoskeleton were seen to protrude to a greater extent on the pillared surface compared to the bare control with cardiomyocytes cultured on the pillars retaining their conductive and contractile properties.

Human mesenchymal stem cells (hMSC) were used to study the effect of protrusion height on various cellular functions; cell adhesion, spreading, cytoskeletal formation and differentiation was investigated using anodized titanium surfaces.[19] By varying the anodizing voltage; surfaces with controlled protrusions, rather than random surface roughness, were created allowing insight into the exact role of topography on cellular response. hMSC adhesion was greater on the structured surface, with protrusions 15 nm in height, compared to the planar control but was found to decrease with further increasing protrusion height. Cell response was also greatest on the 15 nm pillared structure with the mean area of cell spreading nearly twice that of the control.

A similar trend was observed for endothelial cells cultured on 13 nm islands producing highly spread cell morphologies containing a well-defined cytoskeleton.[20] Polymer islands 13, 35 and 95 nm in height were produced through polymer demixing of polystyrene and poly (4-bromostyrene). Their results suggested that the 13-nm island substrate produced the most acute response and the strongest effect on accelerating cell spreading compared with other nanotopographies studied.

In addition to protrusions; pits and pores have been implicated in regulating cell behaviour and function on the aortic heart valve and vascular system.[21, 22] The diameter, spacing and symmetry of pits have been shown to induce a variety of effects on cell adhesion.[23] Introducing a degree of disorder to the pit or pore arrangement appears to improve cell adhesion and function.[24] Shallower pits seem to induce greater cell spreading and attachment

than deep pits or flat surfaces: pits smaller than 30 nm formed focal adhesion structures (paxillin-actin) and activated focal adhesion kinases to a significantly greater degree than the flat surface control or the surface with pits in excess of 30 nm.[25] It is postulated that surface features with a maximum z axis dimension (height or depth) of 50 nm may inhibit the formation of focal adhesion sites necessary for integrin mediated adhesion to substrates.[26–28]

The impact of nanoscale grooves upon cell geometry and the expression of a cell–cell coupling protein of cardiac tissue constructs has been elegantly demonstrated using a capillary lithography based approach.[29] Neonatal rat ventricular myocytes were cultured on a nanopatterned PEG hydrogel containing grooves 50 nm wide, 200 nm high and a ridge of 150 nm. The ridges and grooves influence cell behaviour heavily leading to highly anisotropic cell arrays guided by the underlying nanoridges. Filopodial extension in the groove results in adhesion proteins and actin filaments becoming aligned parallel to the groove direction; with the organisation of actin filaments or microtubules being identified as the first step in contact guided cell alignment. However, when pharmaceutical agents were deployed to destroy actins or microtubules; cells still displayed contact guidance with a positive correlation between focal adhesion proteins and cell alignment.[30]

The dimension of the groove is critical in influencing cell behaviour; however, no clear consensus has been reached regarding the absolute dimensions of grooves for optimal cell control (Fig. 1). It is likely to be cell specific and dependant on whether cell-cell contact has been achieved or if cells are being cultured in isolation. Groove depth appears to be an important parameter; shallow nanogratings result in an increased interfacial area leading to improved cell adhesion and spreading with grooves as small as 35 nm deep shown to induce cell alignment.[31] This effect diminishes when feature size becomes negligible (<35 nm).

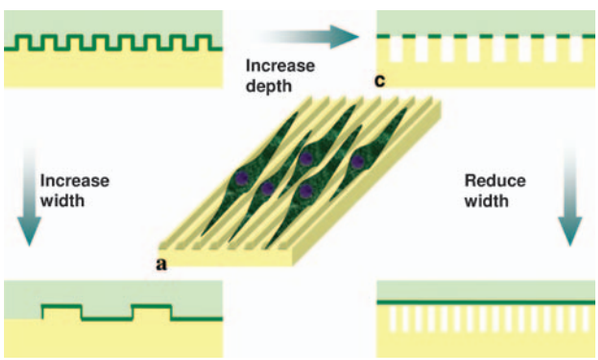

Fig. 1. Schematic illustration of cell response to a grooved substrate. a) Cells align and elongate along the direction of the groove. b) Grooved substrate leads to an increased interfacial area resulting in improved adhesion and spreading. c) Increased depth results in cells unable to reach the groove leading to elongation in the direction of the groove but no spreading perpendicular. d) Reducing the width of the groove allows the cells to bridge over the gap. e) Increased width results in cells sensing a planar surface with a step, diminishing the impact of topography on cell function.

If the depth of groove is increased then the cell can no longer reach the groove resulting in elongation in the direction of the ridge but retardation in the direction perpendicular. This can lead to a smaller cell size resulting in slower proliferation rate and even apoptosis.[32, 33] Increasing the groove or ridge width excessively will lead to the cells sensing each ridge/groove as a planar surface separated by a step which neither initiates integrin activation and clustering nor increases the surface area to facilitate focal adhesion formation. In contrast, decreasing the groove width whilst increasing the groove depth leads to cells bridging the ridge without descending into the groove. Epithelial cells cultured on grooves 150 nm deep formed bridges over grooves 330 nm–950 nm wide yet descended into grooves 2.1 μm wide.[34]

The cell specificity of responses to substrate topography has been demonstrated in EC, whereby EC from different anatomical locations (human umbilical vein endothelial cells (HUVEC), human dermal microvascular endothelial cells (HmVEC-d), human aortic endothelial cells (HAEC) and human saphenous vein endothelial cells (HSaVEC-c)) were cultured on topographically patterned substrates resulting in unique and distinct behaviour for each cell type.[35] Whilst important advances have been made with ample evidence available establishing the connection between nanostructures and cellular response, the mechanistic understanding of this relationship is still lacking. Many studies are still qualitative; coupled with variations in cell types, disparities in nanoscale features and different experimental protocols all make it difficult for in-depth analysis.

3. SCAFFOLD ASSEMBLY AND FABRICATION

In addition to the surface morphology of the scaffold; for a tissue engineered construct to be successful it must be able to maintain a large mass of viable cells in a 3D environment. Whereas 2D biomaterials are a potent tool to elucidate the regulatory mechanisms governing cell-substrate interactions; 3D structures allow the reconstruction of complex tissues. Integrin binding and the formation of focal adhesions in 3D tissues is substantially different from their binding and formation in 2D culture with 3D structures *in vivo* strongly influencing cell shape, affecting the differentiation process (Fig. 2).[36, 37] To achieve this aim, a high surface area-to-volume ratio and a porous environment for mass transport of nutrients and metabolic waste products is required. The coupling of a porous scaffold with nanostructured substrate offers a powerful tool for regenerating tissue, allowing the manufacturing of scaffolds which mimic the ECM in dimension and scale. These biomimetic scaffolds can be produced primarily through three manufacturing techniques: electrospinning, phase separation and self assembly, with Table II providing a summary of the main features of these techniques.

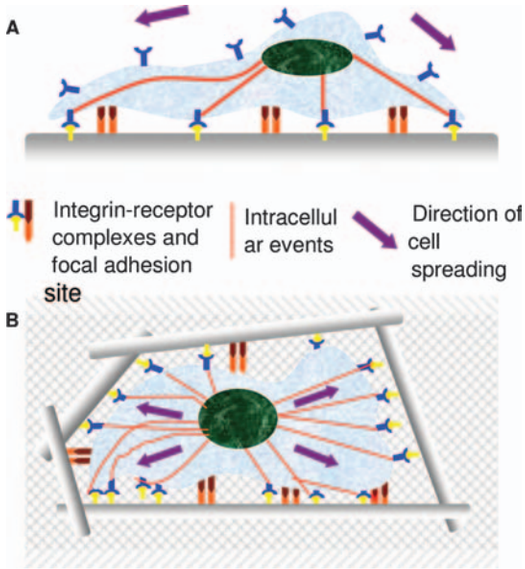

Fig. 2. Cell adhesion and spreading on 2 dimensional (A) and 3 dimensional (B) substrates. Cells binding to 2D substrates flatten and spread whereas a 3D substrate provides many more adhesion sites for the formation of focal adhesions, due to the increased surface area available. The cell senses its environment and responds via a series of intra and extra cellular signalling which can affect its motility, function and spreading. The more biomimetic, 3D environment, is thought to be essential in maintaining a large mass of clinically viable cells with the correct phenotype required for the formation of functional tissue.

3.1. Electrospun Fibres

A highly versatile and inexpensive method of mimicking the native ECM fibrous network is through electrospinning.[38, 39] In this method (Fig. 3), an electrostatic force is applied between the positively charged polymer solution and the substrate. When the electrostatic charge overcomes the surface tension of the droplet, a polymer jet is formed (Fig. 3(A)), which then elongates and thins. As the solvent evaporates from the jet, an electrically charged polymer is left behind. These solidified fibres are then collected on a grounded surface (Fig. 3(B)). Due to the simplicity of this method, electrospinning has been widely used by a variety of research groups. A range of materials – biodegradable and non-biodegradable, synthetic and natural polymers – can be electrospun, ranging from silk fibroin and collagen to polyurethanes and polyesters.[40–42] This technique allows for the control of thickness, composition and porosity of nanofibre meshes with a relatively simple experimental set up. Fibres with a diameter of a few micrometers down to as small as ~3 nm can be developed, resulting in significantly larger surface areas.[43] Porosities in excess of 90% and pore sizes ranging from a few microns to tens of microns can be produced resulting in effective cellular infiltration and allowing the effective mass transport of nutrient and waste products to and from cells.[44, 45] Blends of different materials can be used to augment the mechanical and/or biological behaviour of the scaffold.[46, 47] Furthermore, the fibres can be functionalised with a wide range of ECM proteins and bioactive agents resulting in a scaffold which has ECM like physical and biochemical properties.[46, 47] Through providing a more biomimitec environment for cells to grow upon; cell adhesion, proliferation, migration and differentiation were all shown to improve on electrospun fibres for

Table I. Influence of nanoscale surface features on cell behaviour. *Keys*: PGS: polyglycerol sebacate; Ti: titanium; PMMA: poly(methyl methacrylate); TiO_2: titanium dioxide; Al_2O_3: alumina; PCLLGA: poly(ε-caprolactone-r-l-lactide-r-glycolide); HSC: hematopoietic stem cells.

Substrate	Cell type	Nanoscale feature and size	Comments	Ref
PEG	Primary cardiomyocytes	Well defined array of pillars 150 nm wide and 400 nm high	↑cellular adhesion, retained their conductive and contractile properties	[18]
PGS	C2C12 muscle cells	Doubled ridged gratings 10 μm wide × 10 μm deep × 10 μm spaced at 170 μm intervals and pores with dimensions 150 μm×150 μm and 280 μm × 150 μm	Cells penetrated pores and aligned parallel to gratings.	[115]
Ti	hMSC	Surface protrusions 15 nm in height, 28 nm wide and 40 nm spacing	↑cellular adhesion and spreading	[19]
PS and poly (4-bromostyrene)	EC	Islands 13 nm, 35 nm and 95 nm in height	↑cellular adhesion and spreading	[20]
PMMA	hMSC	Pits 100 nm deep and 120 nm in diameter in both ordered and disordered arrays.	↑cellular adhesion and function on disordered array	[24]
PEG	Neonatal rat ventricular myocytes	Grooves 50 nm wide, 200 nm high and a ridge of 150 nm	↑cellular adhesion, spreading and function	[29]
Ti	EC	Grooves 150 nm in height and 750 nm pitch	↑cellular adhesion	[116]
TiO_2	HSC	Pits 15 nm wide, 1.5 μm deep and 15 nm spaced	↑cellular adhesion, proliferation, migration, and differentiation	[117]
Al_2O_3	SMC	200 and 10 nm pores	no response in cell adhesion, an alteration in cell morphology, ↑cell proliferation for cells grown on 200 nm-pore surfaces than on 20 nm-pore surfaces	[118]
PCLLGA	Human vSMC	Microchannels 160 μm long, 300 μm wide with gaps of 40 μm	Cells proliferate well initially, indicative of synthetic phenotype, but change to a contractile phenotype upon confluence.	[119]

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Table II. Common scaffold processing techniques for tissue engineering.

Fabrication method	Feature size	Advantages	Disadvantages
Electrospinning[39]	≥3 nm	Highly versatile, range of sizes available, cheap and experimentally simple set-up, can be applied to a number of materials	Can only create fibres, not nanopatterned surfaces. Limitations in size and shape of scaffolds produced.
Phase separation[54]	Fibre size 50–500 nm, pore size range from nm–μm	Highly porous, simple experimental set-up, versatile, can combine with other techniques for greater control over macro and micro structure. Permits incorporation of bioactive agents.	Randomly orientated fibres and pores. So far, no reports of organised fibres and pore structures. Macropores sometimes not interconnected.
Molecular self assembly[62]	Dependant on molecular design	3-dimesional structures, molecular control of substrate, versatile, can be used with sensitive biomolecules, range of morphologies and sizes available, offers properties and functionalities not possible with conventional organic synthesis	The non-trivial engineering of molecules that will self-assemble, mechanical stability, degradation not heavily studied. Longer prep time in certain circumstances

a variety of cell types.[48–51] Cells tend to grow in the direction of the fibre alignment which is particularly pertinent for EC as it mimics the morphology of EC *in vivo* under blood flow.

3.2. Phase Separation

Phase separation involves the thermodynamic mixing of a homogenous polymer-solvent solution into a polymer-rich and polymer-poor phase, usually achieved by exposing the polymer-solvent solution with another immiscible solvent or by cooling the solution. A wide variety of porous structures, including nanofibrous structures, can be manufactured with this technique through the fine-tuning of kinetic and thermodynamic parameters.[52, 53] Process parameters such as polymer concentration, temperature, the types of polymers and solvents all influence final structure.[54] High molecular weight polymers, or an increase in polymer concentration and/or viscosity all lead to a decrease in porosity, and thus an increase in mechanical properties. A major advantage of phase separation method is its simplicity and the lack of need for any specialised equipment. As it is a mould based technique, scaffolds with complicated shapes, or pore structures can be manufactured relatively easily (Fig. 4). The phase separation process resembles a porous structure embedded in a 3D fibrous network (Fig. 5). The fibrous network consists of fibres ranging form 50 to 500 nm and display porosities up to 98%.[55] The versatility of the phase inversion method allows itself to be combined with other processing techniques, such as particulate leaching or 3D printing, to design complex 3D structures with well-defined pore morphologies.[56, 57] By combining phase separation with a second manufacturing technique; control over the macropore structure can be administered, in addition to the generation of ECM resembling nanofibres, which can lead to the more efficient cellularisation of the scaffold and the mass transport of nutrients and metabolic waste products. The resulting high surface area-to-volume ratios result in enhanced protein adsorption and improved cellular functions including adhesion proliferation and migration.

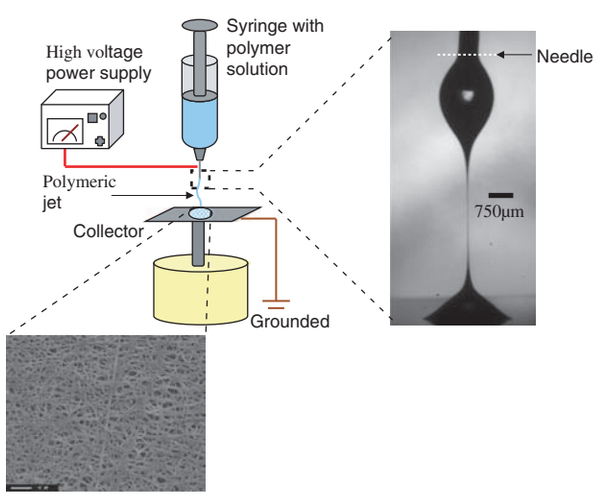

Fig. 3. Schematic Diagram of the electrospinning process for the production of polymeric nanofibres. A polymer solution is held in a syringe and pumped through a metal needle. A high voltage supply is connected to the needle, producing a fine jet of polymer solution (A). This dries out in transit, resulting in fine fibres which are collected on an earthed target (B).

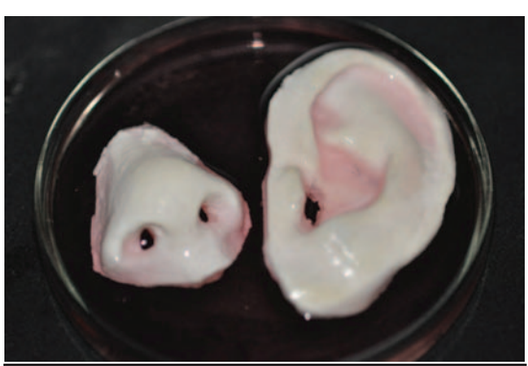

Fig. 4. Complex anatomical structures of nose and ear produced via the phase separation process with controlled porosity and mechanical rigidity using a POSS-Nanocomposite polymer developed and patented by authors.

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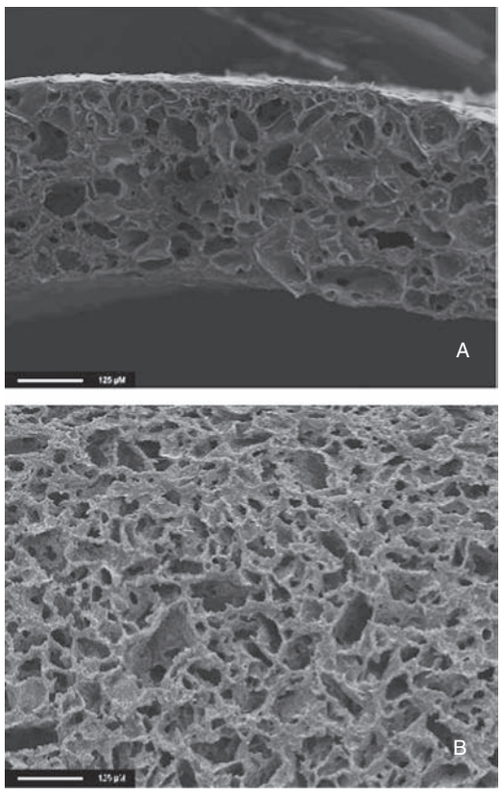

Fig. 5. 3-dimensional porous POSS-PCU scaffolds produced by phase separation. A) Cross section of scaffold demonstrating interconnected nature of the pores. B) Surface displaying a porous, textured and roughened surface of scaffold.

Both synthetic and natural polymers have been used in this way to manufacture 3D scaffolds.[58, 59]

3.3. Molecular Self Assembly

Molecular self-assembly is unique in its ability to form a wide range of diverse nanostructures. It involves the spontaneous organisation of molecules into more energetically stable conformations favoured by hydrophobic, Van der Waal and electrostatic interactions as well as hydrogen bonding resulting in a final supramolecular structure ordered on multiple length scales.[60–62] This technique allows for the molecular control of the materials whilst fabricating 3D scaffolds providing the potential to mimic the complex signalling machinery of the ECM. Several critical structural features are required for self-assembling molecules; for instance, long alkyl tails are required to confer the hydrophobicity which drives self-assembly; a long enough linker region which provides the flexibility to the hydrophilic head; and cysteine residues which can polymerise the reaction via disulfide bonds.[63] An elegant example of collagen, a key component of the native ECM, self assembly was demonstrated using peptide amphiphiles held together in a staggered array by disulfide bonds.[64] The hydrophobic head of the peptide amphiphiles formed the triple helical structure whilst the hydrophilic tail reorganises and stabilises the self assembled 3D structure of the scaffold. Scaffolds can be functionalised with self-assembled peptide amphiphiles which act as cell adhesive ligands such as Tyr-Ile-Gly-Ser-Arg (YIGSR) and Val-Ala-Pro-Gly (VAPG).[65] The attachment of these peptides can significantly increase the adhesion, spreading and proliferation of EC. It was also found that these particular ligands reduced platelet adhesion compared to the collagen control, which for vascular applications, is highly desirable. Self-assembly can be initiated in a number of ways including pH, concentration, temperature, electrostatic interactions and the introduction of metallic ions.[66–69]

4. CARDIOVASCULAR TISSUE ENGINEERING

The field of cardiovascular tissue engineering is expanding at an exponential rate. The following section will aim to provide recent of examples of the use of biomaterials, both synthetic and natural, structured at the nano-scale in the regeneration of vascular, valvular and myocardial tissue, summarised in Table III.

4.1. Vascular

Diseases of the blood vessels – arteries, veins and lymph vessels – are a principle component of CVD and a major cause of mortality. In severe cases the only treatment option is for bypass surgery – re-routing blood round the blockage. Whilst synthetic grafts have proven to be suitable for replacing large calibre vessels, patency rates have been largely disappointing in the replacement of vessels 5 mm or smaller in diameter.[70] Autologous vessels remain the conduit of choice for small diameter applications; however, they are not always available and failure rates remain high. Failure has been attributed primarily to thrombus formation, due to the inherent thrombogenicity of the synthetic surface, and intimal hyperplasia as a result of a mechanical mismatch between the elastic artery and rigid graft.[71, 72]

For a cardiovascular graft to be patent in the long term it must resist narrowing of the lumen by intimal thickening and possess thromboresistant properties with a functional endothelium. The endothelium is the thin layer of cells that line the interior surface of blood vessels, maintaining vessel integrity with various dynamic mechanisms preventing intimal hyperplasia and thrombosis. Materials which promote the adhesion and growth of endothelial cells are much sought after. A number of studies have demonstrated that EC adhesion growth and function is improved on rough surfaces.[73, 74] Poly(lactic-co-glycolic acid) (PLGA) was treated with sodium hydroxide (NaOH) and cast onto silastic moulds resulting in random and uncontrollable

Table III. Examples of nanomaterial based cardiovascular tissue engineering strategies.

Fabrication method	Scaffold material	Feature size (nm)	Cell type	Comments	References
Chemical etching	PLGA	~200	EC and SMC	↑ cell density, ↑ cell function	[74–76]
Electrospining	PCL	~250	Primary cardiomyocytes	↑ expression of cardiac specific proteins	[105, 106]
	PGA	~5	Cardiac stem cells	↑ cellular adhesion	[107]
	Decellularised heart vale + poly-4-hydroxybutyrate	—	MSC	↑ ECM deposition	[96]
	Polyurethane	~880	EC	Functioning EC attachment successful	[80]
	Gelatine, elastin, PCL and poliglecaprone composite	0–2400	EC	EC had normal function, ↓ reduced platelet adhesion	[120, 121]
	Polylactide fibers and silk fibroin-gelatin composite	139–1413	3T3 mouse fibroblasts and HUVECs	↑ cell adhere, spread, and proliferate. ↓ macrophages and lymphocytes adhesion *in vivo*	[122]
	Poly(L-lactid-co-ε-caprolactone) collagen	100	EC	Confluent layer of EC, maintained phenotypic expression of PECAM-1. Patent after 7 weeks *in vivo* in rabbit.	[85, 123]
Phase separation	PLGA	120–240 μm	A10 cell line	Collagen modified scaffold resulted in greatest cell adhesion. Cells aligned along microtubules.	[124]
	Poly(ester rethane)urea	12–232 μm	Muscle-derived stem cells	Confluent layer of von Willebrand Factor-positive cells observed	[125]
Self-assembly	Fibrin gel	—	human microvascular endothelial cells	Magnetically guided assembly. Cell adhered and spread with excellent cell-substrate alignment	[126]
	Heparin binding peptide amphiphiles	6–7.5	*In vivo* rat cornea angiogenesis assay	Peptide sequence: LRKKLGKAXBBBXXBX, where X is a hydrophobic amino acid and B is a basic amino acid. Significanlty increased angiogenesis with heparin-PA	[127]

surface features 200 nm in size. Both vascular smooth muscle cell (SMC) and EC densities were improved on treated PLGA surfaces possibly due to an increase in the adsorption of fibronectin and vitronectin, key proteins for mediating cell density on nanostructured PLGA.[75] Cellular function was also shown to improve; cells grown on nano-structured surfaces were observed to have very long filopodia protruding from the cell body allowing the cell to scout the surrounding area and interact with the nanometre structures.[76] Furthermore, an increase in matrix metalloproteinases (MMPs), enzymes linked to cell movement and adhesion to substrata, was observed from the supernatant of EC cultured on nano-structured surfaces.[77] EC migration was studied on rough surface using a nanocomposite of polyurethane doped with gold nanoparticles as a model system.[78] The rougher surface resulted in activation of the focal adhesion kinase (FAK) and P13K/AKT signalling pathways, resulting in cytoskeletal changes and an upregulation of eNOS, indicating greater EC migration and proliferation.

Tubular scaffolds for tissue engineering a blood vessel have been manufactured from materials as diverse as silk fibroin to poly(L-lactide-*co*-ε-caprolactone) and polyurethanes through electrospinning.[79–81] These constructs are often limited by the size in which they can be manufactured leading to poor burst strengths. However, by aligning the nanofibres, greater mechanical strength and modulus of nanofibre can be achieved and some degree of control over direction of cell growth can be administered.[82, 83] Further functionalization of the scaffold with ECM proteins such as gelatin and collagen, appeared to improve EC and SMC adhesion, growth and functionality.[84] The expression of EC specific surface markers such as von Willebrand Factor (vWF), CD31, CD54 and CD106 indicate normal cell function is maintained *in vitro*.[85] Electrospinning can be further utilised to overcome the inherent problem of cellular infiltration into scaffold pores, by concurrently electrospraying cells whilst electrospinning the polymer.[86] SMC's were uniformly integrated into the scaffold both radially and circumferentially using this technique. The scaffold appeared to be strong and flexible with reasonable dynamic compliance and burst strength values.

Our lab made use of the phase separation method to manufacture a small diameter vascular graft from a novel nanocomposite, polyhedral oligomeric silsesquioxane poly(carbonate-urea)urethane (POSS-PCU) (Fig. 6).[87] The amphiphilic, lipid like, nature of the POSS-PCU nanocomposite resulted in it having anti-thrombogenic properties by both repelling platelet surface adsorption and

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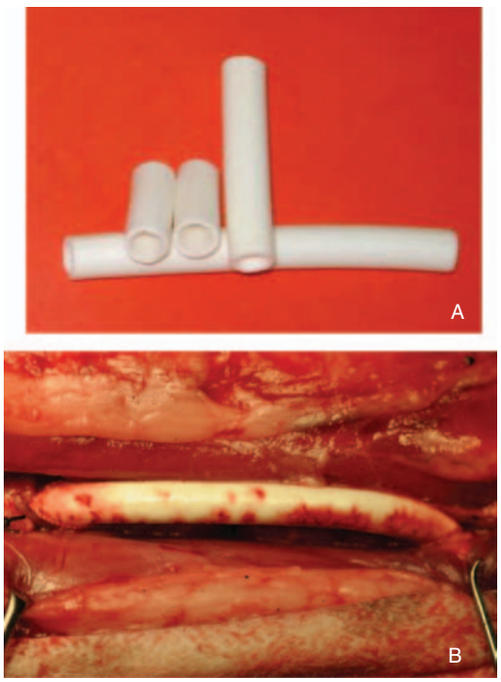

Fig. 6. POSS-PCU vascular graft produced by the phase separation method and implantation of this graft in sheep carotid artery, undergoing pre-clinical trials.

lowering the binding strength of platelets to the nanocomposite polymer.[88] The improved tensile strength of POSS-PCU allows the fabrication of a porous graft capable of endothelialisation, without compromising its mechanical integrity. The conduits produced through phase separation have unique viscoelastic properties resulting in pressure-responsive radial compliance characteristics similar to that of biological microvessels.[89] This would minimise compliance mismatch between graft and host artery over physiological pressure ranges, thereby reducing the incidence of intimal hyperplasia.[90]

As blood vessels are load baring structures, their mechanical properties are critical for a successful therapy. A major drawback of many tissue engineering constructs is insufficient radial strength leading to poor bursting pressures. Interestingly, blood vessels constructed through the self assembly approach result in superior mechanical properties. The self assembly approach consists of culturing human umbilical vein SMC (hUVSMC) and dermal fibroblasts (hDF) *in vitro* into a cell sheet which is then rolled around a mandrel and cultured to form a tissue engineering blood vessel with a similar medial and adventitial structure to the native vessel.[91] EC cells can then be seeded onto the luminal surface to form a functioning endothelium. The vessels produced through self-assembly have the tensile strength to be used as a viable graft; and compliance values, over the physiological loading range, comparable to the native vessel and are thus a promising candidate for clinically viable TEBV.[92] Indeed, self-assembled TEBV have been shown to be antithrombogenic and mechanically stable *in vivo* for a period of 8 months in a rat model resulting in the formation of a confluent endothelium and vasa vasorum formation.[93]

4.2. Heart Valve

Heart valve prostheses are amongst the most widely used biomedical devices and face an ever growing demand.[94] However, currently available prosthetic valves lack the ability to grow, repair and remodel in an *in vivo* environment; in addition to problems associated with calcification, thrombosis, tearing and biodegradation.[95] A tissue engineered heart valve has the potential to overcome a number of these drawbacks.

A decellularised heart valve has been proposed as the ideal scaffold for tissue engineering heart valves as they provide the natural valve architecture and optimal conditions for cell culturing. There are some major limitations to the use of decellularised scaffold; principle amongst them is the loss of all mechanical integrity. To counter this problem, a decellularised heart valve was coated with electrospun poly-4-hydroxybutyrate – a biodegradable biomaterial – and then seeded with mesenchymal stem cells (MSC).[96] The hybrid scaffold displayed improved mechanical properties and the ECM like morphology of the electrospun fibres provides a biomimitec surface for culturing MSC. The scaffold has been further modified to include bFGF loaded chitosan nanoparticles in a bid to stimulate MSC proliferation.[97] A significant increase in collagen and 4-hydroxyproline was noted for the bFGF containing scaffold, suggesting that the inclusion of bFGF enhances the formation of ECM components leading to an improvement in the mechanical strength of the valve.

A promising, wholly synthetic, heart valve scaffold has been developed in our lab using POSS-PCU (Fig. 7).[98] The addition of the POSS nanoparticle alleviates many of the traditional problems associated with polyurethane's; namely, biodegradation, calcification and, as previously mentioned, thrombosis. POSS-PCU displayed significantly improved biodurability and stability when exposed to a variety of degradative solutions.[99] The hydrophobic nature, and improved mechanical performance, of the POSS-PCU heart valve also led to reduced calcification when exposed to calcium solution in a bespoke *in vitro* accelerated physiological pulsatile pressure system for a period of 31 days.[100] Furthermore, the roughened surface morphology means a greater surface area of polyurethane is available for adhesion, growth and proliferation of endothelial cells.[101]

A hydrogel composed of polyvinylaclohol (PVA) and bacterial cellulose nanofibers of <100 nm has also been mooted as a possible biomaterial for tissue engineering heart valves.[102] It was hypothesised that whilst the PVA would provide the elasticity required by heart valves,

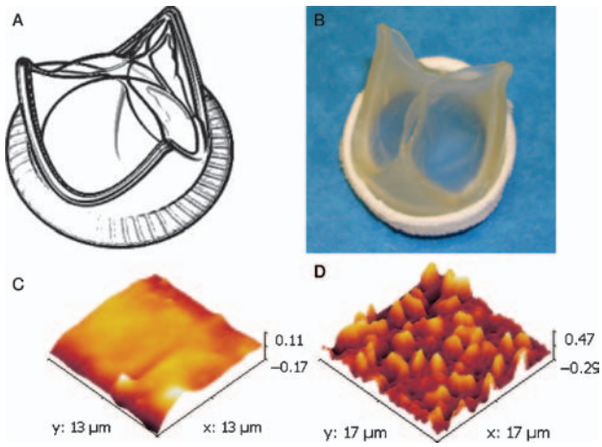

Fig. 7. A) Trileaflet valve design with complex geometry and additional reflection on the leaflets to improve performance and durability. B) Prototype valve fabricated from POSS–PCU nanocomposite with a Dacron suture ring. AFM images of the surface topography of PCU (C) and POSS-PCU (D). The POSS nanocomposite cage can be seen clearly protruding from the film surface giving the polymer a rough, textured surface more conducive to endothelialisation.

the introduction of bacterial cellulose would provide the stiffness; mimicking the role of elastin and collagen, respectively, in native tissue. The nanocomposite hydrogel displayed stress/strain behaviour comparable with porcine aortic heart valves and efforts have been made to optimise leaflet design through computational simulations.[103]

4.3. Myocardium

Heart failure contributes to the death of 300 000 people and leads to over 1 million hospitalisations annually in the US.[104] Cardiac myocytes are terminally differentiated cells and cannot regenerate following injury. With a chronic shortage of transplantable hearts, the ability to repair or engineer myocardium is highly attractive.

Electrospun scaffolds have shown great promise in supporting cardiomyocytes *in vitro*. The fibres provide support, analogous to the ECM, by providing isotropic and anisotropic cues for growth allowing cells to grow into and pull on the fibres.[105] A nanofibrous PCL mesh was seeded with cardiomyocytes from neonatal Lewis rats and cultured *in vitro* for 14 days.[106] The mesh started beating after 3 days and expressed cardiac specific proteins – α-myosin heavy chain, connexin43 and cardiac troponin I – suggesting that functional contracting cardiac grafts can be generated. In order to get a dense, 3D graft, with the ability to provide enough function; individual meshes were overlaid to create a multilayered, thick graft.[45] The authors reported that the individual layers adhered well and morphological and electrical communication was established between the layers with the construct beating in sync.

Cell sourcing remains a critical problem as it is difficult to obtain autologous cardiomyocytes for transplantation. Cardiac stem cells (CSC), with the ability to differentiate into cardiomyocytes, hold great promise. CSC were seeded onto collagen scaffolds incorporating poly(glycolic) acid nanofibres and were cultured *in vitro*.[107] A greater number of CSC adhered to the scaffold incorporating the nanofibres than the control with no fibres. An interesting alternative to the use of cardiomyocytes is the controlled delivery of granulocytes colony-stimulating factor (G-CSF) to promote myoblast differentiation towards the myocardiocyte lineage.[108, 109] By functionalising electrospun fibres with G-CSF, an ECM mimicking scaffold with the ability to release G-CSF was produced.[110] Cardiomyocyte like phenotype was only partially induced in skeletal myoblasts, however, this study provided an interesting alternative to currently used approaches in cardiac tissue engineering.

The high surface area and ECM like topography of electrospun nanofibres has also been utilised for *in vivo* regeneration through injectable self assembling peptide nanofibres.[111–114] These self assembled peptide nanofibres were found to create microenvironments conducive to progenitor cell recruitment within the myocardium.[112] A secondary injection of exogenous cells within the peptide microenvironment resulted in recruitment of α-sarcomeric actin/Nkx2.5–positive cells. Furthermore, an additional significant advantage of self-assembling peptides is that they can be engineered to be incorporate growth factors and other signalling molecules capable of controlling cellular fate. The controlled release of platelet derived growth factor (PDGF) from self-assembled peptide nanofibres, for a period of 14 days, led to reduced cardiomyocyte death and infarct size following infarction.[113] In a similar fashion, the sustained release of insulin-like growth factor-1 (IGF-1), in conjunction with local injections of clonogenic cardiac progenitor cells, led to a reduction in infarct size and improved the recovery of myocardial structure and function.[114] Protease-resistant stromal cell derived factor-1 chemokine was anchored to the self-assembled nanofibres in an effort to attract endogenous stem cells.[111] Whilst the local delivery of chemoattractants for stem cells is a popular strategy for regeneration, it is often handicapped by rapid diffusion from the site of injection. Anchoring the chemokine to the self assembling peptides alleviates this major drawback. Increased cellular recruitment and capillary tube formation was observed in the test subjects, in addition to improved systolic function one month after infarction.

5. CONCLUSIONS

The nanoscale design of biomaterials has led to highly promising technologies capable of improving surgical management of tissue loss. Greater control over cellular interactions at the material interface can be implemented, and with improvements in cell and developmental biology, greater control over the human body's response to exogenous materials can be exerted. Whilst tissue engineering

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approaches to repair or regenerate cardiovascular tissues hold great potential, they are still in their infancy and numerous challenges remain to be overcome before they can become a clinical reality. Suitable cell sources need to be identified and the rules governing cell growth and differentiation on biomaterials need to be understood. For the exciting possibility of tissue engineering the entire heart, scaffolds capable of providing the necessary flux of oxygen and nutrients to densely packed cells in whole organs need to be developed. To overcome these problems, a multidisciplinary approach needs to be taken with life scientists working hand in hand with engineers, material scientists and mathematicians.

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