Synthetic polymer scaffolds for tissue engineering

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The field of tissue engineering places complex demands on the materials it uses. The materials chosen to support the intricate processes of tissue development and maintenance need to have properties which serve both the bulk mechanical and structural requirements of the target tissue, as well as enabling interactions with cells at the molecular scale. In this *critical review* we explore how synthetic polymers can be utilised to meet the needs of tissue engineering applications, and how biomimetic principles can be applied to polymeric materials in order to enhance the biological response to scaffolding materials (105 references).

Introduction

Tissue engineering (TE) aims to generate replacement biological tissues and organs for a wide range of medical conditions involving tissue loss or dysfunction. The clinical scope of TE is enormous, with therapeutic potential in many of the ageing and lifestyle diseases prevalent in Western populations—such as heart disease, diabetes, cirrhosis and osteoarthritis—as well as a host of other major afflictions, including spinal cord injury and disfigurement. TE typically involves implanting cells into some form of supporting structural device—termed a scaffold—and allowing the cells to remodel the scaffold into natural tissue, before implanting it into a patient's body. In some cases this middle step can be omitted and the scaffold can be placed directly into the recipient, utilising a compartment of the host's own body as a bioreactor. This raises the possibility of cell harvest, scaffold seeding and implantation occurring in a single surgical event. Taking this approach one step further, the scaffolding material is sometimes inserted *without cells*; regeneration then relies on the recruitment of native cells into the implant and the subsequent deposition of an extracellular matrix (ECM) (Fig. 1).[1–3] Whichever approach is taken, the scaffold itself is critical to the success of the implant, and in many cases actively directs the behaviour of the cells within.

The roots of TE extend at least as far back as the 1970s with several independent attempts to create skin substitutes using cultured cell sheets, or collagen and glycosaminoglycans (natural components of many tissues, including skin).[4] However, a shift in emphasis from that of using natural scaffolds towards the use of synthetic polymers signalled the genesis of the field as we know it today. Vacanti *et al.* experimented with different synthetic degradable polymers to try to create functional tissue *ex vivo*,[5] and in 1991 the team implanted the first tissue engineered device to be used in a human.[6] In this procedure a synthetic polymer scaffold, seeded with the patient's own cartilage cells, was introduced into a patient with a congenital sternal deficiency (Poland's syndrome). For many scientists, the modern understanding of the term 'tissue engineering' dates back to a 1993 paper by Langer and Vacanti,[7] in which they described TE as 'an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological

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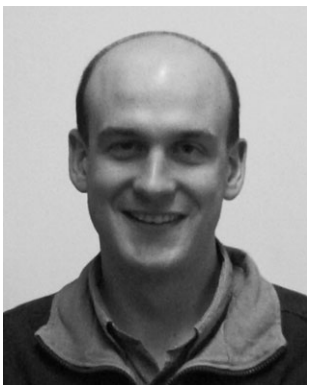

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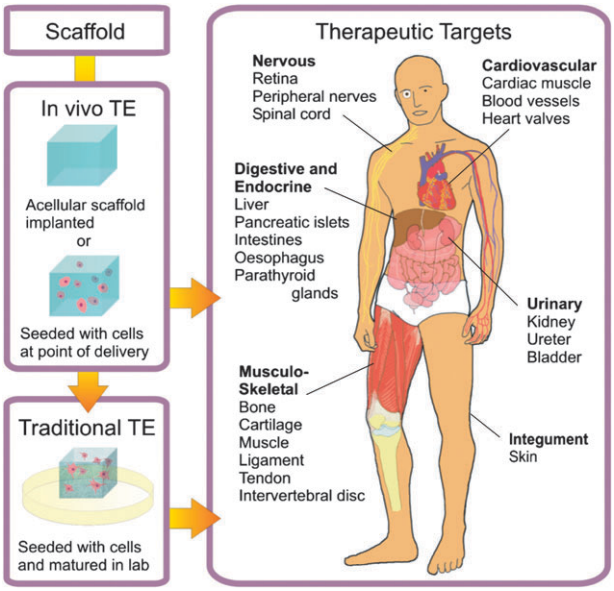

Fig. 1 TE approaches. In traditional TE, cellularised scaffolds are given time to mature *in vitro* before being introduced into the patient, while for *in vivo* TE, the scaffold is implanted directly into the patient, with or without the addition of cells. These approaches are currently being applied in therapy for a range of tissues and organs, including those indicated (not an exhaustive list).

substitutes that restore, maintain or improve tissue function.' This landmark paper drew together threads from different lines of research to consolidate and extend pre-existing ideas on tissue engineering, whilst catapulting the term and concepts associated with it to a higher level of awareness among scientists.

There are considerable scientific challenges involved in bringing TE products to the clinic in a cost effective manner, including generating large numbers of cells from small samples, and achieving adequate vascularisation to implanted material. Furthermore, financial and quality control issues have impacted the translation of products from laboratory to clinic. But despite these challenges, significant progress has been made towards addressing the central aims of TE. TE constructs for skin replacement were one of the earlier breakthroughs: TransCyte®, a biosynthetic covering produced by Advanced Tissue Sciences Inc., was approved by the Food and Drug Administration (FDA) in 1997, followed by several related materials.[4] The first clinical trial of a TE organ was recently carried out by Atala and his team, who carried out reconstructive surgery on seven patients with end-stage bladder disease, using TE bladders.[8] Here, 3D moulded polymers were seeded with the patients' own cells expanded from biopsy. Thirty-one months later the engineered bladders displayed histologically normal, trilaminar walls, and capacity and compliance were improved. Meanwhile, a growing number of patients are benefiting from TE techniques in clinical trials of vascular grafts[9] and cartilage,[10] among others. Advances in scaffold design, as well as in cell culture and surgical techniques, promise to consolidate and extend these successes to a wider range of tissues and anatomical sites, and ultimately to large numbers of patients.

Broadly speaking, the main demands on biomaterials for TE scaffolds are that they serve the bulk mechanical and structural requirements of the target tissue, and enable molecular interactions with cells that promote tissue healing. In the first respect, synthetic polymers are very attractive candidates as their material properties are typically more flexible than those of natural materials. It is reasonably straightforward to control the mechanical and chemical properties of synthetic polymers; they can be non-toxic, readily available and

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relatively inexpensive to produce, and in many cases can be processed under mild conditions that are compatible with cells. For these reasons, they have found widespread application in TE, but they lack the biological cues inherent in many natural materials that can promote desirable cell responses. This review is concerned with how synthetic polymers can be chosen and modified to fulfil the essential requirements of TE scaffolds, concentrating first on the control of material properties, then on ways in which materials may be functionalised to enhance biological response.

Synthetic polymers for TE

The first requirement of any biomaterial is biocompatibility. Several synthetic polymers are already known to be biocompatible, are FDA licensed for certain applications within the body and are well established in TE. Poly(ethylene glycol) (PEG, or poly(ethylene oxide) (PEO) at high molecular weights) is an extremely hydrophilic polymer, with excellent solubility in a range of solvents and high solution mobility (Fig. 2). It is used extensively in TE, particularly as a component of hydrogels due to its ability to imbibe water, and thanks to its protein repellent effect, it can be useful as a non-interfering background upon which specific biological

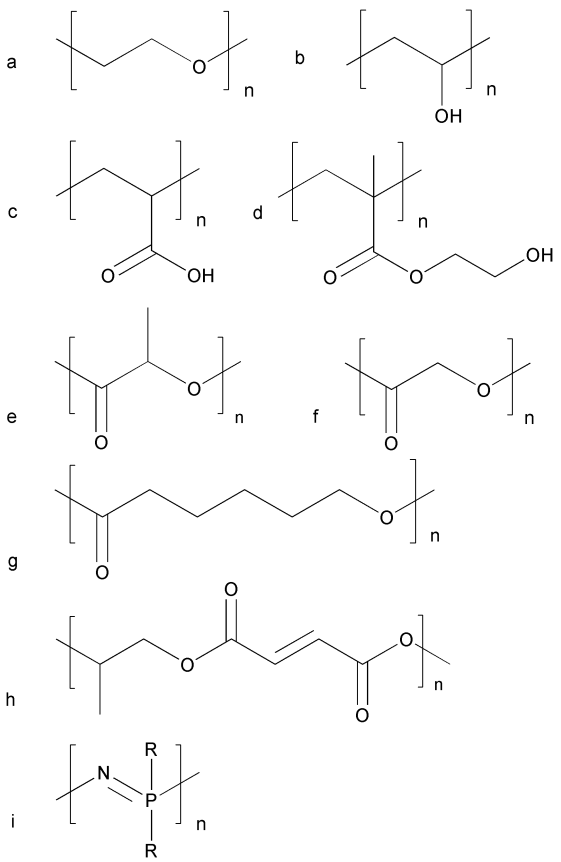

Fig. 2 Structures of some polymers commonly used in tissue engineering. a: poly(ethylene glycol) (PEG), b: poly(vinylalcohol) (PVA), c: poly(acrylic acid) (PAA), d: poly(2-hydroxyethyl methacrylate) (PHEMA), e: poly (lactic acid) (PLA), f: poly(glycolic acid) (PGA), g: poly(caprolactone) (PCL), h: poly(propylene fumarate) (PPF), i: poly(phosphazene) R = alkoxy, aryloxy or amino groups.[17]

cues can be built up (*vide infra*). Poly(vinyl alcohol) (PVA), and poly(acrylic acid) (PAA) and its derivatives (such as poly(2-hydroxyethyl methacrylate) (PHEMA)) (Fig. 2) have also been applied, although it is important to note that the use of non-degradable polymers is restricted: they must either be confined to permanent implants, chemically modified (*e.g.* by the introduction of ester linkages) or used in blocks of low M_n to ensure elimination from the body.[11]

The use of non-permanent scaffold materials that, over time, become completely replaced by natural extracellular matrix is central to the TE approach. The objective is to implant a scaffold that can persist in a robust state for sufficient time to allow for the formation of new tissue, but which will ultimately degrade and become replaced by this tissue. The most widely used synthetic degradable polymers are poly(α-hydroxy acids), *e.g.* poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL) and their copolymers (Fig. 2). These polyesters degrade by hydrolysis, eventually releasing oligomers or monomers that feed into natural metabolic pathways.[12,13] They have been particularly prevalent as they already had a range of medical applications (*e.g.* in degradable sutures, stents, wound dressings) and have FDA approval in those devices.[14] However, these polymers are hydrophobic, which can be disadvantageous in tissue regeneration applications, due to poor wetting and lack of cellular attachment and interaction. Recently plasma treatment of the polymer surface has gone some way to overcoming this issue, although the effects may not be permanent.[15] Poly(α-hydroxy acids) are also known to undergo autocatalytic degradation, whereby the accumulation of acidic breakdown products accelerates the rate of hydrolysis.[16] Implants placed inside the body therefore tend to degrade rapidly from within, leaving a capsule of high M_n polymer on the outside and an acidic core which is detrimental to cells. Blending the polyester with buffering agents may help to retard this process (as with the use of alkaline bioceramics for bone TE).

Some of the limitations of poly(α-hydroxy acids) can be overcome by using alternative degradable polymers with a variety of desirable features. To name a few, polycarbonates produce less acidic degradation products, poly(fumarate)s can be crosslinked due to the double carbon bond in their backbone (Fig. 2) and α-amino acids/peptides can be directly attached to poly(ester amide)s.[17] Poly(urethane)s are particularly promising alternatives to polyesters and can be suitable for both hard and soft TE applications.[18,19] Most are non-degradable, but recently degradable poly(urethane)s have been developed, including an example from Guelcher *et al.* which has a compressive strength similar to that of non-degradable poly(methyl methacrylate) (PMMA) bone cements.[20] Poly(anhydride)s and poly(orthoester)s degrade by surface erosion, which has made them suitable for drug delivery and could also be beneficial in TE, although their use within TE has thus far been limited. A relatively new (and promising) class of polymer is the poly(phosphazene)s, attractive due to their structural adaptability which affords a high level of control over degradation, crystallinity and other characteristics (Fig. 2).[17] Interest is growing in expanding the range of polymers (and polymer combinations) used in TE in order to find optimum solutions for individual applications, and the

development of high-throughput microarrays for screening of cell–biomaterial interactions could accelerate this process.[21] The importance of matching polymers to specific applications is given further significance by recent results which suggest that small chemical functional groups may even be used to influence stem cell differentiation.[22]

Controlling degradation

The rate of hydrolytic degradation of ester linkages is affected by a multitude of factors. In general, actions which increase the penetration of water accelerate the rate of hydrolysis—such as using, or blending with, a more hydrophilic polymer. Two important considerations are the polymer's glass transition temperature (T_g) and crystallinity, both of which reflect the ability of water to access the polymer chains. A high T_g corresponds to relatively limited molecular motion and low free volume within the polymer network, meaning that less space is available for water molecules to penetrate. This can be achieved by stiffening the polymer chain, for example by incorporating backbone phenol moieties, or bulky side groups.[23] A high degree of crosslinking or chain branching can also limit movement. Conversely, flexible C–O–C backbone linkages increase molecular motion, and the inclusion of short branches leads to an increase in free volume due to the higher number of chain ends.[23] Both of these factors can therefore reduce T_g and accelerate hydrolytic degradation.

Similarly, a high degree of crystallinity limits hydration through the tight, ordered packing of polymer chains. Crystallinity is reduced by actions which disrupt packing, such as the inclusion of short side chains or random copolymerisation, and is also heavily influenced by polymer stereochemistry. Poly(L-lactic acid) (PLLA) and poly(D-lactic acid) (PDLA) can co-crystallise to form a stereocomplex. The polymer chains form left- and right-handed helices, respectively, and pack tightly side by side to form a racemic crystal.[24,25] This species shows a higher melting temperature than either of the enantiomers (230 °C compared to 175 °C) and also improved resistance to hydrolysis.[25] Processing conditions can also affect crystallinity, for example rolling or extrusion can facilitate the parallel alignment of polymer chains.[25] Further influences include the device size and morphology, and the local pH conditions.[16]

A major drawback of random chain hydrolysis is that in many cases it can lead to a dramatic deterioration in material strength. One solution is to use scaffolds that undergo controllable degradation, for example where enzyme-cleavable peptide segments are incorporated into the polymer network.[2,26–29] In living tissues, cells inhabit an extracellular matrix (ECM) which, although highly tissue specific, consists largely of soluble and insoluble (mostly fibrous) proteins, and hydrated polysaccharides. Cells migrate through the matrix either by adapting their morphology to manoeuvre a path of least resistance or by clearing a trail using secreted, locally activated or membrane bound proteases such as matrix metalloproteases (MMPs) and plasmin.[28] This natural process can be recreated in biomaterials by incorporating matrix proteins such as collagen or fibrin (which naturally harbour protease-cleavable sequences), but synthetic systems have also been designed in which enzyme-cleavable motifs provide this functionality. West and Hubbell developed ABA copolymers, consisting of a central poly(ethylene glycol) (PEG) block (B) with two acrylate end-capped oligopeptides (A) which included cleavage sites either for MMP-1 (target site APGL) or for plasmin (target site VRN).[29] Photopolymerisation of the acrylate end groups yielded hydrogels that degraded in the presence of the targeted protease. Later, a simpler synthetic strategy was developed whereby a vinyl-sulfate terminated PEG block was crosslinked with a cysteine-containing peptide by a Michael-type addition reaction.[27] The crosslinking peptide, GC**YKNR**CG**YKNR**CG-NH_2, contained three cysteine residues and two plasmin substrate sites (shown in bold). The rate of invasion of cells into this type of hydrogel can be altered by changing the amino acid sequence, which tunes the enzymatic sensitivity of the MMP substrate peptide crosslinkers.[26] Lower M_n PEG gives a higher crosslink density and a finer mesh, which also slows down the movement of cells into the gels, and at sufficiently high crosslink density it is possible to exclude cells completely.[26]

Tissue engineering scaffolds

In addition to the degradation mode and kinetics of the polymer, the form of the engineered device is critical. Ideally, damaged tissue should be treated so as to minimise further injury to the surrounding tissue. One minimally invasive way to achieve this is to *inject* a polymer matrix into the site of damage in the form of a hydrogel—possibly carrying with it the necessary cells to effect regeneration. Hydrogels are highly hydrated networks of hydrophilic (often based on PEG or PAA), crosslinked polymer chains. A gel with covalent crosslinks is known as a chemical hydrogel, commonly produced by reacting functionalised polymers with small molecule/polymer crosslinking functionalities, for example, thiols will crosslink with acrylate or vinyl sulfone groups,[27] and amines are used to crosslink aldehydes or activated ester groups (Fig. 3).[30] Crosslinking of acrylate groups can also be achieved photochemically (*i.e.* by UV exposure).[31]

Conversely, physical hydrogels have non-covalent crosslinks, such as electrostatic interactions, hydrogen bonds or crystallised segments (Fig. 3). Hydrophobic interactions also lead to gelation, for example by the self-assembly of amphiphilic block and graft copolymers into micelles, worms or lamellae, followed by further aggregation and ultimately gelation.[32] The exploitation of such physical hydrogels is extended through the use of biological molecules, species whose structures are typically enforced by such non-covalent interactions. All the major classes of biopolymers can be grafted to polymer chains leading to gelation by a variety of different interactions (Fig. 3). Examples include complementary oligonucleotide sequences,[33] and oppositely charged peptides that dimerise into coiled-coil superhelices.[34] Heparin, an ECM polysaccharide that binds a multitude of proteins, has been conjugated to PEG by Yamaguchi and co-workers, and the solution gelled by the addition of heparin binding growth factor proteins.[35]

An ideal injectable system would be delivered as a viscous solution that would mould precisely to fill irregularly shaped defects before gelling, *in situ*, under mild conditions. This may

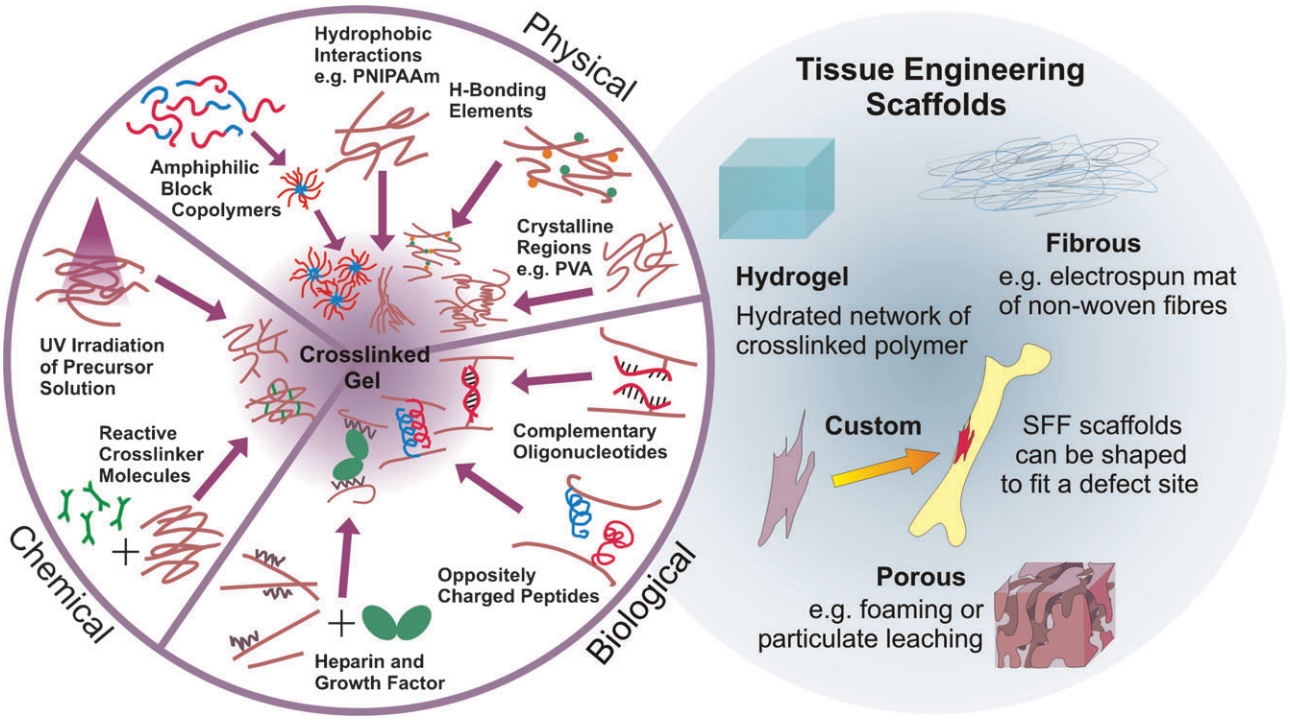

Fig. 3 (Left) Crosslinking within hydrogels. Permanent, covalent links between polymer chains can be created by UV irradiation or through the use of reactive groups, with or without initiators, creating 'chemical' gels. Physical crosslinks are reversible bonds based on a variety of non-covalent interactions, and this approach can be extended through the use of biological agents such as peptides. (Right) In addition to hydrogels, TE scaffolds can take fibrous, porous or custom morphologies.

be achieved—to an extent—by the simultaneous delivery of reactive precursors from separate syringe barrels. Unfortunately the kinetics of chemical crosslinking can be inappropriate—either too fast to allow the mixture to flow or slower than would be convenient in an operating theatre.[36] Moreover, cell surface features may be attacked or become otherwise involved in the reaction. Conversely, by their very nature physical hydrogels do not have such aggressive reactivity. Furthermore, they are formed in equilibrium reactions which can be controlled by the solvent, salt concentration, pH or temperature. Therefore the careful design of crosslinking groups can yield materials which gel when introduced to a physiological environment. Thermally reversible polymers which undergo sol–gel transitions with increasing temperature are promising materials in this respect (reviewed recently in this journal[37]). Many such materials contain poly(*N*-isopropylacrylamide) (PNIPAAm); the combination of hydrophilic amide and hydrophobic isopropyl groups gives it a unique temperature sensitivity. Below the lower critical solution temperature, hydrogen bonding between the amide and water leads to its dissolution, whereas at higher temperatures polymer–polymer and water–water interactions become dominant and the isopropyl groups dehydrate and aggregate.[36] An alternative approach is to synthesise copolymers from blocks with different solubilities in aqueous media; using this strategy PEG is usually coupled to hydrophobic blocks such as copoly(lactic acid–glycolic acid) (PLGA), PCL or poly(propylene fumarate) (PPF). The properties of the gels are adjusted by varying the block length and M_n of the copolymers, thereby coaxing the sol-to-gel transition temperature into a physiologically useful range and optimising mechanical performance.[32]

In some cases the level of mechanical support required of a scaffold may exceed that provided by a hydrogel matrix. Various engineering approaches can be employed in these instances to produce solid implants that are more resistant to high forces (Fig. 3). The inclusion of an interconnected network of pores for cell movement and mass transport of nutrients is also an essential design feature of these scaffolds. One method to generate pores is particulate leaching wherein a polymer melt or solution is poured over a bed of granular porogens (usually NaCl, sugar or paraffin) which are leached out once the polymer has solidified.[38–40] Foaming techniques are an alternative, in which gas bubbles are produced by chemical reaction or the expansion of CO_2.[18] Supercritical or high pressure CO_2 plasticises the polymer prior to decompression, so the escape of gas causes the polymer to solidify while creating porosity. Sintering solid microspheres can also create solid scaffolds with an open structure. On the other hand, fibrous scaffolds are produced *via* electrospinning[41] and phase separation;[42] the latter technique can be combined with particulate leaching to produce a macroporous architecture.[38] Furthermore, when a thermal gradient is applied during phase separation, solid walled scaffolds with microtubular architectures are created.[43] Teng *et al.* have used this method to produce the outer shell of a multicomponent scaffold for spinal cord repair, the central core of which was produced by salt leaching. Neural stem cells were introduced into the inner portion, while the aligned microtubules were intended to serve as guidance channels for regenerating nerve axons.[44]

Technologically advanced options include a range of solid freeform fabrication (SFF) techniques. Some of these are computer assisted systems whereby the layer-by-layer deposition of materials, including, potentially, cells, into a pre-specified 3D shape is achieved either through printing or by the extrusion of a polymer melt. Alternatively laser beams can be used to solidify polymers into complex shapes by photopolymerisation or sintering as they sweep over polymer liquids or layers of powder, respectively.[45,46] These technologies afford precise control over scaffold architectures, and when coupled to established medical imaging techniques they can produce solid TE devices fitted to the shape and dimensions of individual defect sites.[46] Some of these advanced options carry a set of more specific material requirements than the simpler techniques, such as low T_m and high thermal stability for melt extrusion.[47] In some cases the polymer and manufacturing method are incompatible and this is overcome by producing negative moulds (*i.e.* species that can be dissolved or otherwise removed following casting with the appropriate polymer).[45] The choices, therefore, are broad in terms of both polymers and fabrication processes: in order to select method and material from such an array, one needs to consider the range of properties required of the implant. In turn, to a large extent these properties are determined by the tissue which it is intended to replace.

Recreating tissue mechanics

Traditionally, biocompatibility and mechanical properties have been first considerations in selecting materials for TE scaffolds, and they remain central to the success of any implant on several levels. Many of the target tissues for TE exist to perform an essentially physical task, whether that be as part of the musculoskeletal, cardiovascular, integumentary or other system. Artificial constructs which recreate a specific set of mechanical properties can support the immediate mechanical needs at the implant site—and one needs only to consider the example of an engineered heart valve to appreciate how essential this can be. It is desirable that the physical properties of a construct resemble those at the implant site as closely as possible, so that it may tolerate the forces acting on it and minimise shear by deforming with its surroundings. Additionally, this ought to ensure that appropriate physical stresses and strains are transmitted to the cells within, for whom such stimuli may be a profound determinant of their physiological function.[48] However, the mechanics of living tissues are the product of a whole array of matrix components—their structures, abundance, organisation and interplay—and working to the complex mechanical specifications laid down by nature with our current toolkit is challenging.

In some cases the best way to recreate these properties may be to perform *in vitro* conditioning, in which some aspect of the *in vivo* mechanical environment is simulated, prompting cells to deposit an ordered matrix (a promising approach for TE heart valves, for example[49]). Often, however, the material itself may be optimised to possess mechanical properties approximating those of the target tissue. Each tissue presents its own set of demands: cortical bone, for example, is notable for its high strength and toughness while constructs for soft tissues such as muscle or skin need to be flexible and elastic. Furthermore, most tissues exhibit anisotropy in their mechanical properties. The tensile properties of tendons, for example, are enormously stronger in the direction of force transmission than they are perpendicular to it, thanks to the axial alignment of collagen fibres. This parallel arrangement can be mimicked in electrospun scaffolds by replacing a flat collecting plate with a rotating collector, the speed of which determines the degree of fibre alignment.[50] Alternatively a mat of randomly oriented fibres can be annealed at high temperatures under strain.

Hydrogels have potential in many soft tissue applications due to their high water content. To produce mechanical properties suitable for soft tissues, the extent of crosslinking needs to be controlled: too many crosslinks leads to a brittle structure and limits swelling; too few produces a material that is too weak to provide the necessary support. At the optimum range, a hydrogel will be both strong and elastic. PVA hydrogels crosslinked by thermal cycling are unusual in that their stress–strain relationships display an exponential shape similar to that of many natural tissues.[51] Their physical crosslinks consist of crystallites which form and grow during each thermal cycle, resulting, in effect, in a nanocomposite in which the crystalline regions reinforce an amorphous matrix. By stretching the sample after a single cycle the primary crystallites, which have dimensions of just a few nanometres, can be made to align along the direction of stress, and further cycling leads to their growth. Increasing the initial strain applied to the gel leads to a higher degree of crystallite alignment and therefore anisotropy, whereas the stiffness in both directions increases in proportion to the number of thermal cycles as the volume fraction of crystallites continues to grow. Millon *et al.* applied strain during thermal cycling to produce an anisotropic PVA hydrogel with a stress–strain relationship very similar to that of porcine aorta in both the longitudinal and perpendicular directions (Fig. 4).[51]

Physical hydrogels can be useful as *in situ* gelling TE scaffolds, but they tend to be less strong than chemical gels, and even with optimisation may lack the requisite mechanical properties. Chemical crosslinking is often achieved by

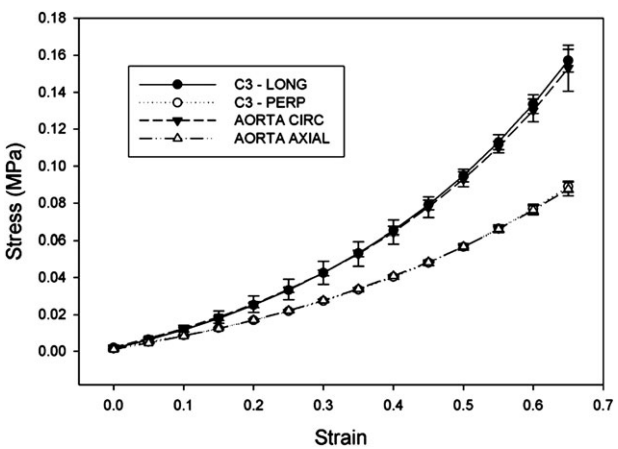

Fig. 4 Stress–strain curves of porcine aorta and anisotropic PVA hydrogels, showing a close match in both directions. Reprinted with permission from ref. 51. Copyright (2006) Wiley Periodicals.

photopolymerisation of acrylate functionalities; however, the photoinitiator toxicity and heat effects (when rapid gelation is required) are not ideal for *in vivo* use.[31] To overcome these limitations, some groups have introduced both chemical and physical crosslinks, in a way that improves both the strength of the hydrogel and the reaction conditions, allowing for rapid gelation by physical means followed by slow chemical curing.[36] Hiemstra *et al.* have described a system wherein stereocomplexation (physical) and photopolymerisation (chemical) are combined in PLA hydrogels. They used eight-arm star block copoly(ethylene glycol-D-lactic acid) and copoly(ethylene glycol-L-lactic acid) with methacrylate end groups. The poly(D-lactic acid) and poly(L-lactic acid) portions formed the stereocomplex (*vide supra*) which itself assisted the photopolymerisation process. This was apparently due to the accumulation of the hydrophobic photoinitiator within the stereocomplex, which permitted the use of a low concentration of photoinitiator.[31] The storage moduli of the resulting 'stereo-photohydrogels' was drastically increased compared to equivalent hydrogels formed solely by stereocomplexation or photopolymerisation—31.6 kPa *versus* 5.6 and 1.8 kPa, respectively, in one instance.

To achieve the high strength required for bone TE, composite materials comprising synthetic polymers (usually PLA, PGA, PCL or crosslinked PPF) and well dispersed reinforcing particles have been applied. If nanofillers are used, rather than conventional microscopic or macroscopic additives, the strengthening effect may be greater still.[52] Nanoparticles introduced directly into a polymer solution will usually precipitate or phase separate due to weak interfacial bonding, but a surfactant can aid dispersion. Furthermore, the particles may be functionalised to enable covalent linkage to the polymer network.[53] Large scale improvements in mechanical properties have been achieved by the addition of small quantities of reinforcing material. Recently, Shi *et al.* supplemented PPF with 0.1% wt functionalised single-walled carbon nanotubes (SWNTs) to yield a nanocomposite that was over twice as strong in compression and flexion as pure PPF.[54] It was proposed that covalent linkages formed between the polymer and nanotubes and improved the mechanical coupling between the two phases. However, the strength of this type of composite material still falls far short of the strength and toughness of bone.

More generally, ceramic or glass particles have been used as reinforcement materials. The resulting composites are somewhat reminiscent of natural bone matrix, which consists primarily of hydroxyapatite nano-crystals deposited in between highly ordered collagen-I fibres; these two components render the tissue resistant to compressive and tensile forces, respectively. Furthermore, crosslinks based on electrostatic interactions between negatively charged proteins and the apatite crystals bind the fibres into a strong network. Sarvestani *et al.* prepared a peptide of six glutamic acid repeat units to mimic a glutamic acid rich region in osteonectin (a protein which binds to both hydroxyapatite and collagen with high affinity). An acrylate end group was used to covalently attach the synthetic peptide to a block copoly(lactic acid–fumarate–ethylene glycol) hydrogel, approximately doubling the shear modulus of composites produced with nanoapatite crystals.[52] Such hydrogel based composites are at an early stage of development, and their stiffness remains orders of magnitude below the physiological range, but the strategy of attempting to model natural structures may prove worthwhile.

Modern TE materials increasingly try to incorporate design motifs from nature. Anionic proteins such as osteonectin are implicated in the early stages of bone mineralisation and are proposed to stabilise the amorphous calcium phosphates,[55,56] prompting the use of anionic groups in polymers to improve mineral growth. Carboxylic acid functional groups can be incorporated *via* the use of functionalised monomers,[55,57] by surface modification,[58] or they may be generated during ester hydrolysis.[55] Many phosphorus-containing polymers are also effective at encouraging mineral deposition.[56] Wang *et al.* describe a photo-crosslinked hydrogel prepared from poly(ethylene glycol)-di-(ethylphosphatidyl(ethylene glycol)-methacrylate) and poly(ethylene oxide) diacrylate.[59] Osteoblasts (bone forming cells) within the scaffold produce alkaline phosphatase, an enzyme which cleaves the phospho-ester linkages in the polymer, releasing phosphoric acid. This promotes mineralisation as the phosphoric acid reacts with calcium in the medium to form insoluble calcium phosphate.

Guiding cell behaviour

The impetus to develop composites containing glasses and ceramics has been driven by more than just their improved strength. Bioactive glasses (Fig. 5) have the advantage of chemically bonding to host bone due to the formation of a carbonated hydroxyapatite surface layer under physiological conditions.[16,60,61] Furthermore, some calcium phosphates (the class of material to which bone mineral belongs) may possess osteoinductive potential, *i.e.* promote the differentiation of progenitor cells towards an osteoblastic type.[62] This is perhaps not surprising, as the highly complex structures of extracellular matrices have evolved to support many different requirements, including providing behavioural signals for the cells that inhabit them, as well as providing structural support. Accordingly, TE scaffolds are being viewed less and less as passive cell carriers, and increasingly as structures that can be loaded with information to direct cell function. Cell behaviour is guided by a wealth of external influences including ions, soluble growth factors, ECM elements and the physical properties of the matrix. Just understanding these interactions in all their complexity is a monumental challenge for cell biologists, and recreating them would be an impossible task. However, it is possible to select certain critical influences and present them to cells in order to attempt to control behaviour. Physiology and stem cell research have yielded a wide inventory of molecules that can help to direct cells within TE scaffolds *via* specific receptor–ligand interactions. In native tissues cells attach to substrates by the coupling of binding domains within matrix proteins, such as fibronectin and collagen, to integrin receptors on the cell membrane. This allows force generation for movement, provides behavioural cues, and without it a cell may undergo apoptosis resulting from a dearth of cell-matrix signals. Therefore, the incorporation of adhesion proteins into a scaffold can support the migration, proliferation, differentiation and even the survival

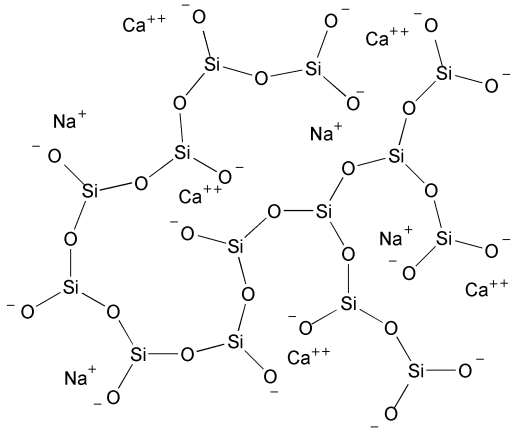

Fig. 5 Two-dimensional representation of the general structure of bioactive glass. Each silica atom is complexed with four oxygen atoms in a tetrahedral arrangement (the fourth bond is out of the plane of the diagram). Depending on the formulation, the glass contains varying quantities of sodium, calcium and other cations. Phosphorus is also present as orthophosphate, which is charge balanced by cations (not shown).[61]

of its cell population. Meanwhile, growth factors are extremely powerful tools for controlling cell differentiation and function. Their potency is such that they need only be used in very small quantities; the technical challenge is to present them in a functional state over an extended period of time.

Bulk modification with bioactive compounds

The bulk incorporation of proteins into polymer scaffolds is often achieved simply by mixing the two solutions before processing.[63,64] A variation on this approach is to allow proteins to diffuse through a hydrogel from one end before crosslinking, resulting in a concentration gradient that may provide cells with directional cues.[65] Alternatively, polymer terminal or side groups can be functionalised to enable grafting of biochemicals.[66] In some cases proteins or peptides can be directly incorporated into the polymer backbone, for example in poly(amido-amines), which are formed by polyaddition between primary or secondary amines and bisacrylamides (Fig. 6).[67] Whichever of these approaches is taken, any processing steps performed after the addition of protein must allow for these sensitive molecules' need for mild conditions.

Where retarded release of growth factor protein is required, strategies include coating loaded fibres with another polymer, for example by using chemical vapour deposition.[64] Another way of achieving coated fibres is by co-electrospinning—a new variation on the electrospinning technique which produces core–shell fibres in a one-step process.[68] A popular technique is to fabricate loaded microspheres using the double emulsion technique, whereby primary (water in oil) and secondary (oil in water) emulsions generate aqueous pockets of dissolved proteins within polymer droplets.[69] The droplets can then be dried into beads, which can be seeded within a scaffold,[40,63] or else an entire scaffold can be fabricated by their fusion (microsphere sintering). As the microspheres degrade, so the pockets of dissolved growth factor are gradually freed.

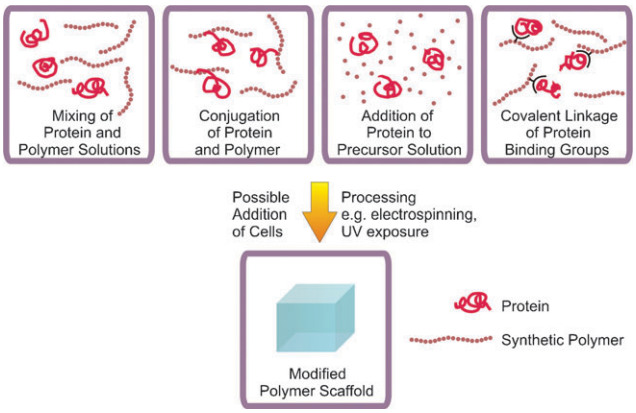

Fig. 6 Bulk modification of polymers. Proteins (or peptides) can be incorporated into polymer scaffolds by mixing of solutions before processing; by direct conjugation of protein to polymer chains; by the addition of protein prior to polymerisation; or by association with covalently linked protein binding groups.

Furthermore, by combining approaches, the sequential release of two or more growth factors may be contrived in order to expose cells to a temporal scheme of events designed to recreate some aspects of developmental pathways.[63]

Growth factor presentation has been taken to new levels of sophistication by research teams who have engineered hydrogels that permit active, *i.e.* cell mediated, release of morphogenetic signals. Domains for matrix binding and/or cleavage by proteases are embedded within the structures of many growth factors, providing 'off the shelf' binding and release activity. Heparin, for example, sequesters numerous morphogens into the matrix by binding them with high affinity until they are liberated by cellular remodelling. Pratt *et al.* have exploited this natural system by attaching peptides with a heparin binding domain to PEG within hydrogels.[27] The peptide associates with heparin *via* electrostatic interactions, and the bound heparin acts as a bridge between the scaffold and the growth factor (Fig. 7). Thus, a range of different growth factors can be indirectly tethered to the polymer matrix. Once immobilised, the growth factor can be released through the breakdown of heparin by cell-derived enzymes. In

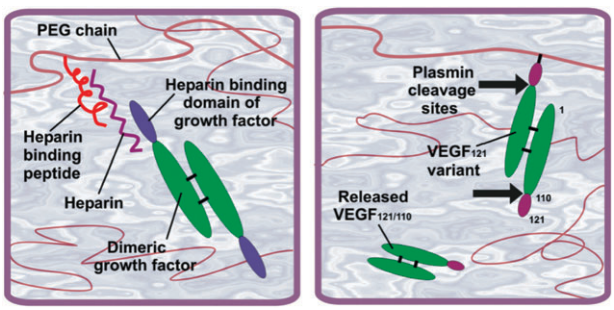

Fig. 7 (Left) General scheme used by Pratt *et al.*[27] to immobilise growth factors within hydrogels *via* a heparin bridge. The growth factor can be released through polymer or heparin degradation, or in some cases by cleavage of protease sites within the growth factor. (Right) Zisch *et al.*[70] conjugated modified $VEGF_{121}$ to PEG. The VEGF could be released through plasmin cleavage at amino acid 110.

this way, release of growth factors can be made to occur in synchrony with cellular remodelling rather than by simple diffusion. Likewise, Yamaguchi *et al.* envisage that their PEG–heparin conjugates, which assemble into gels in the presence of growth factors (Fig. 3), will disperse by ligand-exchange in the presence of cell-derived receptors.[35]

Another notable example of the cell dependent approach is in the use of vascular endothelial growth factor (VEGF), which has a plasmin substrate site downstream of its receptor binding site. Zisch *et al.* introduced a mutant variant of human $VEGF_{121}$ ($VEGF_{121-Cys}$) into PEG hydrogels by conjugation *via* an additional cysteine residue at the C-terminus. As a result, the VEGF could be released by local cellular activity causing cleavage of the plasmin site (Fig. 7).[70] Vascularisation of constructs remains one of the central challenges in TE today: without the ability to rapidly establish adequate blood supplies within scaffolds, TE successes have been more or less restricted to thin or avascular tissues. VEGF itself is a potent angiogenic agent and is a powerful tool for inducing blood vessel formation within engineered tissue; however, it is often seen that the administration of soluble VEGF results in the formation of malformed, leaky new blood vessels with poor hierarchical structure (ref. 71 and references therein). Results from Ehrbar *et al.* suggest that immobilised VEGF may provide the correct micro-environmental cues to procure the development of higher quality vasculature.

Scaffold surface engineering

In contrast to bulk modification is the surface functionalisation approach, in which relevant proteins are adsorbed or covalently bound to the surface of a solid polymer scaffold. Cell attachment to unmodified scaffolds occurs indirectly, *via* a surface layer of adsorbed proteins, and the extent of protein adsorption is largely affected by surface hydrophobicity/hydrophilicity.[72,73] Most of the commonly used degradable polymers are hydrophobic and tend to hold proteins in a non-native conformation, thus failing to present their binding domains in a sterically favourable manner. Surface treatments are therefore generally aimed at increasing the hydrophilicity so that adsorbed proteins can largely retain their normal functionality.[72]

Plasma treatment by air, nitrogen, oxygen, methane or other gases introduces charged groups to the scaffold surface.[74,75] Chemical etching can also be performed, such as the use of NaOH on PLGA and HNO_3 on poly(ether urethane).[39,76,77] Reactive groups can further be introduced by peroxide or ozone oxidation, γ- or UV-irradiation, or by reaction with cerium(IV) salts. Inevitably these harsh techniques involve some degradation of the polymer, and furthermore the effects may be non-permanent. Following surface functionalisation hydrophilic or charged polymers are sometimes grafted, to provide a permanent solution.[78] A technique developed by the Albertsson group enables covalent modification of delicate biodegradable polymers with vinyl monomers (*e.g.* acrylamide, maleic anhydride) without degradation. Their method uses the monomers and photoinitiator in the vapour phase, under UV irradiation, and eliminates the use of solvents.[79] A layer of hydrophilic polymer can also be locked to a surface by the entrapment method. Here, the surface layer

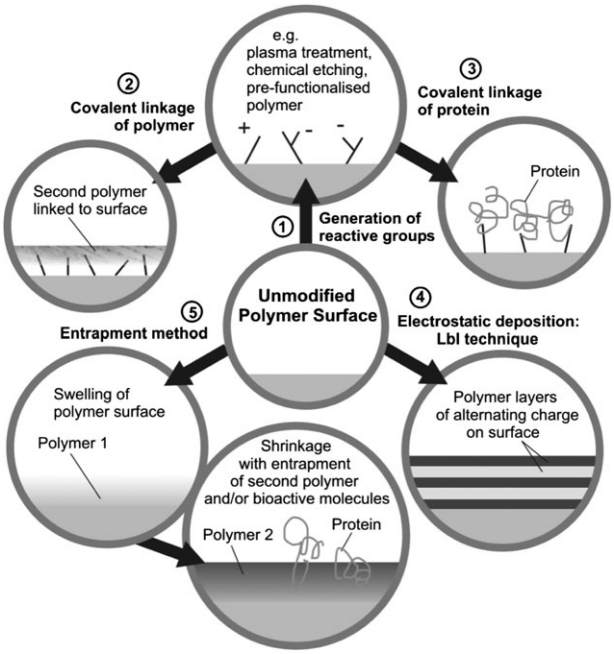

Fig. 8 Scaffold surface modification techniques. (1) The generation of reactive groups at a polymer surface through techniques such as plasma treatment can be followed by the covalent linkage of either a second polymer (2) or proteins (3). (4) Alternatively layer by layer (Lbl) methodology can be used to deposit polymer non-covalently, while (5) polymers and/or proteins can be anchored into a shrinking polymer surface through solvent exchange.

of a polymer scaffold is swollen with solvent; the swelling is reversed through the use of a nonsolvent, and the shrinking mesh of polymer chains entraps a second type of polymer as it contracts (Fig. 8).[80] It is also possible to engineer the spontaneous arrangement of hydrophilic polymers at a surface by using appropriate amphiphilic block copolymers. Lucke *et al.* have shown that segregation of the block types in diblock copoly(LA_EG) can result in a PEG rich surface layer.[81] The layer by layer technique is especially suitable for coating irregularly shaped structures where traditional methods may fail. It has been used by Zhu *et al.* to coat aminolysed PLLA surfaces with multiple bilayers of poly(styrene sulfonate, sodium salt) and chitosan to improve biocompatibility.[82]

Strategies relying on physical associations between polymer surfaces and serum proteins have the disadvantage of non-specificity. By using covalent linkages to conjugate specific proteins to scaffolds, more selected influences can be presented to cells and the chosen molecules may retain their functionality better than adsorbed proteins.[70] The carboxylate groups resulting from air plasma treatment surface groups may be exploited to graft molecules to the surface of polymer fibres or nanofibres, using a suitable coupling agent (*e.g.* carbodiimide).[75] Another option is to employ diamines as linker molecules between polyesters and proteins, one amino group coupling with the scaffold while the second reacts with the glutaraldehyde on the protein.[77] Elsewhere, Jia *et al.* attached an enzyme to nanofibres by electrospinning functionalised polystyrene (containing nitrophenyl end groups) and then immersing the fibres in a buffer solution containing the enzyme.[83]

Use of peptides in polymer scaffolds

Adding full proteins to biomaterials can be difficult to execute as the proteins are vulnerable to denaturation and degradation. A widely used method to improve cell adhesion is to incorporate short peptide motifs derived from binding regions of ECM proteins. Although they may only have a fraction of the activity of the complete protein, they can be included at very high concentration and are easy to synthesise and functionalise. Furthermore, when used in combination with PEG, non-specific protein adsorption can be eliminated and upon this blank canvas only the desired, specific receptor-mediated interactions will occur.

The well-known Arg-Gly-Asp (RGD) motif from various ECM proteins including fibronectin, laminin, collagen I, fibrinogen and vitronectin was being investigated as a means of improving cell adhesion to surfaces as far back as the late 1980s.[84] It has since been incorporated into a wide range of surfaces, scaffolds and hydrogels. The binding specificity of the peptide to cell surface integrin receptors can be improved by using a cyclic peptide conformation, and the flanking peptides are also critical: GRGDS is the most commonly used.[85] The inclusion of a PEG spacer arm may improve cellular response to RGD in terms of both magnitude and specificity by suppressing protein adsorption and presenting the motif to cells in a sterically favourable fashion.[86] A biphasic cell response is noted with varying RGD concentrations: maximum cell migration is seen at intermediate ligand densities, because too few attachment sites fail to adequately support contractile forces, and high concentrations overwhelm the cells' ability to detach from the substrate.[28] Massia *et al.* determined that an average distance of 440 nm between RGD peptides was sufficient to enable fibroblast attachment and spreading, but a significantly higher density—an interpeptide distance of 140 nm—was required for focal contact formation and cytoskeletal reorganisation into stress fibres.[87]

Although RGD peptides have been extensively used, other adhesion sequences have been studied too including IKLLI, IKVAV, LRE, PDSGR and YIGSR from laminin, DGEA from collagen I and GEFYFDLRLKGDK from collagen IV, and also cell-adhesive carbohydrates.[88–90] A Pro-His-Ser-Arg-Asn (PHSRN) sequence also found in fibronectin enhances RGD activity, and the distance between the two motifs is believed to be significant for this synergistic effect. They can be separated by PEG spacers, or by glycine repeats designed to reflect the 40 Å spacing between these domains in the native protein. Benoit and Anseth found that PEG-based hydrogels containing the sequence $RGDG_{13}PHSRN$ improved osteoblast adhesion and spreading compared to those with just RGD,[91] although elsewhere a similar sequence was shown to improve monocyte adhesion in comparison with one with 6 glycine repeats but not over RGD alone.[92] It is important to consider these precise spatial relationships when designing biomaterials. It stands to reason that cells may be expected to respond to features that exist at subcellular length scales, since their own sensing apparatus consists of molecular scale machinery. This applies both to chemical signals and to the physical configuration of the matrix. By incorporating bioactive molecules into scaffolds with nanoscale chemical patterning and architectural features, we can create a synthetic nanoenvironment that communicates with cells on a physical and chemical level.

Nanoscale design elements

Cells inhabit a complex environment rich in topographical and physicochemical cues at length scales from several nanometres to microns. Upon a backdrop of fibrous ECM proteins with nanoscale diameters, the specific binding domains of proteins and growth factors present numerous sites for interaction with cell surface receptors, while the cleavage sequences of proteases await scission by cell-derived enzymes. In this way, cells interact entirely with a landscape of nanoscale physical features. As cells move over a substrate they extend and retract filopodia peppered with integrin receptors, effectively 'feeling' the environment as they migrate. It is thought that many cell types respond directly to nanoscale surface features by changing their phenotype or activity and it has been seen that a number of cell types preferentially align along grooved surfaces.[93,94] In response to this, there have been efforts towards incorporating nanometre range features into TE materials to mimic those found in the natural extracellular environment.[95]

Nanopatterning of the RGD peptide into clusters may imitate ECM adhesion complexes, which are composed of multimeric proteins and offer an array of ligands for interaction with receptors. It has been shown that YRGD peptides, when presented in clusters, support cell migration at considerably lower densities than when evenly spread.[96] It has also been suggested that different behavioural responses—such as adhesion, migration, proliferation and differentiation—are affected differently by different pattern parameters (number of ligands per cluster, distance between clusters, overall ligand density).[97] This sort of patterning could therefore be useful for optimising cell behaviour. Clustering of adhesion peptides is achieved by first decorating polymer molecules with a controlled number of ligands, then mixing them with unmodified molecules in greater or lesser proportions. For example, Maheshwari *et al.* tethered YGRGD ligands to star PEO and then linked modified and unmodified PEO stars to a PEO hydrogel surface.[96] Irvine *et al.* have developed amphiphilic comb polymers with a PMMA backbone and RGD modified PEG side chains that can be mixed with unmodified comb copolymer for ligand clustering.[98] When the polymers are co-dissolved with PLA and spin-coated into thin films, surface segregation can be effected by aqueous annealing. This leaves the backbone running along the solid PLA scaffold surface while the side chains extend, brush-like, into solution, presenting the RGD motifs more effectively. This surface segregation technique may be suitable for modifying complex three-dimensional (3D) scaffolds.[99]

Nanometre scale design elements can, of course, be introduced into TE scaffolds in structural, as well as chemical, features. Nano- and micro-fibrous scaffolds physically resemble protein fibres and cells inhabiting them may assume a more naturalistic morphology in which the cell is involved on all sides in interactions with the matrix. This is in contrast to the flattened shape and highly polarised expression of integrins

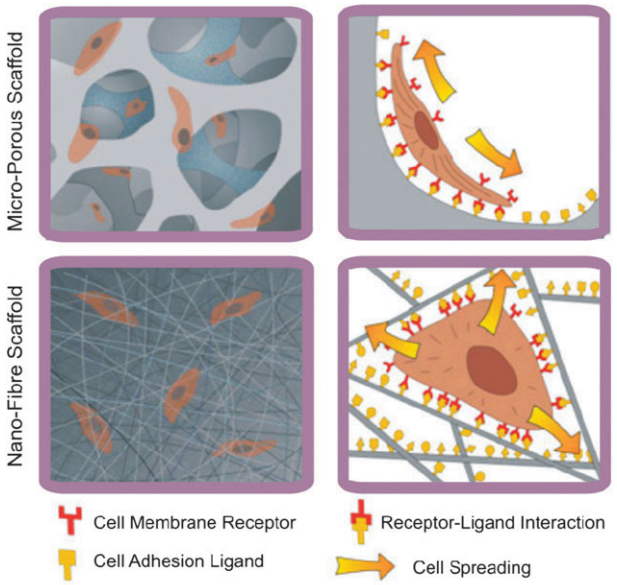

Fig. 9 Cell–substrate interactions. Scaffolds having feature dimensions much larger than the width of a cell effectively present a two-dimensional surface to the cell. If the surface is endowed with adhesion ligands the cell will flatten as it adheres and spreads. In contrast, cells within fibrous scaffolds may be involved in matrix interactions in three dimensions, similar to their experience within the fibrous environment of the natural ECM. More naturalistic cell spreading, occurring in three dimensions, is seen. Modified with permission from ref. 95, Copyright (2005) AAS Publications.

that is seen when cells are dwarfed by their substrate (Fig. 9).[100] 3D nanofibre scaffolds can be fabricated using electrospinning[41] and thermally-induced phase separation,[42] with control over pore size and fibre dimensions. The self-assembly of block copolymers into nanofibres has also been achieved[101] although currently is at a somewhat less advanced stage of development than peptide self-assembly.[102] Increasing the nanoscale roughness of polymer surfaces also improves their resemblance to the physical arrangement of the ECM. This can be achieved by using nanoparticles as the reinforcing phase in composites (including carbon nanotubes[103]), or by chemical etching. The latter is simple to perform on 3D scaffolds, and the response of a handful of cell types to treated surfaces has been analysed. The use of different polymers or cells is sometimes seen to have opposite effects on cellular adhesion—at least in part because the etching process affects not only the surface roughness, but also its chemistry. Decoupling the topographical and chemical changes resulting from these surface treatments remains a major challenge. Thapa *et al.* have used a silicone elastomer to cast impressions of the treated surfaces, and then used these as moulds to produce nanostructured polymers with the same chemical properties as the bulk material. Using this approach, an increase in bladder muscle cell numbers was observed on both PU and PLGA.[76] The differing responses of other kinds of cells to etched and cast surfaces suggest exciting possibilities for controlling the spatial distribution of cell types within TE constructs.[104] With each new discovery of this nature, the potential for sophistication in scaffold design grows.

Conclusions and future perspectives

When TE emerged as a new field in the early 1990s, there was much excitement associated with it. This positivity led numerous efforts to turn nascent concepts into reality, using technology still in its infancy, with often disappointing results. Now, TE is entering a period of fruition, with many promising animal and human trials published and several products approved.[105] Humans are already benefiting from lab built organs. In constructing their tissue engineered bladders, Atala and his team used relatively simple moulded polymers optimised for mechanical compliancy.[8] But as has been discussed, the capabilities already exist to produce far more complex scaffolds. The last decade has seen an increasing use of biomimetics in scaffold design, aimed at producing matrices which engage cells on a molecular level, coaxing them into remodelling a synthetic implant into genuine tissue. Incorporating multiple physical, chemical and biological functionalities to biomaterials will yield synthetic scaffolds which mimic the natural cell environment more closely and which participate in a dynamic, bidirectional exchange of information with cells.

Some of the most technologically advanced examples to date have been hydrogels crosslinked by protease cleavable peptides and decorated with biological functionalities including adhesion ligands. These can be modular systems in which any combination of bioactives can be added to a bioinert polymer background. Notably, the synthetic process used by the Hubbell group allows any thiolated proteins or peptides to be included in PEG hydrogels by reaction with bis(α,ω-vinylsulfone)poly(ethylene glycol) before crosslinking.[3] Elsewhere, Chung *et al.* have produced semi-interpenetrating polymer networks for bone regeneration.[2] They used PNIPAAm for its thermogelling property, and grafted MMP degradable crosslinkers and integrin-binding peptides onto linear PAA chains to allow invasion by osteoblasts. This polymer network supported new bone formation *in vivo* following injection into a treatment site in rat femora—even without the inclusion of cells or costly growth factors.[2] If in some cases, through the use of inductive material scaffolds, one can circumvent the prolonged and expensive process of expanding huge numbers of cells of host or donor origin, this will improve the ease with which effective, sterile products for regenerating functional tissues and organs can be produced.

These examples demonstrate that bioactivity can be conferred onto previously bioinert synthetic polymers, potentially generating materials that combine versatile material properties and inexpensive production with control over cell behaviour. Scaffolds based on naturally derived materials are widely used, including in many commercial products, and will remain an excellent choice for many applications. Other applications, however, are likely to be better served by materials whose mechanical properties and degradation profiles can be closely controlled, and which may be modified in a variety of ways to suit a particular purpose. In this way, taking our cues from nature, we can produce synthetic scaffold materials that actively instruct cells to regenerate tissue in damaged areas. Already the emergence of sophisticated materials with exciting *in vivo* results suggests that in the not too distant future a

plethora of tissue engineered products, for a range of degenerative diseases, will be put to clinical use on ageing populations around the globe.

References

1 M. M. Stevens, R. P. Marini, D. Schaefer, J. Aronson, R. Langer and V. P. Shastri, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 11450–11455.
2 E. H. Chung, M. Gilbert, A. S. Virdi, K. Sena, D. R. Sumner and K. E. Healy, *J. Biomed. Mater. Res., Part A*, 2006, **79A**, 815–826.
3 M. R. Lutolf, F. E. Weber, H. G. Schmoekel, J. C. Schense, T. Kohler, R. Muller and J. A. Hubbell, *Nat. Biotechnol.*, 2003, **21**, 513–518.
4 J. Viola, B. Lal and O. Grad, *The Emergence of Tissue Engineering as a Research Field*, NSF Report, Abt Associates, Cambridge, MA, 2003.
5 J. P. Vacanti, M. A. Morse, W. M. Saltzman, A. J. Domb, A. Perez-Atayde and R. Langer, *J. Pediatr. Surg.*, 1988, **23**, 3–9.
6 C. A. Vacanti, *Tissue Eng.*, 2006, **12**, 1137–1142.
7 R. Langer and J. P. Vacanti, *Science*, 1993, **260**, 920–926.
8 A. Atala, S. B. Bauer, S. Soker, J. J. Yoo and A. B. Retik, *Lancet*, 2006, **367**, 1241–1246.
9 N. L'Heureux, N. Dusserre, A. Marini, S. Garrido, L. de la Fuente and T. McAllister, *Nat. Clin. Pract. Cardiovasc. Med.*, 2007, **4**, 389–395.
10 A. P. Hollander, S. C. Dickinson, T. J. Sims, P. Brun, R. Cortivo, E. Kon, M. Marcacci, S. Zanasi, A. Borrione, C. De Luca, A. Paviesio, C. Soranzo and G. Abatangelo, *Tissue Eng.*, 2006, **12**, 1787–1798.
11 J. K. Tessmar and A. M. Gopferich, *Macromol. Biosci.*, 2007, **7**, 23–39.
12 R. H. Platel, L. M. Hodgson and C. K. Williams, *Polym. Rev.*, 2008, **48**, 11–63.
13 W. Amass, A. Amass and B. Tighe, *Polym. Int.*, 1998, **47**, 89–144.
14 A.-C. Albertsson and I. K. Varma, *Biomacromolecules*, 2003, **4**, 1466.
15 J. Yang, G. Shi, J. Bei, S. Wang, Y. Cao, Q. Shang, G. Yang and W. Wang, *J. Biomed. Mater. Res.*, 2002, **62**, 438–446.
16 K. Rezwan, Q. Z. Chen, J. J. Blaker and A. R. Boccaccini, *Biomaterials*, 2006, **27**, 3413–3431.
17 P. Gunatillake, R. Mayadunne and R. Adhikari, *Biotechnol. Annu. Rev.*, 2006, **12**, 301–347.
18 M. Sokolsky-Papkov, K. Agashi, A. Olaye, K. Shakesheff and A. J. Domb, *Adv. Drug Delivery Rev.*, 2007, **59**, 187–206.
19 I. C. Bonzani, R. Adhikari, S. Houshyar, R. Mayadunne, P. Gunatillake and M. M. Stevens, *Biomaterials*, 2007, **28**, 423–433.
20 S. A. Guelcher, A. Srinivasan, J. E. Dumas, J. E. Didier, S. McBride and J. O. Hollinger, *Biomaterials*, 2008, **29**, 1762–1775.
21 D. G. Anderson, S. Levenberg and R. Langer, *Nat. Biotechnol.*, 2004, **22**, 863–866.
22 D. S. W. Benoit, M. P. Schwartz, A. R. Durney and K. S. Anseth, *Nat. Mater.*, 2008, **7**, 816–823.
23 R. J. Young and P. A. Lovell, *Introduction to Polymers*, Chapman & Hall, London, UK, 2nd edn, 1991, pp. 241–306.
24 H. Tsuji, *Macromol. Biosci.*, 2005, **5**, 569–597.
25 D. Ishii, W.-K. Lee, K.-I. Kasuya and T. Iwata, *J. Biotechnol.*, 2007, **132**, 318–324.
26 M. P. Lutolf, J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields and J. A. Hubbell, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 5413–5418.
27 A. B. Pratt, F. E. Weber, H. G. Schmoekel, R. Muller and J. A. Hubbell, *Biotechnol. Bioeng.*, 2004, **86**, 27–36.
28 A. S. Gobin and J. L. West, *FASEB J.*, 2002, **16**, 751–753.
29 J. L. West and J. A. Hubbell, *Macromolecules*, 1999, **32**, 241–244.
30 Y. Murakami, M. Yokoyama, T. Okano, H. Nishida, Y. Tomizawa, M. Endo and H. Kurosawa, *J. Biomed. Mater. Res., Part A*, 2007, **80A**, 421–427.
31 C. Hiemstra, W. Zhou, Z. Y. Zhong, M. Wouters and J. Feijen, *J. Am. Chem. Soc.*, 2007, **129**, 9918–9926.
32 J. Lee, Y. H. Bae, Y. S. Sohn and B. Jeong, *Biomacromolecules*, 2006, **7**, 1729–1734.
33 S. Nagahara and T. Matsuda, *Polym. Gels Networks*, 1996, **4**, 111–127.
34 C. Wang, R. J. Stewart and J. Kopecek, *Nature*, 1999, **397**, 417–420.
35 N. Yamaguchi, L. Zhang, B. S. Chae, C. S. Palla, E. M. Furst and K. L. Kiick, *J. Am. Chem. Soc.*, 2007, **129**, 3040–3041.
36 S. A. Robb, B. H. Lee, R. McLemore and B. L. Vernon, *Biomacromolecules*, 2007, **8**, 2294–2300.
37 L. Yu and J. Ding, *Chem. Soc. Rev.*, 2008, **37**, 1473–1481.
38 R. Y. Zhang and P. X. Ma, *J. Biomed. Mater. Res.*, 2000, **52**, 430–438.
39 G. E. Park, M. A. Pattison, K. Park and T. J. Webster, *Biomaterials*, 2005, **26**, 3075–3082.
40 M. Lee, T. T. Chen, M. L. Iruela-Arispe, B. M. Wu and J. C. Y. Dunn, *Biomaterials*, 2007, **28**, 1862–1870.
41 W. J. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan and F. K. Ko, *J. Biomed. Mater. Res.*, 2002, **60**, 613–621.
42 P. X. Ma and R. Y. Zhang, *J. Biomed. Mater. Res.*, 1999, **46**, 60–72.
43 P. X. Ma and R. Zhang, *J. Biomed. Mater. Res.*, 2001, **56**, 469–477.
44 Y. D. Teng, E. B. Lavik, X. Qu, K. I. Park, J. Ourednik, D. Zurakowski, R. Langer and E. Y. Snyder, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 3024–3029.
45 T. Weigel, G. Schinkel and A. Lendlein, *Expert Rev. Med. Devices*, 2006, **3**, 835–851.
46 S. J. Hollister, *Nat. Mater.*, 2005, **4**, 518–524.
47 T. B. F. Woodfield, J. Malda, J. de Wijn, F. Péters, J. Riesle and C. A. van Blitterswijk, *Biomaterials*, 2004, **25**, 4149–4161.
48 A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, **126**, 677–689.
49 M. P. Rubbens, A. Mol, R. A. Boerboom, R. A. Bank, F. P. T. Baaijens and C. V. C. Bouten, *Tissue Eng. A*, 2008, **14**, 1–10.
50 W. J. Li, R. L. Mauck, J. A. Cooper, X. N. Yuan and R. S. Tuan, *J. Biomech.*, 2007, **40**, 1686–1693.
51 L. E. Millon, H. Mohammadi and W. K. Wan, *J. Biomed. Mater. Res., Part B*, 2006, **79B**, 305–311.
52 A. S. Sarvestani, X. He and E. Jabbari, *Eur. Biophys. J.*, 2007, **37**, 229–234.
53 S. Haque, I. Rehman and J. A. Darr, *Langmuir*, 2007, **23**, 6671–6676.
54 X. Shi, J. L. Hudson, P. P. Spicer, J. M. Tour, R. Krishnamoorti and A. G. Mikos, *Biomacromolecules*, 2006, **7**, 2237–2242.
55 J. Song, V. Malathong and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2005, **127**, 3366–3372.
56 J. D. Kretlow and A. G. Mikos, *Tissue Eng.*, 2007, **13**, 927–938.
57 C. K. Williams, *Chem. Soc. Rev.*, 2007, **36**, 1573–1580.
58 J. L. Chen, B. Chu and B. S. Hsiao, *J. Biomed. Mater. Res., Part A*, 2006, **79A**, 307–317.
59 D. A. Wang, C. G. Williams, F. Yang, N. Cher, H. Lee and J. H. Elisseeff, *Tissue Eng.*, 2005, **11**, 201–213.
60 L. L. Hench and H. A. Paschall, *J. Biomed. Mater. Res.*, 1973, **7**, 25–42.
61 Z. Zarzycki, in *Glasses and the vitreous state*, ed. R. W. Cahn, E. A. Davies and I. M. Ward, Cambridge University Press, Cambridge, UK, 1991, pp. 37–74.
62 H. Yuan, Z. Yang, Y. Li, X. Zhang, J. D. De Bruijn and K. De Groot, *J. Mater. Sci. Mater. Med.*, 1998, **9**, 723–726.
63 T. P. Richardson, M. C. Peters, A. B. Ennett and D. J. Mooney, *Nat. Biotechnol.*, 2001, **19**, 1029–1034.
64 J. Zeng, A. Aigner, F. Czubayko, T. Kissel, J. H. Wendorff and A. Greiner, *Biomacromolecules*, 2005, **6**, 1484–1488.
65 M. C. Dodla and R. V. Bellamkonda, *J. Biomed. Mater. Res., Part A*, 2006, **78**, 213–221.
66 M. Gonen-Wadmany, L. Oss-Ronen and D. Seliktar, *Biomaterials*, 2007, **28**, 3876–3886.
67 P. Ferruti, S. Bianchi, E. Ranucci, F. Chiellini and V. Caruso, *Macromol. Biosci.*, 2005, **5**, 613–622.
68 Z. Sun, E. Zussman, A. L. Yarin, J. H. Wendorff and A. Greiner, *Adv. Mater.*, 2003, **15**, 1929–1932.
69 A. Taluja and Y. H. Bae, *Int. J. Pharm.*, 2008, **358**, 50–59.

70 A. H. Zisch, M. P. Lutolf, M. Ehrbar, G. P. Raeber, S. C. Rizzi, N. Davies, H. Schmokel, D. Bezuidenhout, V. Djonov, P. Zilla and J. A. Hubbell, *FASEB J.*, 2003, **17**, 2260–2262.
71 M. Ehrbar, S. M. Zeisberger, G. P. Raeber, J. A. Hubbell, C. Schnell and A. H. Zisch, *Biomaterials*, 2008, **29**, 1720–1729.
72 Z. Ma, Z. Mao and C. Gao, *Colloids Surf., B*, 2007, **60**, 137–157.
73 A. Kikuchi and T. Okano, *J. Controlled Release*, 2005, **101**, 69–84.
74 H. R. Allcock, L. B. Steely, S. H. Kim, J. H. Kim and B. K. Kang, *Langmuir*, 2007, **23**, 8103–8107.
75 Z. W. Ma, W. He, T. Yong and S. Ramakrishna, *Tissue Eng.*, 2005, **11**, 1149–1158.
76 A. Thapa, D. C. Miller, T. J. Webster and K. M. Haberstroh, *Biomaterials*, 2003, **24**, 2915–2926.
77 Y. B. Zhu, M. F. Leong, W. F. Ong, M. B. Chan-Park and K. S. Chian, *Biomaterials*, 2007, **28**, 861–868.
78 H. S. Choi, H. Suh, J. H. Lee, S. N. Park, S. H. Shin, Y. H. Kim, S. M. Chung, H. K. Kim, J. Y. Lim and H. S. Kim, *Eur. Arch. Otorhinolaryngol.*, 2008, **265**, 809–816.
79 U. Edlund, M. Kallrot and A. C. Albertsson, *J. Am. Chem. Soc.*, 2005, **127**, 8865–8871.
80 R. A. Quirk, M. C. Davies, S. J. B. Tendler and K. M. Shakesheff, *Macromolecules*, 2000, **33**, 258–260.
81 A. Lucke, J. Tessmar, E. Schnell, G. Schmeer and A. Gopferich, *Biomaterials*, 2000, **21**, 2361–2370.
82 Y. Zhu, C. Gao, T. He, X. Liu and J. Shen, *Biomacromolecules*, 2003, **4**, 446–452.
83 H. F. Jia, G. Y. Zhu, B. Vugrinovich, W. Kataphinan, D. H. Reneker and P. Wang, *Biotechnol. Prog.*, 2002, **18**, 1027–1032.
84 B. K. Brandley and R. L. Schnaar, *Anal. Biochem.*, 1988, **172**, 270–278.
85 R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 7461–7472.
86 D. L. Hern and J. A. Hubbell, *J. Biomed. Mater. Res.*, 1998, **39**, 266–276.
87 S. P. Massia and J. A. Hubbell, *J. Cell Biol.*, 1991, **114**, 1089–1100.
88 J. Salber, S. Grater, M. Harwardt, M. Hofmann, D. Klee, J. Dujic, J. H. Huang, J. D. Ding, S. Kippenberger, A. Bernd, J. Groll, J. P. Spatz and M. Moller, *Small*, 2007, **3**, 1023–1031.
89 L. M. Weber, K. N. Hayda, K. Haskins and K. S. Anseth, *Biomaterials*, 2007, **28**, 3004–3011.
90 M. Heyde, M. Moens, L. Van Vaeck, K. M. Shakesheff, M. C. Davies and E. H. Schacht, *Biomacromolecules*, 2007, **8**, 1436–1445.
91 D. S. W. Benoit and K. S. Anseth, *Biomaterials*, 2005, **26**, 5209–5220.
92 D. R. Schmidt and W. J. Kao, *J. Biomed. Mater. Res., Part A*, 2007, **83**, 617–625.
93 A. I. Teixeira, P. F. Nealey and C. J. Murphy, *J. Biomed. Mater. Res., Part A*, 2004, **71**, 369–376.
94 E. K. Yim, R. M. Reano, S. W. Pang, A. F. Yee, C. S. Chen and K. W. Leong, *Biomaterials*, 2005, **26**, 5405–5413.
95 M. M. Stevens and J. H. George, *Science*, 2005, **310**, 1135–1138.
96 G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells and L. G. Griffith, *J. Cell Sci.*, 2000, **113**, 1677–1686.
97 W. A. Comisar, N. H. Kazmers, D. J. Mooney and J. J. Linderman, *Biomaterials*, 2007, **28**, 4409–4417.
98 D. J. Irvine, A. M. Mayes and L. G. Griffith, *Biomacromolecules*, 2001, **2**, 85–94.
99 D. J. Irvine, A. V. Ruzette, A. M. Mayes and L. G. Griffith, *Biomacromolecules*, 2001, **2**, 545–556.
100 E. Cukierman, R. Pankov, D. R. Stevens and K. M. Yamada, *Science*, 2001, **294**, 1708–1712.
101 G. Liu, L. Qiao and A. Guo, *Macromolecules*, 1996, **29**, 5508–5510.
102 R. J. Mart, R. D. Osborne, M. M. Stevens and R. V. Ulijn, *Soft Matter*, 2006, **2**, 822–835.
103 G. Jell, R. Verdejo, L. Safinia, M. Shaffer, M. Stevens and A. Bismarck, *J. Mater. Chem.*, 2008, **18**, 1865–1872.
104 D. C. Miller, A. Thapa, K. M. Haberstroh and T. J. Webster, *Biomaterials*, 2004, **25**, 53–61.
105 M. J. Lysaght, A. Jaklenec and E. Deweerd, *Tissue Eng. A*, 2008, **14**, 305–315.