Review

New materials for tissue engineering: towards greater control over the biological response

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One goal of tissue engineering is to replace lost or compromised tissue function, and an approach to this is to control the interplay between materials (scaffolds), cells and growth factors to create environments that promote the regeneration of functional tissues and organs. An increased understanding of the chemical signals that direct cell differentiation, migration and proliferation, advances in scaffold design and peptide engineering that allow this signaling to be recapitulated and the development of new materials, such as DNA-based and stimuli-sensitive polymers, have recently given engineers enhanced control over the chemical properties of a material and cell fate. Additionally, the immune system, which is often overlooked, has been shown to play a beneficial role in tissue repair, and future endeavors in material design will potentially expand to include immunomodulation.

Introduction

Cells within our bodies respond to various stimuli presented by the extracellular matrix (ECM), a main regulatory and structural component of tissues that is composed of fibrous proteins, proteoglycans and glycoproteins, and mimicking these cues with synthetic analogs of the ECM (scaffolds) has been a major research topic in the tissue-engineering field [1]. The first tissue-engineering scaffolds were typically designed to exhibit some minimum level of mechanical support and to regulate diffusion of nutrients and waste products between the new tissue and surrounding host tissues [2]. Classic biomaterials (e.g. long-lasting metals, ceramics and polymer composites) have successfully been used to replace the mechanical function of tissues such as teeth, hips, knees, heart valves and intervertebral discs, but their limited ability to modulate the repair and regeneration of host tissues has limited their use for tissue-engineering applications and has motivated the development of new materials [3]. Currently, materials are being designed with the aim to regulate tissue regeneration by different mechanisms, such as controlling specific cell-binding interactions, releasing growth factors, degrading at a controlled rate and responding to environmental cues [1]. The resulting increased functionality of tissue-engineering materials arises from extensive work to covalently modify existing materials and efforts to synthesize new materials.

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In addition to a material's chemical properties, tissue engineers have recognized that structural aspects can have profound influences on cell function, fate and tissue formation [4–6]. This has motivated the development of new fabrication processes that can form biomaterials into specific three-dimensional (3D) micro- and nanostructures in which pore structure, surface area to volume ratio, texture and surface topography are manipulated to control cell shape, alignment and organization [2,7,8]. Furthermore, the mechanical properties (e.g. stiffness) of a material and methods of modulating the distribution of mechanical cues transferred to cells by the material have been of extreme interest in tissue engineering because they have been shown to strongly affect cell phenotype, function and patterning [9–11]. However, the roles of architecture and mechanical signals in tissue morphogenesis are beyond the scope of this article and are reviewed elsewhere [5,9,12].

This review will provide an overview of the types of materials (more specifically, polymeric materials) that can modulate tissue regeneration via direct and indirect chemical control over transplanted or host cells. The first section will briefly summarize some of the traditional materials used in tissue engineering, including their functionalization with bioactive molecules and degradation, and the second half will discuss alternative materials that are being developed. Despite extensive research efforts that have investigated how materials directly affect the cells of a developing or healing tissue, there has been limited focus to date on how materials affect other host processes, such as inflammation and immune modulation, which are likely to be key factors in successful tissue regeneration. Although inflammation is often viewed as a negative event, it is a key component of processes such as angiogenesis [13] and tissue remodeling. Consequently, modulating the inflammatory response via material properties is potentially a powerful tool for driving tissue morphogenesis. Thus, the third section will discuss the role of inflammation in wound repair and regeneration and the potential of using materials to manipulate the host immune response to obtain a beneficial outcome for tissue-engineering applications. Finally, continuing challenges and future directions in material development will be discussed.

Traditional materials

Purified ECM components or decellularized ECMs derived from animals are logical scaffold choices in tissue

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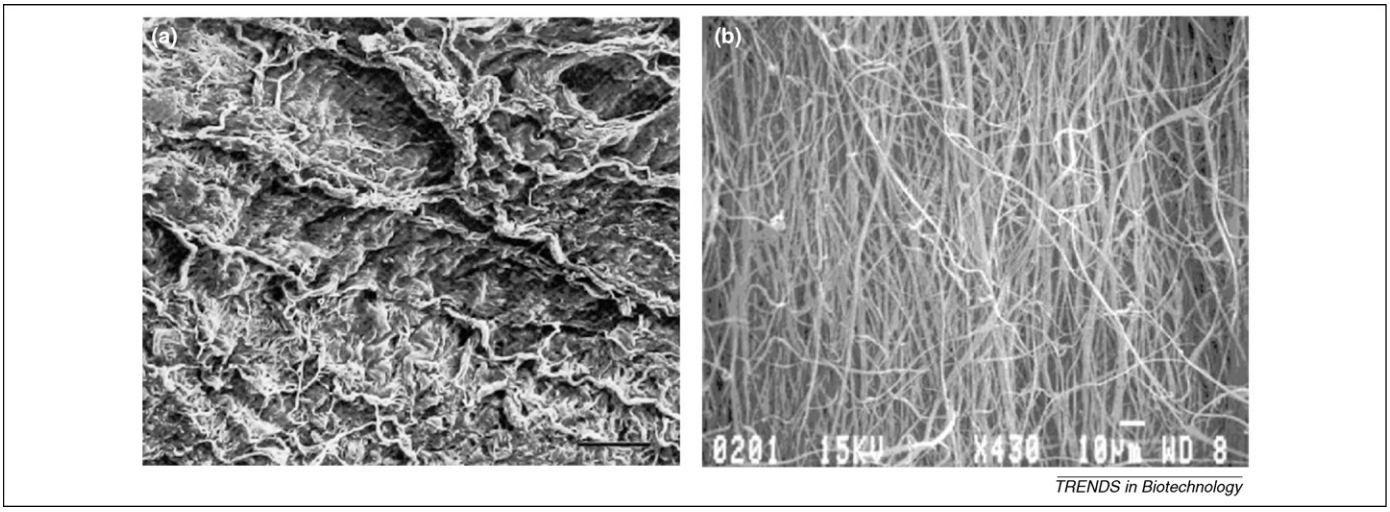

Figure 1. ECM-derived materials. **(a)** Scanning electron micrograph (SEM) of the mucosal surface of intact small intestine submucosa. Reprinted, with permission, from Ref. [14]. © Mary Ann Liebert, Inc. **(b)** SEM of collagen type I (derived from calf skin) that has been electrospun into fibers to create a well-defined, reproducible scaffold material. Reprinted, with permission, from Ref. [16]. © American Chemical Society. The scale bars represent 10 μm.

engineering because they retain relevant aspects of the complex structure and chemical composition of the ECM. Decellularized ECM (Figure 1a) [14] has already been successfully developed into commercial products for soft-tissue repair, as shown in Table 1 [15], but also has the potential for immunogenicity, disease transfer and wide variability, making it less reliable as a therapeutic device. By contrast, single purified ECM components [e.g. collagen, hyaluronic acid (HA) and fibrin] can be combined with other ECM components and processed to create more well-defined, standard materials that are potentially less immunogenic and have a similar structure to native ECM (Figure 1b) [16]. These materials have been successfully used as substrates for cell adhesion and tissue repair with promising results [17]. Other naturally derived materials that mimic the gel-forming nature of the ECM, such as alginate and chitosan, have also been commonly used in tissue engineering. Alginate and chitosan, which are glycans extracted from brown algae and the exoskeleton of shellfish, respectively, have gained popularity because of their biocompatibility, ease of processing and ability to encapsulate cells and bioactive molecules [18–20]. However, because of their weak mechanical properties, hydrogels might not be suited for applications that require a high elastic modulus. On the contrary, silkworm- and spider-silk fibers have been used for centuries in high-strength biomedical applications, particularly as sutures [21]. Because of their mechanical properties and ability for side-chain modification with growth and adhesion factors, they have been used, although not as widely, in applications such as bone and ligament repair [21].

A variety of biodegradable synthetic polymers, including poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(ethylene glycol) (PEG), polycaprolactones, polyorthoesters, polyanhydrides and polycarbonates have also been extensively characterized and widely used to fabricate tissue-engineering scaffolds [22]. Their ease of chemical synthesis on large scales, combined with the Federal Drug Administration (FDA) approval of several of these polymers has motivated the application of synthetic polymers in the tissue-engineering field. Additionally, the ability to co-polymerize, combine and control the molecular weight of many of these polymers has given tissue engineers the flexibility to tailor the mechanical and degradation properties of a material for specific applications, particularly those at tissue interfaces, which might require gradients of material properties. For example, to mimic the anterior cruciate ligament (ACL)–bone interface, a triphasic scaffold has been developed that consists of a soft-tissue phase formed from a highly degradable poly(D-L-lactide-co-glycolide) (PLGA) mesh fused to a fibrocartilage phase of more-slowly-degrading PLGA, which itself was fused to a stiffer bone phase made of a PLGA and bioactive glass composite [23]. The soft-tissue and bone phases were seeded with fibroblasts and osteoblasts, respectively, and over several days the scaffold

Table 1. Examples of extracellular matrix materials that have been developed into commercial products

Product	Species and tissue of origin	Device composition	Processing methods	Sterilization method
GraftJacket ® (Wright Medical Technology)	Human dermis	Single layer	Cryogenic, proprietary	None, regulated as a tissue transplant by the FDA
Restore ® (DePuy Orthopaedics)	Porcine small intestine submucosa	10 layers, supplied dehydrated	Peracetic acid, vacuum-dried, minimally processed	Electron-beam radiation
CuffPatch™ (Biomet Sports Medicine)	Porcine small intestine submucosa	8 layers, supplied hydrated	Vacuum-dried, chemically crosslinked (carbodiimide)	25-kGy gamma irradiation
TissueMend ® (TEI Biosciences)	Fetal bovine skin	Single layer	Proprietary	Ethylene oxide
Permacol ® (Tissue Science Laboratories)	Porcine dermis	Single layer, supplied hydrated	Chemically crosslinked (isocyanate)	Gamma irradiation

Adapted from Ref. [15].

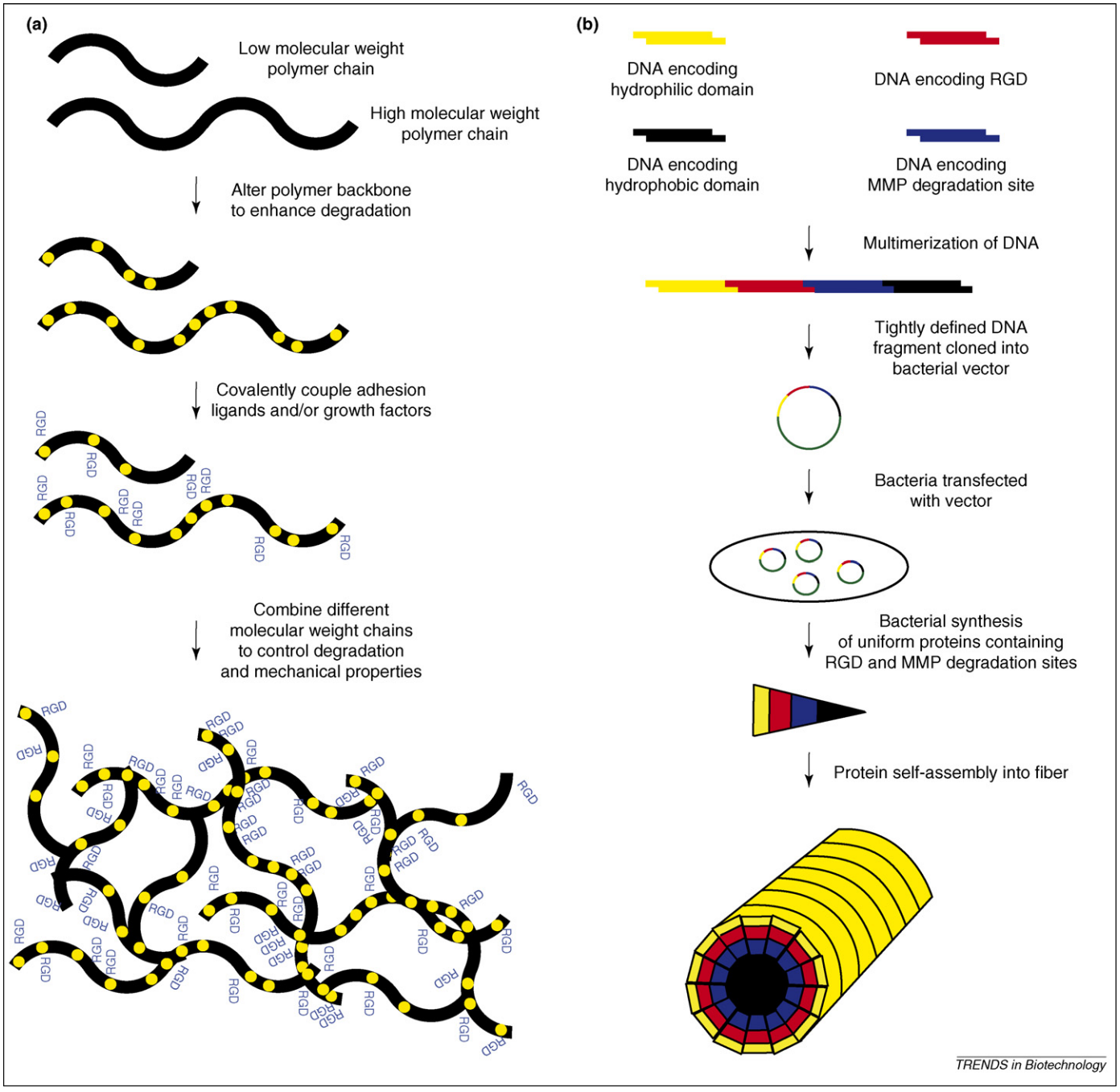

Figure 2. Representative examples of approaches to scaffold fabrication. **(a)** One typical approach to designing tissue-engineering scaffolds involves covalent modification of traditional polydisperse materials to control degradation and infer a specific mechanism of cell adhesion. In this example, the polymer backbone can be oxidized to facilitate degradation and subsequently modified with cell-adhesion ligands at random locations to support cell attachment. Chains of different molecular weight can then be crosslinked to form a scaffold with controlled mechanical and degradation properties. **(b)** Alternatively, synthetic proteins with a tightly defined structure (e.g. precise numbers and locations of adhesive and degradation domains) and uniform molecular weight can be synthesized. First, DNA fragments coding the desired amino acid sequences must be isolated and ligated or designed *de novo*. The new gene is then inserted into a DNA vector and transfected into a bacterial host that synthesizes the material. The synthetic protein can be used either directly or combined with other polymers.

supported cell growth while maintaining phase-specific matrix deposition [23]. Although synthetic materials provide tissue engineers with large flexibility in material design, they do not have an intrinsic mechanism for interacting with cells, and cell adhesion is typically mediated by non-specific cell adhesion [24]. This limits their use in applications that require defined control over cell–matrix interactions, but this can be achieved by functionalizing these matrices with bioactive molecules, as discussed below.

Material functionalization

Simple chemical modification can transform biologically inert materials into materials that can actively direct cell biology and expand the range of properties available from traditional materials (Figure 2a). Chemical modification of synthetic polymer materials with entire ECM molecules or relevant peptide or glycan fragments can be used to mediate specific mechanisms of cell adhesion, and the association of normally soluble cues (e.g. growth factors) with the synthetic ECM can provide a further level of control over

tissue morphogenesis [25]. Some of the most widely utilized peptide fragments are cell adhesion domains of ECM proteins, such as arginine-glycine-aspartic acid (RGD) (derived from fibronectin) and tyrosine-isoleucine-glycine-serine-arginine (YIGSR) (derived from laminin), which are frequently coupled with amide linkages to carboxylic-acid-containing polymers using carbodiimide chemistry [18]. It has long been appreciated that the specific peptides used to modify the material as well as its density are critical because they dictate which specific surface integrin receptor is used by the cell for adhesion, how many bonds are formed between the cell and substrate and the extent of subsequent intracellular signaling [26]. In addition, recent studies have shown that the nanoscale organization of proteins or peptides presented by the material might also affect many aspects of cell behavior, including proliferation, migration and differentiation [27,28], further demonstrating the importance of peptide presentation in dictating cell fate. For example, closely spaced nanopatterned islands of RGD ligands supported greater pre-osteoblast focal adhesion kinase phosphorylation and cell spreading, whereas more widely spaced RGD islands supported pre-osteoblast differentiation [29]. The mechanisms underlying these effects are currently not fully understood. However, the development of new tools that are able to quantify the interactions between cells and specific ligands, as well as the traction forces exerted on these bonds [30–33], are likely to be pivotal in elucidating these mechanisms and contributing to a more rational design of cell–material interactions in the future. New tools have demonstrated that the type and amount of adhesion ligand, as well as the mechanical properties of the substrate, are of equal importance in controlling cell behavior. For example, the use of a fluorescence resonance energy transfer-based technique revealed that the proliferation and differentiation of pre-osteoblasts on an RGD-modified matrix correlated to the magnitude of force that cells generated to cluster cell-adhesion ligands and that this was dependent on the mechanical stiffness of the adhesion substrate [30]. It is apparent that the fate of cells in growing tissues relies heavily on the adhesion ligands presented by the matrix, and the development of methods to functionalize materials with these molecules is central in recapitulating these matrix effects and supporting the growth of functional tissue.

Control of material degradation

Another critical factor that can control tissue morphogenesis is the degradation rate of the scaffold. Regulating the degradation rate of tissue-engineering materials can facilitate scaffold remodeling and replacement by resident and host cells, enable the infiltration of blood vessels and control the release of matrix-associated growth factors and morphogens to enhance tissue regeneration. A variety of strategies have been employed to regulate scaffold degradation, including the addition of side chains to sensitize or desensitize the polymer to hydrolysis [34], the chemical altering of main chains to generate controlled numbers of functional groups in the polymer backbone that are susceptible to hydrolysis (e.g. partially oxidizing alginate) [35] or the co-polymerization of macromers that have different degradation profiles [36]. The release of growth factors and morphogens that have been encapsulated in polymers to manipulate the fate of both transplanted and host cells is governed by diffusion through the polymer and polymer degradation [37]. For example, use of an alginate hydrogel to deliver vascular endothelial growth factor-A_{165} (VEGF-A_{165}) in a sustained manner enhanced blood vessel formation and tissue perfusion in a mouse ischemic hindlimb model more than using a bolus dose of VEGF [38]. The sustained release of VEGF was tuned by controlling the biodegradation of the hydrogel via a bimodal molecular weight distribution of alginate and by partially oxidizing the polymer chains [38]. Recently, materials have been further developed to enable the sequential release of factors that direct multiple steps of tissue formation [39,40]. Although scaffold degradation plays an important role in modulating tissue growth, it is important to keep in mind the biocompatibility of degradation products, which might elicit undesired inflammatory responses or induce toxicity in the body. Thus, the discovery and synthesis of biodegradable materials as well as methods to control material degradation are an important area of study in the field of tissue engineering.

Alternative materials

Traditional tissue-engineering materials demonstrate the power of materials to regulate tissue formation, but they are commonly composed of a mixture of chemical structures with high polydispersity and have only limited responsiveness to environmental cues. These limitations have motivated the exploitation of different classes of polymers and the development of recombinant-gene-expression approaches to create 'designer materials' with tightly defined physical, chemical and biological properties. Unlike traditional materials, many of which are either in clinical trials or in commercial products, these newer materials are typically at the stage of *in vitro* or preliminary small animal studies.

Peptide-based materials

The ability to custom-design proteins by taking advantage of nature's protein synthesis machinery allows material scientists to genetically engineer novel, well-defined and multifunctional materials [41]. Peptides and proteins self-assemble into distinct structures (e.g. β-sheets and α-helices) because of Van der Waals and ionic interactions at the molecular level. Depending on the amino acid sequence, the same set of amino acids can create a virtually unlimited range of protein materials with various structures. Protein-based materials can be derived by cloning sequences from organisms that naturally produce the protein or, for more controlled material properties, by engineering plasmids that code only the desired amino acid sequences (often consisting of repeated amino acid motifs) [42]. These approaches have been exploited to create tailor-made hydrogels, stimuli-sensitive polymers and materials with controlled biorecognition, crosslinking, degradation, structure and mechanical properties (Figure 2b) [41]. For example, modified human collagen proteins consisting of tandem repeats of the collagen II domain, which is associated with chondrocyte migration, were synthesized to support greater chondrocyte prolifer-

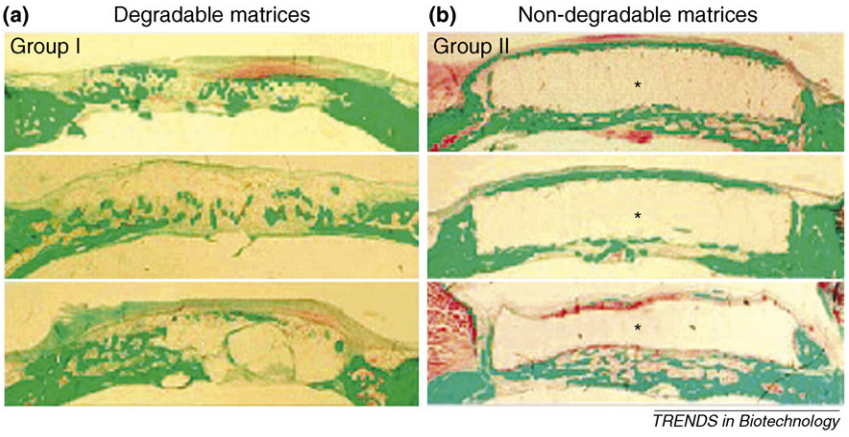

Figure 3. The importance of scaffold degradation in guiding tissue regeneration. Degradable peptide materials **(a)** and non-degradable peptide materials **(b)** encapsulating bone morphogenic protein (BMP) were placed in rat calvarial defects *in vivo*. Peptide materials containing degradation sites were resorbed and supported bone growth (stained in green) throughout the matrix, whereas non-degradable matrices remained intact and only supported bone growth at the bone–scaffold interface (hydrogel marked with *). This study demonstrates the potential of peptide-based materials and the importance of scaffold degradation in guiding tissue regeneration. Reprinted, with permission, from Ref. [49]. © American Chemical Society.

ation and ECM production than wild-type collagen II [43]. Spider silks were also genetically engineered to contain altered amino acid motifs that controlled the quantity of β-sheet structures in the resulting proteins and enhanced the mechanical properties of the silk [44]. Additionally, the self-assembly of various peptides into nanofibers has been utilized to form scaffolds for 3D cell culture and controlled cell differentiation [45], to create elastin-mimetic tri-block copolymers with predictable mechanical properties for soft-tissue-engineering applications [46] and to guide the pattern of hydroxyapatite nucleation and growth for hard-tissue applications [47]. To further increase the functionality available in synthetic peptides, unnatural amino acids containing reactive alkenes, alkynes or halogens can be incorporated to allow for chemical modifications after the material is synthesized [42]. This is achieved by conjugating unnatural amino acids to suppressor transfer RNAs (tRNAs) that bind to stop codons engineered into the DNA [48] or by substituting natural amino acids with close structural analogues [42]. The protein-based material could further encode specific proteolytic sites that facilitate material degradation as cells infiltrate and replace the scaffold. A striking example of this is the co-polymerization of PEG polymers with proteins encoding RGD peptides and plasmin and matrix metalloproteinase (MMP) degradation sites. The proteins were crosslinked via the PEG groups and contained biologically active motifs to control cell-binding interactions, cell-mediated degradation and subsequent bone morphogenic protein (BMP) release to heal critical-sized defects in a rat calvarial defect model [49]. Degradable matrices were completely resorbed over time and supported bone growth throughout the defect, but non-degradable matrices only supported bone formation at the surface of the scaffold, which remained intact (Figure 3) [49]. Clearly, the ability to synthesize different amino acid sequences to create tightly defined, custom materials that can meet the biological complexity required for tissue regeneration makes protein-based materials an attractive option for tissue-engineering scaffolds. However, producing materials via recombinant gene techniques is relatively complex and expensive, and the entire system must be redeveloped to enable even small changes in the protein (e.g. molecular weight or amino acid substitution).

DNA-based materials

Like peptide-based materials, DNA is increasingly being investigated as a biomaterial because one can control material properties by defining sequences of building blocks, in this case nucleotides [50]. Single-stranded DNA molecules with specific nucleotide sequences can self-assemble into predictable duplex conformations because of base pairing (Figure 4a) [51]. Additionally, the presence of specific cations during self-assembly has been shown to provide another level of structural control by manipulating electrostatic interactions that influence the conformation of DNA. For example, DNA cross motifs (Figure 4b) have been shown to assemble into two-dimensional (2D) crystal lattices in the presence of Mg^{2+} (Figure 4c), but assemble into nanofibers in the presence of Ca^{2+} (Figure 4d) [51]. The negative charge of DNA can also be utilized to attract Ca^{2+} ions and induce mineralization to create composite materials [51]. Furthermore, 3D DNA hydrogels could be used to encapsulate cells and proteins *in situ* by using efficient, ligase-mediated reactions that crosslink double-stranded DNA [52]. Lastly, in addition to being used as a bulk material, DNA could be a valuable crosslinking agent because it allows control over the crosslink length (typically up to 100 bases or 34 nm) [53] and the subsequent melting temperature [54], thus dictating the mechanical and rheological properties of the resulting material. These properties make DNA-based materials potential candidates for bone regeneration, as well as other applications that might benefit from DNA's ability to mineralize calcium ions, and as scaffolds for non-invasive tissue-engineering applications.

Electrically conductive materials

Electrically conductive materials are increasingly being investigated in tissue engineering because of their potential for generating electrical signals in muscle and neural

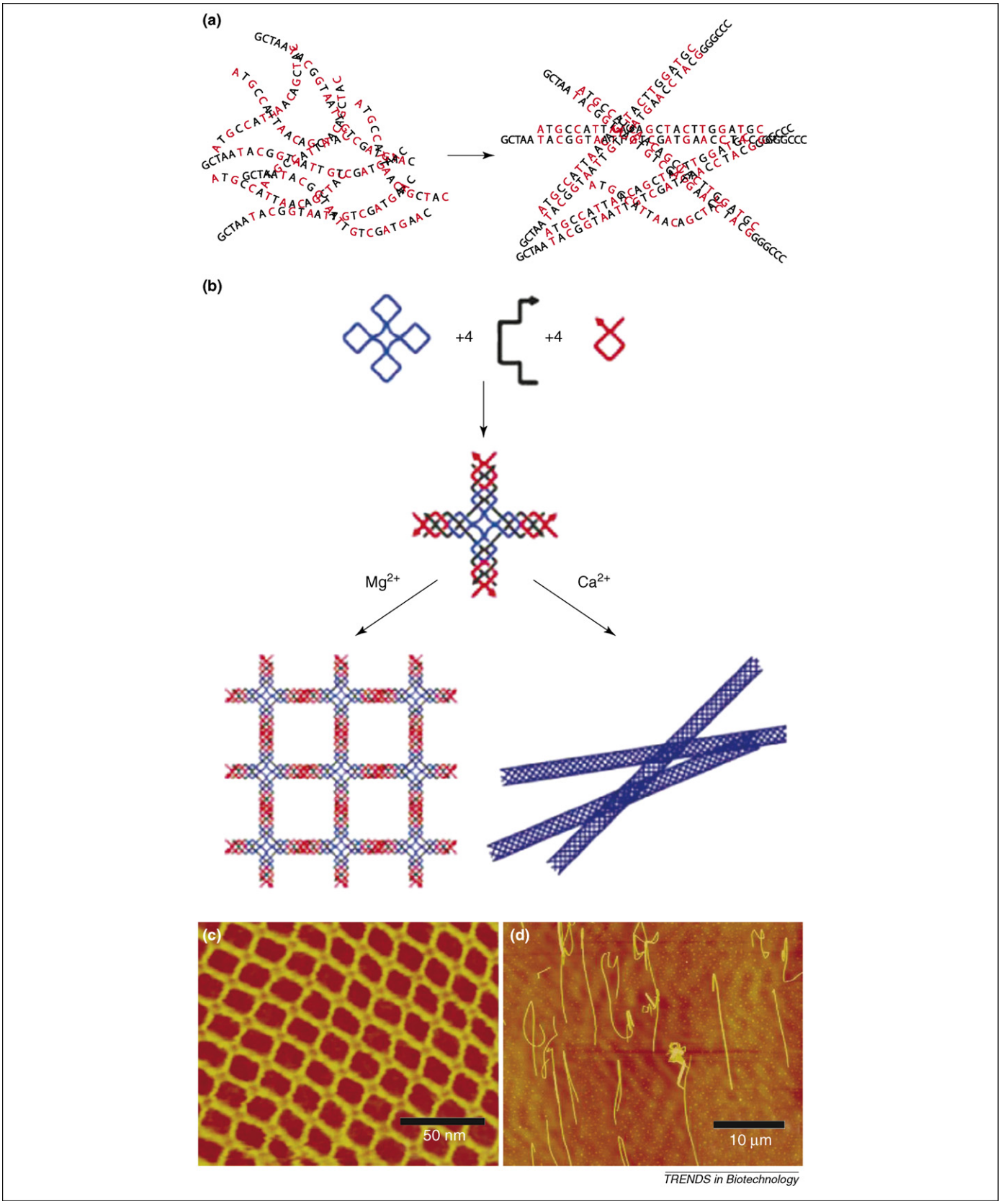

Figure 4. Self-assembly of DNA-based materials and the role of divalent cations in determining the subsequent structure. **(a)** DNA fragments will spontaneously self-assemble because of base pairing of the nucleotides adenine (A) with thymine (T) and guanine (G) with cytosine (C). **(b)** Complex structures can result from self-assembly of specific DNA cross motifs formed from single-stranded DNA. A large-scale ordering is dependent on the specific cations present in solution. The arrows on the black and red strands indicate the 3′ end. **(c)** Atomic force microscopy image of Mg^{2+}-dependent DNA self-assembly into regular 2D lattices. **(d)** Atomic force microscopy image of Ca^{2+}-induced nanofiber formation. (b), (c) and (d) reproduced, with permission, from Ref. [51].

tissues and their ability to contract and relax in response to applied voltages. Electroactive polymers (EAPs), which include conjugated polymers and dielectric elastomers, are capable of mechanical actuation induced by an external electric field, and consequently they have been studied for applications as artificial muscles and responsive prosthetics [55,56]. Conjugated polymers, such as polypyrrole-(PPy) and polyaniline-based actuators, rely on ion and solvent transport to induce mechanical displacement. They can function in bodily fluids and require low voltages (<1V) to induce large strains (>30%) [55], and their commercial applications have been successfully demonstrated as blood vessel connectors and microvalves for treating urinary incontinence [55]. In addition to replacing muscle function, EAPs might also be used to impart mechanical signals to adherent cells and manipulate their organization and differentiation [57]. The EAP's oxidation state has also been shown to directly control cell phenotype and behavior [58], and electrochemical triggering can control the release of bioactive molecules encapsulated within EAPs [59]. Additionally, conjugated polymers can be synthesized in degradable forms [55], which could allow growing tissues to replace the polymer over time.

EAPs have received particular interest in neural-tissue engineering, where electrical signals are crucial, and it was shown that they improved neurite outgrowth from injured neurons [60]. PPy, which is synthesized in an oxidized state, has been the most widely studied material in this field because it is biocompatible, inexpensive to synthesize and easily polymerized with negatively charged dopant ions and biomolecules to alter its surface characteristics [55,60]. For example, to stimulate the regeneration of axons, additional molecules such as nerve growth factor (NGF) can be incorporated onto the surface of PPy without significantly reducing its conductance, thus providing electrical and biological stimulation to neurons [61]. Overall, the properties of EAPs make them versatile materials for a potentially broad range of tissue-engineering applications.

Stimuli-sensitive polymers

Stimuli-sensitive polymers, which also include EAPs, are a general category of materials that respond to cues in their surroundings. Developing tissues are dynamic systems that need to respond to changes in the local or systemic environment (e.g. pH, hormones, mechanical cues), but polymers traditionally used in tissue engineering have limited sensitivity to external stimuli. This has spurred the development of responsive materials, termed smart materials. Smart materials can be designed to undergo structural, often reversible, changes in response to environmental factors (e.g. temperature, pH, electric field, solute concentrations, light) that would allow them to provide functionalities on-demand, such as phase transition, alteration in shape or release of encapsulated growth factors or cells [62,63]. One of the most widely studied smart materials is the hydrogel poly(N-isopropylacrylamide) (PNIPAAm) and its copolymers, which can swell and shrink in response to temperature change. A variety of PNIPAAm copolymers have been synthesized that exhibit striking properties, including autonomous chemomechanical oscillators that respond to ATP [64] or glucose [65]. Shape-memory polymers are also of considerable interest because these materials can be deformed, 'frozen' into a temporary shape and, with an appropriate trigger, returned to their initial shape driven by the force of preferred entropic and internal energy states [66]. Because of their ability to recover large deformations in response to heat, light or solvents, they have the potential to be used as stents or tissue-engineering scaffolds that could be introduced endoscopically into the body in a compact form and then, once in place, be triggered to re-assume their more complex, space-filling form (Figure 5) [66,67]. Finally, stimuli-sensitive polymers have been used for drug-delivery vehicles, including insulin release systems that have been developed to respond to high glucose levels [62] and growth-factor release systems that respond to the local mechanical environment [68]. This concept can be further extended to controlling the delivery of multiple bioactive

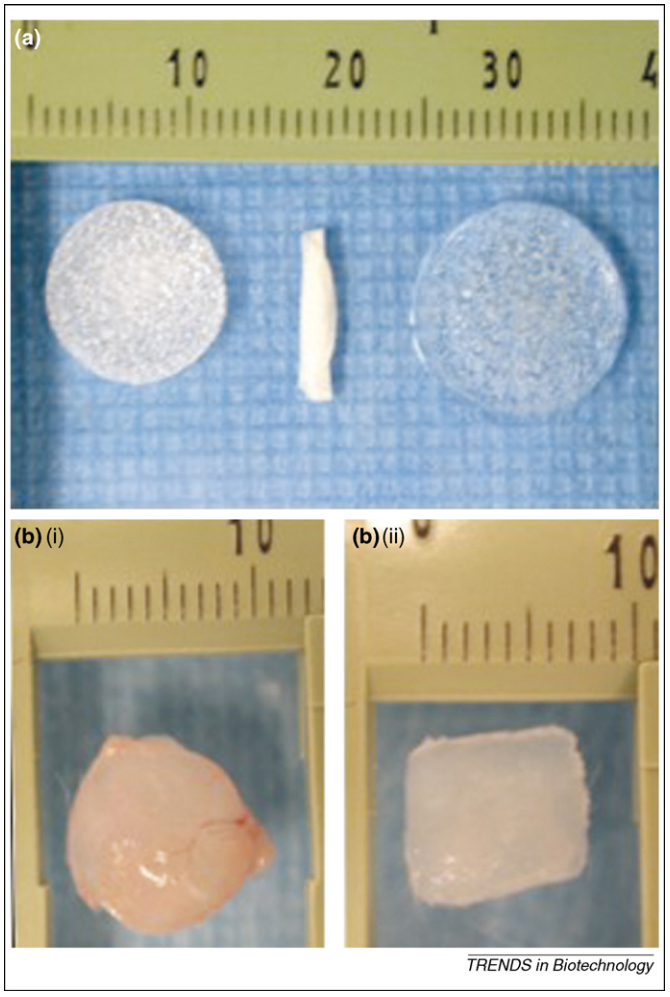

Figure 5. The use of shape-memory materials in tissue engineering. **(a)** Appropriate covalent cross-linking of alginate scaffolds with an original shape of a circular disc (left) allows the lyophilized scaffolds to be mechanically compressed (center); the ability of the material to then rapidly re-assume its original shape and size upon rehydration is demonstrated by the disc on the right. **(b)** Shape-memory alginate discs of circular and rectangular shape were compressed and introduced into mice with a catheter, then rehydrated *in vivo* with media containing bovine articular chondrocytes. After eight weeks the scaffolds were harvested, and they demonstrated the formation of cartilaginous tissues in the shape of the original scaffolds: a circular disc (i) and a rectangular disc (ii). These results demonstrate that tissue growth can be guided into desired geometries via the minimally invasive delivery of shape-memory materials. Reproduced, with permission, from Ref. [67]. © Lippincott Williams and Wilkins.

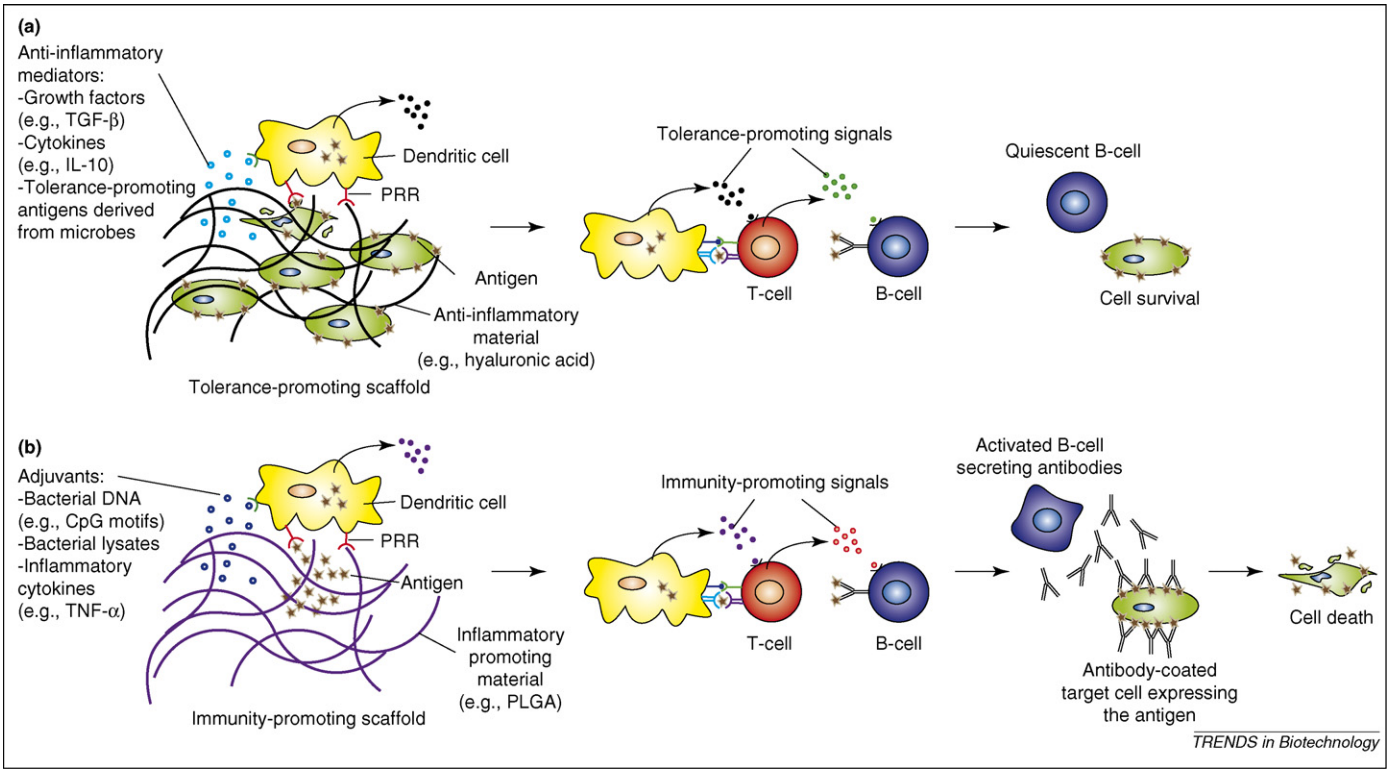

Figure 6. Potential of materials to control the immune response. **(a)** Materials can promote tolerance to specific antigens and cells by directly signaling antigen-presenting cells (APCs), such as dendritic cells (DCs), or by releasing growth factors or cytokines that promote tolerance. After antigen internalization, DCs can activate effector cells, such as T and B cells, to promote tolerant responses to the associated antigen. This approach could potentially be used to promote the survival of transplanted cells. Abbreviation: PRR, pattern recognition receptor. **(b)** Alternatively, materials might promote a destructive immune response by directly providing immunity-promoting signals or by releasing soluble factors. Activated APCs can then induce destructive immune responses, for example through B cells secreting antibodies specific for the antigen, which subsequently lead to the antigen's targeting and removal. This approach could be used to combat infectious diseases and cancer.

agents in response to environmental stimuli to optimize tissue morphogenesis. Although many of these materials are not biodegradable, their ability to respond to external stimuli make them valuable learning tools for tissue engineers. A challenge in the field will be to synthesize smart materials with controllable degradation properties and safe byproducts.

Materials for controlling inflammation and the immune response

To date, attention in tissue engineering has focused on modulating the fate of transplanted- and host-cell populations that directly participate in the rebuilding of tissues. The immune system has been generally regarded as a negative regulator of wound healing, but recent research suggests that immune responses could be actively modulated to drive regeneration. Clearly, a chronic foreign body response (FBR), which is characterized by inflammation and fibrosis, needs to be avoided because it has been shown to impede tissue regeneration. However, it has been recently suggested that an acute FBR might play a positive role in vascularization [13]. This result implies that modulation rather than avoidance of the immune response might enhance tissue remodeling. Furthermore, dendritic cells (DCs) and effector cells of the adaptive immune system have been shown to actively promote regeneration of the newt lens [69], and macrophages have been shown to secrete factors that promote regeneration of the rat optic nerve [70]. Antigen-presenting cells (APCs), such as DCs and macrophages, are continuously 'sampling' (binding and internalizing) their surroundings via pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and C-type lectin receptors (CTLRs) [71]. This suggests that cues from the local environment (e.g. materials) can direct the function of immune cells and might be able to initiate a therapeutic immune response (Figure 6). Notably, several materials have been shown to interact with APCs via PRRs, and a further understanding of these interactions might be useful for the design of improved materials for tissue-engineering applications. For example, alginate, which is composed of alternating mannuronic acid (M) and guluronic acid (G) residues, has been suggested to elicit higher levels of inflammation with an increasing M content via interactions between M residues and the TLR-4 and TLR-2 receptors on monocytes [72,73]. By controlling the M and G content in alginate materials, one might be able to modulate the inflammatory response to improve the healing and regeneration of tissues. HA is also an interesting material in regards of its immunomodulatory properties because it exhibits anti-inflammatory behavior and has also been associated with scarless wound healing in the fetus [74]; future research efforts might elucidate and exploit the underlying mechanism. Aside from naturally occurring materials (e.g. proteins and carbohydrates), which evolutionarily are more likely to be recognized by the innate immune system, man-made materials might also be able to interact with PRRs to alter the immune response. This has been suggested by data indicating that DCs increased expression of the pro-inflammatory cytokines tumor necrosis factor-α and interleukin-

6 when they were cultured on PLGA *in vitro* and that this effect might be mediated by TLRs [75]. PLGA's ability to enhance the immune response might make it a useful material in tissue-engineering and drug-delivery applications that are aimed at boosting the immune system, such as the delivery of antigens for vaccination purposes. This concept might be further extended to develop *in vivo*-based DC vaccines to reverse aberrant immune responses (e.g. allergy and autoimmunity) and to combat infectious diseases and cancer (Figure 6) [76–78].

Controlling other parameters in material design, such as ligand–receptor interactions, also has the potential to significantly affect the immune response. For example, it was shown that *in vitro*, T cells became fully activated when cultured on materials that supported T cell clustering of tethered ligands, but they could not be activated when ligand clustering was inhibited [79]. Another interesting approach is to mimic pathogens that are able to evade the host immune system through signaling of PRRs on the surface of APCs to avoid inflammatory responses that are detrimental to tissue regeneration. For example, helminth parasites, *Mycobacterium tuberculosis* and HIV-1 have been shown to interact with specific CTLRs to suppress inflammation in the host [80]. Interestingly, bacterial cellulose has been shown to integrate well within host tissue without inducing chronic inflammation when used as a scaffold for tissue repair, and this promising result demonstrates the potential of imitating or exploiting pathogens in scaffold design [81]. Additional studies have shown that sugars organized into repetitive arrays (such as those found on helminth parasites) illicit strong anti-inflammatory T-cell responses compared with single sugars [82]. This research opens up the challenges of elucidating the effects of ligand valency on APC receptor activation and deciphering the downstream signaling pathways. For example, PRR activation is shown to regulate nuclear factor-κB [83] and mitogen-activated-protein-kinase signaling cascades [84,85], and understanding how these pathways are affected by the type and strength of PRR activation will help to improve the functionalization of materials to control the immune response.

In addition to utilizing traditional materials or discovering new materials that can chemically signal the immune system, the alternative materials described above could be utilized for immunomodulation. For example, protein and DNA engineering could be used to encode PRR ligands within a material to promote an inflammatory or anti-inflammatory response depending on the application. In the case of protein-based materials, engineering enzymatic sites into the material to modulate material degradation could control the duration of antigen presentation. Additionally, smart materials could be used to moderate the release of cytokines and growth factors to stimulate the immune system for improved tissue healing or enhanced immunity. These tissue-engineering materials are potentially powerful tools that could be applied in a broad range of applications.

Conclusions and future perspectives

Materials for tissue engineering have significantly progressed over the years, from being initially viewed as biologically inert structural supports to platforms capable of providing signals to cells and tissues and orchestrating regeneration. Genetic engineering, advances in chemical synthesis and exploitation of peptide and oligonucleotide self-assembly have allowed tissue engineers to use a bottom-up approach in combining multiple properties to tailor materials for specific applications. Current trends suggest that biomaterial development will continue to create more life-like multi-functional materials that are able to simultaneously provide complex biological signals (chemical, structural and mechanical), replace mechanical function and respond to environmental stimuli. A continuing challenge for this approach will be to find ways of exploiting these sophisticated tools without unduly complicating large-scale production for clinical research.

The role of the immune response is an important factor that is often overlooked when designing and choosing materials for tissue-engineering applications. As the immune mechanisms that influence wound repair are further elucidated, tissue engineers might be provided with another parameter they can potentially control using material design.

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