EXPERT OPINION

Delivering bioactive molecules as instructive cues to engineered tissues

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Introduction: Growth factors and other bioactive molecules play a crucial role in the creation of functional engineered tissues from dissociated cells.
Areas covered: This review discusses the delivery of bioactive molecules – particularly growth factors – to affect cellular function in the context of tissue engineering. We discuss the primary biological themes that are addressed by delivering bioactives, the types of molecules that are to be delivered, the major materials used in producing scaffolds and/or drug delivery systems, and the principal drug delivery strategies.
Expert opinion: Drug delivery systems have allowed the sustained release of bioactive molecules to engineered tissues, with marked effects on tissue function. Sophisticated drug delivery techniques will allow precise recapitulation of developmental milestones by providing temporally distinct patterns of release of multiple bioactives. High-resolution patterning techniques will allow tissue constructs to be designed with precisely defined areas where bioactives can act. New biological discoveries, just as the development of small molecules with potent effects on cell differentiation, will likely have a marked impact on the field.

Keywords: biomaterials, drug delivery, growth factors, tissue engineering

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1. Introduction

Tissue engineering has emerged as a promising interdisciplinary approach to addressing the need for organ replacement and regeneration. It combines principles from engineering, materials and life sciences to create biological substitutes that restore physiological functions [1]. Cells are usually seeded in 3D porous biomaterials that serve as scaffolds providing mechanical support to the growing tissue and defining its boundaries. The cells then secrete their own extracellular matrix (ECM) proteins while the scaffold degrades. During recent years, the approach of seeding cells within porous biomaterials has been used to engineer tissues or organs for nearly every part of the body, including the heart, liver, blood vessels, brain and eyes [2-6]. The potential of engineered tissues has already been demonstrated by the successful restoration of the function of defective complicated organs such as the heart and liver in animal models [7-9].

In living creatures, cells grow on an ECM composed of an intricate meshwork of fibers of proteins such as collagen and elastin. Proteins such as laminin and fibronectin provide specific binding sites that interact with integrins on the cell surface and promote cell adhesion. The ECM stores and releases protein factors that are critical for survival and differentiation of embedded cells. Release usually occurs in response to local stimuli such as a change in pH or enzyme activity [10,11]. To mimic this environment it is, therefore, important not only to engineer synthetic

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Article highlights.

- Bioactive molecules incorporated inside tissue engineering scaffolds as instructive cues regulate many aspects of cell phenotype and behavior, and can accelerate differentiation and organization into functional tissues.
- While many bioactive molecules of use in tissue engineering are biomacromolecules, there is increasing awareness of the role of small molecules to modulate developmental pathways.
- Biomaterials and drug delivery systems can affect cells in the scaffold through their inherent material properties or through spatially and/or temporally controlled drug release.
- The drug delivery system has to conserve the drug's bioactivity during the desired time frame of release.
- There is a broad array of biomaterials and drug delivery systems to consider. Selection requires consideration of specific goals and of the compound(s) to be delivered. In the past, most drug delivery systems provided continuous release of single compounds. Increasingly, multiple compounds are being released in specified temporal sequences. More advanced drug delivery systems will allow fine control over the deployment of bioactive molecules *in vivo* (e.g., triggered release) or respond to changes in their biological environment.
- Better understanding of pathways governing cell differentiation and tissue organization will define new molecular targets and identify new pharmacological lead compounds to be used in drug delivery systems.
- Drug delivery technologies imported from other scientific fields (e.g., nanoelectronics) will allow even more sophisticated control over drug delivery in engineered tissues.

This box summarizes key points contained in the article.

and natural biomaterials functioning as a supporting matrix, but also to provide an environment with the desired biochemical and physical cues [12,13].

This article will focus on the delivery of bioactive molecules, particularly growth factors (GFs), to affect engineered tissues. We will describe the main physiological goals of delivering bioactives, along with illustrative examples of the delivery of compounds to achieve those goals. The major emphasis will be on the spectrum of materials and techniques with which drug delivery techniques can be employed in tissue-engineered constructs.

2. Bioactive molecules for triggering physiological processes in tissue engineering

In vivo, the microenvironment surrounding a cell is rich in biomolecular signals from other cells and the ECM. These can be autocrine signals that affect the same cell that released the molecule, paracrine signals that affect cells close by and endocrine signals that affect remote targets [14]. The ECM and bioactive molecules are interrelated. The ECM itself releases soluble ligands and presents specific motifs (e.g., integrins) that affect cell adhesion, proliferation and other functions. The cross talk between cells and ECM is affected by the physical properties of the ECM [15].

The principal bioactive molecules types used in regenerative medicine include GFs, cytokines, hormones and transcription factors; they can either act immediately on cells or be stored within the ECM for future use. Their individual functions are described in the following section and an overview is given in **Figure 1**.

2.1 Maintaining cell viability

During the process of *ex vivo* tissue engineering, cells are isolated from their natural environment and cultured in conditions that may jeopardize tissue assembly [16]. Their placement into a biomaterial scaffold without their usual cell–cell or cell–matrix interactions may result in cell death and the formation of a thin nonfunctional tissue. It is, therefore, important to provide external cues that maintain cell viability. The normal cell microenvironment meets this need by continuously releasing survival factors such as erythropoietin, which has protective effects in a variety of tissues, including the brain, kidney and heart by preventing apoptosis [17]. Other biomolecules such as transforming growth factor β (TGF-β), hepatocyte growth factor (HGF) and insulin-like growth factor I (IGF-I) have also been shown to activate cell survival signaling pathways independently or in synergism with other molecules [18-21].

In tissue engineering, GFs can either be added to the culture medium [22] or be incorporated into systems that can slowly deliver the GFs to the cultured cells [7]. For example, an IGF-1 controlled-release system was incorporated into engineered cardiac tissues to improve cell viability in thick 3D constructs, where diffusion of oxygen and nutrients is limited and biological wastes tend to accumulate. GF release was able to activate the cardioprotective signaling pathways Akt and ERK1/2 and to maintain cell viability [7].

Similar approaches have been used to maintain the viability of stem cells, which have enormous therapeutic potential. For example, mesenchymal stem cells (MSCs) have the ability to repair bone and cartilage [23]. Theoretically, these cells could be isolated from the bone marrow of a patient, expanded *in vitro* and seeded on 3D matrices. They could subsequently be reintroduced into a patient in undifferentiated, progenitor or terminally differentiated states to restore tissue function. Maintaining their viability during the cultivation period is critical. To address this issue, researchers introduced GFs into the 3D matrix. For example, epidermal growth factor (EGF) covalently immobilized on a biomaterial induced greater cell spreading and survival in MSCs than did free EGF [24]. MSC adhesion and viability were also enhanced in 3D hydrogel composites capable of controlled release of IGF-1 [25].

2.2 Determining cell fate

A major challenge in tissue engineering is to develop new 3D scaffolds that can induce cell proliferation and instruct cells to

differentiate to a desired lineage at the right time. Stem cells are defined by their ability to self-renew and differentiate into multiple cell types. A stem cell's fate is determined by a very specific local 3D microenvironment called the stem cell niche. This may consist of growth-modulating soluble factors, the ECM and interactions among stem cells themselves or with host cells [26]. Soluble factors such as GFs and cytokines are very important for regulating the character of cells in that niche [27], as are their associated signaling pathways. As one example among many, recent studies have demonstrated that fibroblast growth factors (FGFs) can play a crucial role in the proliferation of hematopoietic stem cells, MSCs, neural stem cells and embryonic stem cells [28-31]. FGF-2 and -4 can promote the proliferation of bone marrow mesenchymal stem cells (MSCs) by activation of the ERK1/2 and PI3K-Akt signaling pathways. This cascade mediates the induction of the transcription factor, c-Jun [32]. Other studies have suggested that signaling by Wnt, a group of secreted lipid-modified signaling proteins, plays a vital role in the regulation of MSC proliferation [33]. The proliferative effect is achieved by the upregulation of cyclin D1 and c-Myc, both of which drive cell cycle progression to promote growth [34]. Please see the following reviews for further information on signaling [35], how it propagates [36] and chemical means of manipulating it [37].

The literature on the determination of cell fate by GFs and signaling pathways is extensive, complex and beyond the scope of this review (please see [38] for further elaboration on this subject). It is a particularly important subject matter for the design of compounds that can modulate cell differentiation [39] (see for example, Sec. 2.6).

2.3 Creating blood vessel networks

The fact that oxygen can only diffuse about 100 μm into tissues places a limitation on the potential thickness of engineered tissues [40]. Vascularization, the process of new blood vessel formation from endothelial cells and their progenitors [41,42], is, therefore, critical to ensure a supply of nutrients and oxygen, particularly to thick tissues *in vivo*. A variety of GFs have proven to be useful for the stimulation of neovascularization. Among them, vascular endothelial growth factor (VEGF) has been the best studied [43]. It is involved in branching and remodeling the vasculature during embryonic development and creating new vessels after injury or in bypassing blocked vessels, even in adults [43]. Other key signaling molecules include transforming growth factor-β 1 (TGF-β1) and platelet-derived growth factor-BB (PDGF-BB), which contribute either independently or in synergy with VEGF to the formation of functional blood vessels [44]. The incorporation of these factors within engineered tissues has shown great promise in recent years.

VEGF immobilized onto porous 3D collagen scaffolds has been shown to enhance the vascularization process by promoting the invasion and assembly of vascular endothelial cells [45]. Similarly, release of bFGF from porous composite scaffolds accelerated matrix vascularization after implantation in the rat peritoneum [46]. Efforts have been made to promote the assembly of a mature blood vessel network by mimicking the temporal order of the GF cascade active in angiogenesis. That process involves the initiation of the process by VEGF, followed by vessel stabilization by PDGF-BB-mediated smooth muscle cell and pericyte recruitment and, finally, vessel remodeling with ECM induced by TGFβ-1 [47,48]. Those three GFs have been released sequentially from a single alginate-based tissue engineering scaffold [44].

Adenoviral vectors have also been used to deliver angiogenic GFs in engineered tissues. Cultures of stem cells transfected with adenovirus encoding the cDNA of VEGF combined with endothelial cells in a 3D scaffold enhanced the formation of a vascular network [49].

2.4 Cell recruitment

An important advantage of forming an engineered tissue with autologous cells is that it is less likely to be attacked by the host immune system, which could impact implant function or destroy it. While it is possible to harvest autologous cells from the host and then culture them on a desired scaffold, that has the disadvantage of requiring two procedures. An alternative approach is to recruit the desired host cells to the scaffold after implantation, by virtue of the fact that cells are able to sense remote cues and migrate toward the signaling site. For example, the migration of endothelial progenitor cells (EPCs) from the bone marrow to ischemic tissues is regulated by a complex interplay between cytokines/chemokines, proteinases and cell adhesion molecules [50]. Stromal cell-derived factor 1 (SDF-1) is the most important chemokine involved in stem cell and EPC migration. It has been shown that its concentration in the blood affects the degree of cell mobilization [51]. Ischemia triggers secretion of SDF-1, leading to the recruitment of progenitor cells to the site of injury [52]. Based on this biological function, SDF-1 has been used to recruit endothelial progenitor cells to an engineered cardiac patch transplanted on the omentum. The subsequent release of VEGF promoted the organization of homing pericytes and smooth muscle cells to functional blood vessels [7]. Similarly, elastin/agarose composite scaffolds that release bFGF caused significantly higher myofibroblast infiltration compared with those without GFs [53]. Hepatocyte growth factor (HGF), a pleiotropic cytokine of mesenchymal origin, is known to be a chemoattractant of mesenchymal and neural stem cells. Hyaluronic acid–gelatin gels releasing HGF for more than 3 weeks promoted the migration of human bone marrow stem cells into scaffolds [54].

2.5 Anti-inflammatory activity

Two major problems in the field of tissue engineering are the adverse foreign body response and implant-related infections. Transplantation of an engineered tissue can trigger

immunological attack by a variety of processes including inflammatory processes that are part of the normal wound-healing response. These processes can destroy the implant and/or lead to the formation of fibrotic tissue around it. This problem has been addressed by continuous release of anti-inflammatory agents into the borders of the engineered tissue [55].

While scaffolds can be made with pores small enough to keep inflammatory cells and mediators out, there is a pore size below which nutrient influx will be attenuated and efflux of wastes and perhaps a desired cell product (e.g., insulin) is impeded. Unfortunately, relatively low-molecular-weight pro-inflammatory cytokines can diffuse in if the pores are large enough to allow those processes. A recent strategy to protect encapsulated islet cells from diffusible, pro-inflammatory cytokines (IL-1 β, TNF-α and INF-γ) involved the covalent modification of a hydrogel backbone with a peptide that blocked the interaction between graft cells and inflammatory mediators (an inhibitory peptide for the cell surface IL-1 receptor). Cell viability and function were improved in the peptide-modified scaffolds and resulted in prolonged glucose-stimulated insulin release [56]. Other approaches to preventing inflammation include delivery of anti-inflammatory drugs such as ibuprofen [57] or dexamethasone [58] from the scaffold.

2.6. Small-molecule compounds

The majority of the literature regarding bioactive molecules as applied to tissue engineering relates to GFs and other macromolecules, or fragments thereof. However, a number of small molecules are known to affect a wide range of cellular activities, including production of ECM components (e.g., by ascorbate), differentiation and reprogramming of fate [59] [37,39]. The list of such compounds is growing rapidly. They have been discovered by a range of processes including empirical observation, rational design and high-throughput screening (e.g., for parameters such as modulation of differentiation signaling pathways, morphogenesis, etc.). Thus, small molecules have been synthesized that induce angiogenesis *in vitro* and *in vivo* [60]. High-throughput screening approaches have been used to screen libraries of small molecules that affect cell behavior [61]. Such approaches have identified small molecules that can promote or inhibit differentiation of osteogenic differentiation of hMSCs. By such means, it was found that ascorbate – in addition to its effects on ECM production [62,63] –directs embryonic stem cells toward cardiomyogenesis [64]. Factors have been discovered that enhance neurogenesis [65,66]. Assays have been developed to identify small molecules useful in adipogenesis from hMSCs [67]. Pluripotent stem cells – both human embryonic stem cells and human-induced pluripotent stem cells – have been made to form neural crest cells by modulating the activity of developmental signaling pathways, using small-molecule compounds [68]. Such molecules should be amenable to the same drug delivery techniques described for macromolecules. For example, small-molecule incorporation into hydrogel scaffolds affected hMSC protein and gene expression, morphology and differentiation [69]; small-molecule motifs were identified that encouraged differentiation down specific pathways.

3. Delivery systems in engineered tissues

In engineered tissue constructs, drug delivery systems are an important tool to provide biochemical and physical cues by releasing or presenting bioactive molecules in the microenvironment, aiming to guide cell function [70]. Such systems can be designed to simulate the physiological presentation of bioactive ligands such as RGD [71] or bone morphogenic protein-2 (BMP-2) [72] or to release single or multiple bioactive molecules such as VEGF and PDGF [73,74].

Drugs can elute directly from the scaffold itself, to which they are attached by covalent conjugation, electrostatic complexation or other processes [75-77]. Alternatively, they can be engineered separately and added to the scaffold matrix [78-81]. Critical parameters include i) the loading capacity of the scaffold, ii) the distribution pattern of the bioactive substance within the scaffold, iii) binding affinity and release kinetics and iv) stability and biological activity of the biomolecules [82]. Apart from continuous controlled-release systems, precise spatiotemporal control of drug release can be obtained by regulating platform composition, shape and architecture or by using pulsatile or triggerable systems [83].

Moreover, scaffolds can be modified with molecules intended to affect specific cell populations or cellular locations: one example is juxtacrine signaling of scaffold-conjugated BMP-2 on osteoprogenitor cells [84]. It can be envisaged that subcellular targeting could be achieved using innovative strategies: recently gold nanowires have been used to deliver TNF-α to specific subcellular locations using electrophoretic and dielectrophoretic forces [85].

3.1 Commonly used materials for delivery in engineered tissues

3.1.1 Synthetic polymers

Both synthetic and biologically derived materials have been extensively explored for use in drug delivery systems for tissue engineering. Synthetic polymers are ideally non-immunogenic, nontoxic and bio-resorbable. Among these degradable materials, the α-hydroxy acids, lactic and glycolic acid (co-) polymers (**Figure 2A, 2B and 2C**) are the most widely used synthetic polyesters. The size of the drug delivery vehicle, the molecular weight, the crystallinity, the monomer composition of the polymer and the implantation site govern the degradation rate of these materials; consequently, drug release can last from days to months [86,87]. These materials have acidic degradation products, which may result in a local pH decrease in close proximity to cells or GFs [88]. A potential challenge in the use of these hydrophobic polymers for encapsulation of proteins is the use of

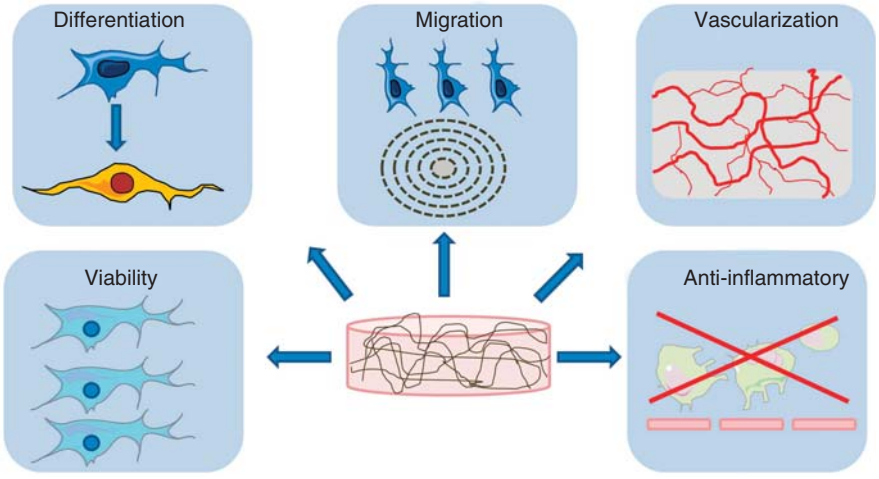


Figure 1. Ideally, a scaffold for tissue engineering should maintain cell viability, support differentiation, cell migration and vascularization. It should have some anti-inflammatory properties to prevent damage through the host's immune system.

organic solvents such as dichloromethane or acetone, which can compromise the encapsulated proteins [89]. Furthermore, the encapsulation of hydrophilic molecules can be difficult. Some of these problems can be tackled by using proper protein stabilization and/or encapsulation techniques (e.g., incorporating protein particles or using the double emulsion technique, see 3.2.2) [90]. Although the polymer itself is hydrophobic, aqueous domains or 'pores' are created on exposure to water, controlling the transport of dissolved drug through interconnected channels to the outside [91]. Degradation occurs simultaneously throughout the matrix (bulk degradation) and results in sudden loss of mechanical strength.

PEG has been copolymerized with lactic and glycolic acid (co-) polymers, to obtain more hydrophilic polymers (**Figure 2D**). Polymers with various monomer compositions and water solubilities have been synthesized yielding materials with distinct properties (crystallinity, water-solubility, biodegradability); they can be classified according to their block structure (for example into di-, tri- or multiblock copolymers). They have been used to fabricate various drug delivery vehicles such as micelles and nano- or microparticles: For example, microspheres were made from PEG–PLGA in a one-to-one monomer ratio; they degraded slowly and released VEGF over 28 days at therapeutic levels [92].

With the polyfumarates (**Figure 2E**), degradation can be fine-tuned by the proper choice of polymer molecular weight and cross-linking density [93]. This can be advantageous for applications such as bone tissue engineering, where sudden loss of mechanical strength is undesirable. Cross-linked poly (propylene fumarate) and similar materials have been used for GF delivery and as scaffolds or implant materials, due to their injectability, biodegradability and biocompatibility *in situ* [94].

Polyanhydrides (**Figure 2F**) degrade mainly by surface erosion through hydrolytic cleavage of labile anhydride linkages. Erosion occurs layer by layer, maintaining the shape of the polymer, and zero-order release kinetics are often observed [95]. In porous microspheres made of poly (anhydride-co-imide)-based polymers, overall microsphere erosion was slow and governed by the solubility of the released monomeric building block. Protein release occurred during the slow dissolution of the individual hydrophobic monomers sticking together [96].

Poly(caprolactone) (**Figure 2G**) is used in many tissue engineering application ranging from porous scaffolds in bone tissue engineering [97] to antibiotic-releasing meshes for prevention of postoperative infections [98] and as a material for vascular stents [99]. It is a semicrystalline biodegradable polyester with a low melting point and glass transition temperature. At room temperature it is a rubbery material and can be used as a monolithic, biodegradable material for diffusion-controlled drug delivery.

Poly(ortho ester)s can be classified into four polymer families (**Figure 2H**, **2I**, **2J**, **2K**) [100]. Autocatalyzed hydrolytic degradation by incorporated acids or released acidic monomers can be used to enable pH-triggered drug delivery using acid-labile ortho esters [101]. For example, poly (ortho ester) microspheres were engineered to achieve rapid release of DNA vaccines at the low pH of phagosomes, to generate an optimal immune response and to successfully suppress the growth of tumor cells [102]. Cleavage of hydrolytically labile ortho ester linkages is limited to the exposed surface, rendering the polymer very stable under physiological conditions and amendable to use as an injectable or solid scaffold for zero-order drug release [103]. For example, poly(ortho ester)s were used to deliver tetracycline over 10 – 14 days to inflamed periodontal pockets [104].

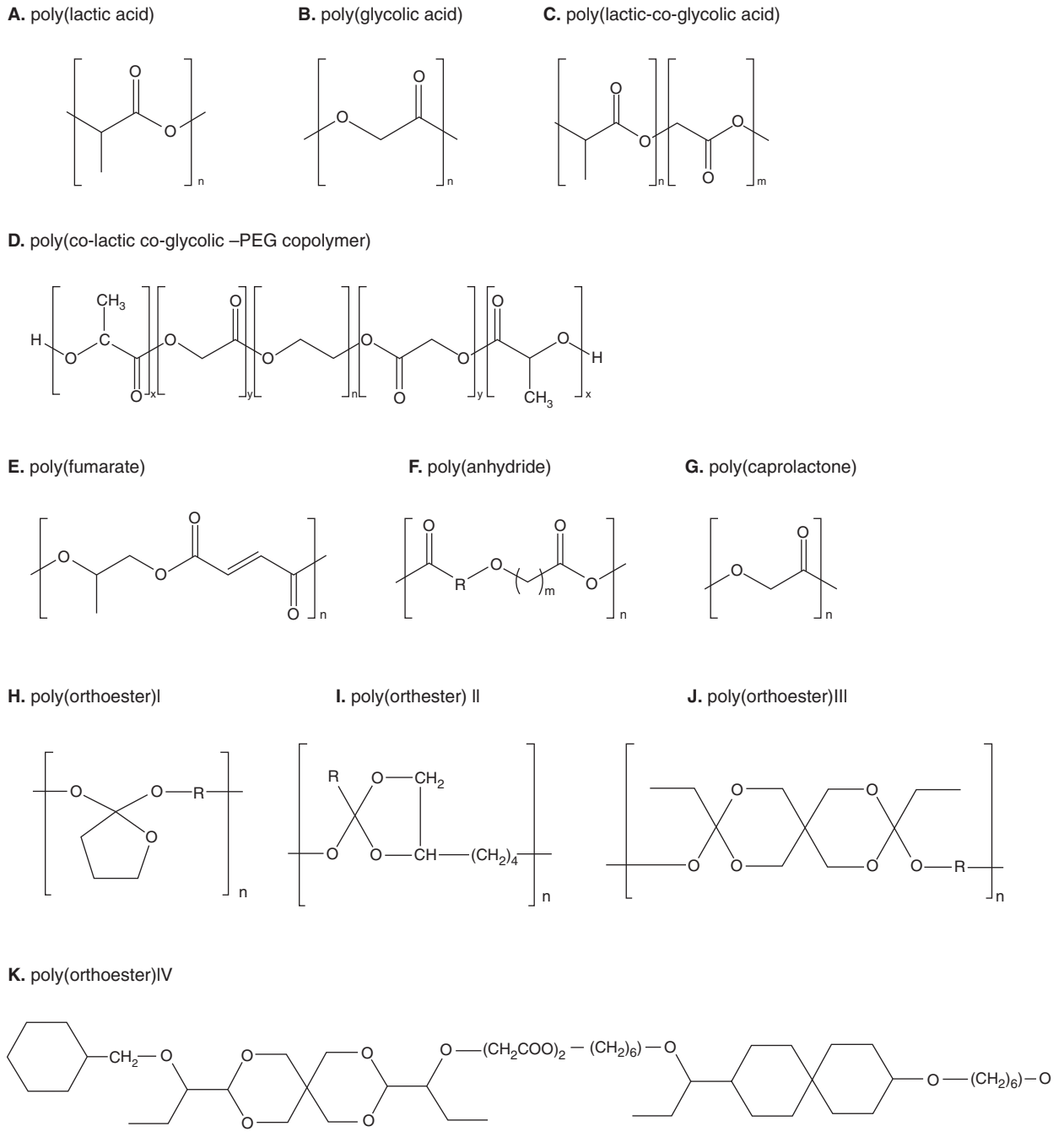
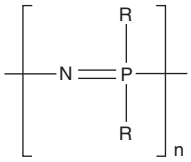


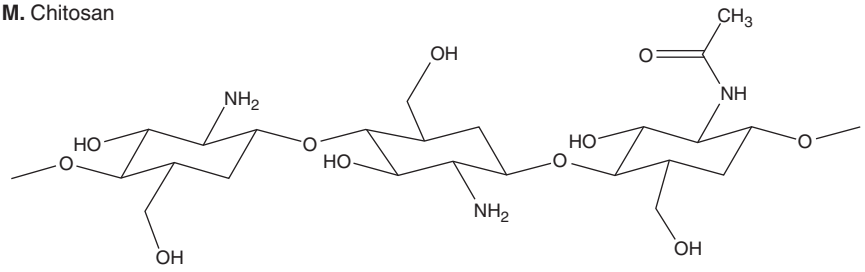
Figure 2. Chemical structure of some synthetic (2a – 2l) and naturally derived polymers (2m – 2o): 2a) poly(lactic acid) polymer, 2b) poly(glycolic) polymer, 2c) poly(lactic-co-glycolic acid) polymer, 2d) PLGA–PEG–PLGA triblock co-polymer, 2e) poly(propylene fumarate) as an example of a polyfumarate, 2f) poly(anhydrides), 2g) poly(caprolactone) polymer, 2h – 2k) poly-(ortho ester) polymers 1 – 4, 2l) poly(phosphazenes), 2m) chitosan, 2n) alginate, 2o) hyaluronic acid.

Poly(phosphazenes) (**Figure 2L**) are a class of inorganic polymers with a backbone consisting of alternating nitrogen and phosphorous atoms linked as a repeating (-RR'P = N-) unit. They form permeable matrices and are biodegrade to harmless products [105]. Properties of poly(phosphazenes) can be fine-tuned by altering the nature of the side groups (mostly substituted with amines of low pKa or activated alcohols [106]. Most of the poly(phosphazenes) are hydrolytically stable. They degrade by bulk and surface erosion, with rates governed by bond lability, water permeability of the polymeric matrix, solubility of the degradation products and environmental factors such as pH and temperature [106].

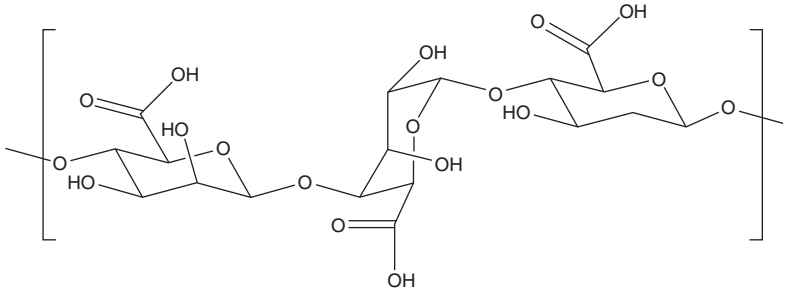
L. poly(phosphazenes)



M. Chitosan



N. Alginate



O. Hyaluronic acid

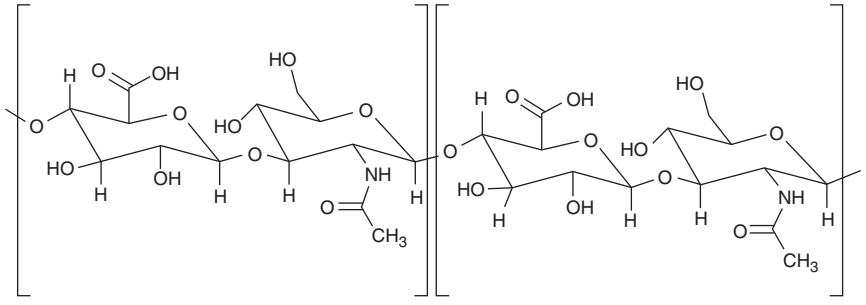


Figure 2. Chemical structure of some synthetic (2a – 2l) and naturally derived polymers (2m – 2o): 2a) poly(lactic acid) polymer, 2b) poly(glycolic) polymer, 2c) poly(lactic-co-glycolic acid) polymer, 2d) PLGA–PEG–PLGA triblock co-polymer, 2e) poly(propylene fumarate) as an example of a polyfumarate, 2f) poly(anhydrides), 2g) poly(caprolactone) polymer, 2h – 2k) poly-(ortho ester) polymers 1 – 4, 2l) poly(phosphazenes), 2m) chitosan, 2n) alginate, 2o) hyaluronic acid (continued).

3.1.2 Naturally derived polymers

Synthetic materials have a limited ability to provide structures for cell adhesion. This can be improved by adsorption or covalent attachment of functional biomolecules on their surfaces or by blending with natural polymers [107]. Alternatively, scaffolds and delivery systems can be based on natural biopolymers, which incorporate many of the cues essential for cell growth and tissue formation. Several protein- and sugar-based polymers have been used successfully as scaffolds and drug delivery systems (reviewed by [12,108,109]).

Although natural polymers have advantages in providing biological recognition cues for cells (receptor-binding ligands, incorporated protease cleavage sites), there are concerns regarding immunogenicity, purity and pathogen transmission. However, batch to batch variation of proteins can be overcome by recombinant technologies, which allow production of monodisperse and well-defined polymers.

3.1.2.1 *Protein-based polymers*

Collagen is a major protein component of the ECM and it is widely used in tissue engineering. Several collagen-based scaffolds are on the market, especially for skin replacement [110]. It has high mechanical strength, low antigenicity, a slow degradation rate and tissue reaction to it is generally benign. It consists of fibrils formed by three polypeptide chains consisting of Gly–X–Pro or Gly–X–OHPro amino acid repeats, twined around one another to form triple helices [111]. Acid or alkaline digestion of collagen produces gelatins with a range of net charges and isoelectric points. This allows flexibility in enabling polyion complexation of a gelatin carrier with either positively or negatively charged biomolecules [112]. For example, sustained delivery of bFGFs was achieved for at least 2 weeks by ionic complexation into gelatin microspheres [113].

Silk fibroin is a protein commonly produced by silkworms and it is composed of fibroin H-chain, fibroin L-chain and fibrohexamerin at a molar rate of 6:6:1 [114]. Silk is a mechanical robust biomaterial that is lightweight, strong and elastic. Silk fibroin is used as a scaffold for drug and cell delivery in bone, cartilage and vascular engineering and in wound-dressing applications [115]. A silk scaffold has been used to establish a 3D cartilage cell culture system [116].

Fibrin is a protein matrix produced by polymerization of fibrinogen after cleavage by the serine protease thrombin and further cross-linking by the transglutaminase activity of factor XIIIa. It can be obtained from autologous or a single allogeneic source using the CryoSeal$^{(R)}$ system or alternatively from cryoprecipitated pooled blood plasma. It has attracted interest in tissue engineering because it can be rapidly invaded by cells and remodeled by cell-associated proteolytic activity (e.g., plasmin and matrix metalloproteinases). The molecular properties of fibrin-based matrices such as their fibrillar structure and their amenability to covalent modifications with adhesion molecules have been used to influence the angiogenic behavior of human umbilical vein endothelial cells (HUVECs) *in vitro*, leading to angiogenesis [117].

Elastin-like natural and recombinant proteins [118] have attracted interest for use in tissue engineering due to their self-assembly properties and their high elasticity, which allows tissues to resume their original shape after stretching or contraction. Elastin-like proteins are artificial polypeptides, derived from Val–Pro–Gly–Xaa–Gly (VPGXG) pentapeptide repeats found in human tropoelastin, that reversibly coacervate above a critical temperature [119]. Composite scaffolds consisting of collagen and elastin-like proteins have been developed for soft tissue engineering applications [120]. Elastin is made by linking the soluble precursor tropoelastin in a reaction catalyzed by lysyl oxidase, to make an insoluble, durable cross-linked array.

3.1.2.2 *Polysaccharide-based polymers*

A second major class of natural polymers is based on polysaccharides, built from simple sugar monomers. These are linked by glycosidic bonds between hydroxyl groups, yielding either linear or branched polymers. Differences in the monosaccharide composition, chain shapes and molecular weight dictate their physical properties including solubility, gelation and surface properties. Chitosan, alginate and hyaluronic acid are examples of polysaccharides used in tissue engineering and drug delivery applications.

Chitosan (**Figure 2M**) is a cationic polymer comprising copolymers of ß(14) glucosamine and *N*-acetyl-D-glucosamine. It is derived from chitin and has a molecular weight between 10 and 1000 kDa. The polymer has hydroxyl or amino groups available for chemical modification or cross-linking. The polymer behaves as a weak poly-base due to the large quantities of amino groups in its chain, which allows pH-sensitive swelling behavior upon protonation of amine groups [121]. Besides applications in tissue engineering, chitosan hydrogels or particle formulations have been proposed as tissue adhesive drug carriers for the delivery of chemotherapeutics or other small-molecule drugs. For example, an injectable hydrogel formulation containing paclitaxel was developed for local chemotherapy applications [122].

Alginates (**Figure 2N**) are polysaccharide block copolymers composed of regions of sequential ß-D-mannuronic acid monomers (M-blocks), regions of α-L-guluronic acid (G-blocks) and regions of interspersed M and G units [12]. The length of the M- and G-blocks and sequential distribution along the polymer chain varies depending on the source of the alginate. Alginates undergo reversible gelation in aqueous solution under mild conditions through interaction with divalent cations such as Ca^{2+}. The calcium ions bind cooperatively between the G-blocks of adjacent alginate chains creating ionic interchain bridges. This gentle gelation procedure preserves cell viability or drug bioactivity, and has led to these polymers being used as cell transplantation vehicles [123]. For example, they have been used to transplant relatively fragile pancreatic islets for type I diabetes treatment [124]. Injectable alginate-based materials have also been used for the sequential delivery of IGF-1 and HGF after myocardial infarction. Electrostatic complexation of those GFs to sulfated alginates also protected them against proteolytic degradation [125].

Hyaluronic acid (**Figure 2O**) is a non-sulfated glycosaminoglycan. It is of interest for tissue engineering, since it is a major macromolecular component of the intercellular matrix of most connective tissues. It is a linear polysaccharide that consists of alternating disaccharide units of α-1,4-D-glucuronic acid and ß-1,3-*N*-acetyl-D-glucosamine, linked by ß(1→3) bonds. Hyaluronic acid has useful

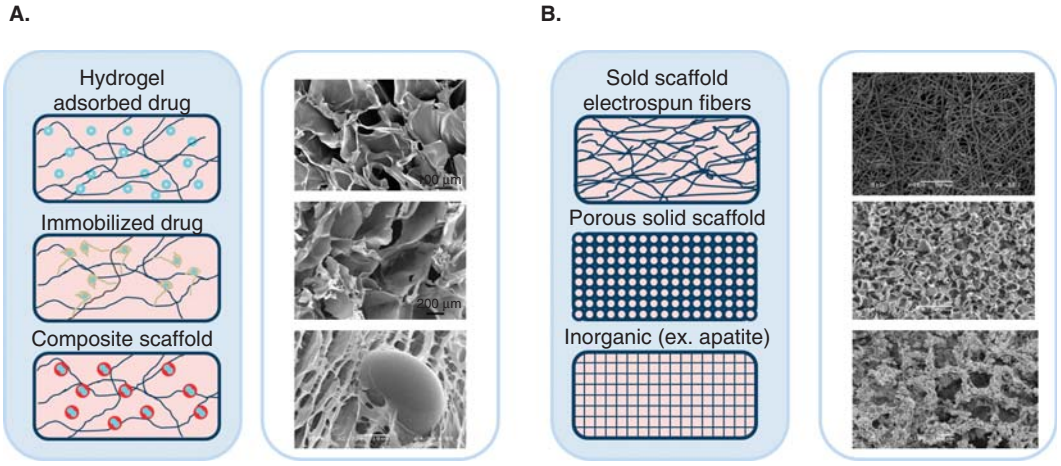


Figure 3. A. Scaffolds can be based on hydrogels containing the drugs free or immobilized via non-covalent (e.g., electrostatic complexation) or covalent interactions. In composite scaffolds, the hydrogel-based matrix contains a second drug delivery systems (particles or fibers). **B.** Solid scaffolds can be based on electrospun fibers or porous materials obtained by particle leaching. Furthermore, scaffolds can also be made from porous inorganic materials such as hydroxy apatite or composite materials.

viscoelastic properties and has physiological roles in lubrification and shock absorption. Hyaluronic acid is used in preclinical investigations as a drug and cell carrier for cartilage, bone or vascular engineering, and FDA-approved hyaluronic acid formulations are used clinically for the treatment of osteoarthritis [126]. Interactions of hyaluronic acid with specific receptors (CD44 and CD168) have been shown to be effective in maintaining stem cells in a viable and undifferentiated state, which is important for stem cell propagation [127].

3.2 Continuous-release systems

3.2.1 Controlled release from the scaffold matrix

Hydrogel matrices are physically or chemically cross-linked water-soluble polymers, which swell to form a gel after exposure to water. Cross-linking of the polymer chains can be mediated through hydrophobic/electrostatic interactions or through divalent ions or chemical (covalent) cross-linkers [128]. The polymer content is usually below 10% and water represents the main component of the hydrogel [129]. This composition allows good diffusivity of oxygen and nutrients for cells growing in the scaffold. Therapeutic agents can be encapsulated within the tortuous pores of the biomaterial. Release of a drug occurs by diffusion through the porous, hydrophilic scaffold and depends on the hydrogel mesh size and the hydrodynamic radius of the drug molecule. Considerable burst release is often seen. Increasing the cross-linking density by using more cross-linking agent [130], incorporating additional reactive groups [131] into the polymer chains or decreasing the molecular weight of the monomer can slow drug release [132]. Hydrogel degradation or dissipation can also affect release kinetics.

Hydrophobic polymers can either be cast as scaffolds – into which drugs can be incorporated directly – or can be contained within hydrogels as a variety of drug delivery systems.

3.2.1.1 Electrostatic interactions

Drug cargos can be reversibly adsorbed to scaffolds by electrostatic interactions, as occurs between the negatively charged acidic gelatin and the positively charged TGF-β1 [133]. Polysaccharide-derived hydrogels can have interactions with proteins that mimic protein–polysaccharide interactions in the ECM [134]. For example, heparin has a dense negative charge due to its acidic sulfate and carboxylic groups, which allows multivalent binding to a range of GFs such as EGF, VEGF, IGF, bFGF, IL-6 and others [135]. The electrostatic complex provides protection of the GF against degradation and slows release over time. Both natural and synthetic polymers have been modified (physically or chemically) with heparin and used to deliver heparin-binding GFs. For example, bFGF was complexed electrostatically with heparinoids (low-molecular-weight heparins), then combined with lactose-modified chitosan. After subcutaneous injection, the resulting hydrogel degraded in about 20 days and induced neovascularization and fibrous tissue formation [136]. Similarly, nerve growth factor (NGF) has been delivered in fibrin-based matrices containing immobilized heparin-binding peptide, which in turn immobilized heparin. These matrices contained an excess of heparin-binding sites that controlled the diffusion-based release of β-NGF and enhanced neurite extension by up to 100% relative to unmodified fibrin [137]. Synthetic molecules have been designed to have the same properties without having the heterogeneity and potential impurities of heparin. For

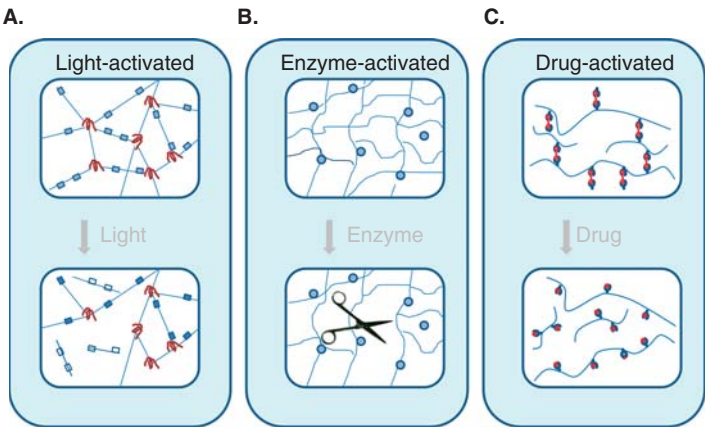


Figure 4. Stimulus-responsive drug delivery for tissue engineering. Examples include **A)** light-, **B)** enzyme-, and **C)** drug-induced breakdown of the hydrogel matrix. **A)** The photodegradable *o*-nitrobenzylether was acrylated to form a photodegradable cross-linker and photo-releasable tether, which readily polymerized to light-degradable materials [191]. **B)** Protease-degradable peptide sequences are integrated into a non-degradable PEG hydrogel to mimic the degradable extracellular matrix [183]. **C)** Addition of the antibiotic novobiocin leads to the dissociation of coumermycin–gyrase-B di-complexes and dissolution of the hydrogel [178].

example, the sulfated tyrosine sequence $SY(SO_3)DY(SO_3)G$ has a much higher affinity to VEGF than heparin [138].

The uronic acids in alginate or hyaluronic acid have been sulfated, to reproduce – and exceed – the high affinity of heparin-binding proteins to heparin/heparan sulfate [135,139]. Sequential release of VEGF, PDGF and TGF-β1 from an alginate-sulfate/alginate scaffold was achieved based on differential affinity for the three GFs, as discussed in Section 2.3 [44].

Self-assembling membranes consisting of hyaluronic acid and peptide amphiphiles with a heparin-binding domain have been used for sustained delivery of bFGF-2 and VEGF. First, heparin was bound non-covalently to the peptide sequence on the membrane surface. Second, the GF was bound to the heparin via heparin-binding domains. By this strategy, bFGF-2 and VEGF were incorporated within the membrane structure prior to self-assembly and then released over 14 days. These bioactive membranes enhanced angiogenesis relative to controls, in the chick chorioallantoic membrane (CAM) assay [140]. These non-covalent immobilization strategies tend to preserve bioactivity since they do not require modification of the GF itself, while prolonging drug release.

3.2.1.2 *Covalent bonding*

Covalent immobilization of bioactive compounds to functional groups on polymeric scaffolds by chemical conjugation can increase loading, extend their presence on the surface, enhance stability, reduce the amount of GF required and present them in an orientation with improved bioactivity [141]. For example, covalent conjugation increased attachment of BMP-2 to glass surfaces compared with simple adsorption [142]. Similarly, covalent binding of TGF-β2 to fibrillar collagen via difunctional PEG [143] led to a prolongation of its pharmacological effect (cell proliferation) *in vitro* and *in vivo*. The 2- and 3D distribution of proteins immobilized within a scaffold can be controlled by the availability of functional groups on the scaffold. For example, high spatial resolution 3D patterning of stem cell differentiation factors can be achieved in a hydrogel by attachment to covalently bound ligands [144].

Variant forms of VEGF have been covalently immobilized within fibrin gels with peptide sequences sensitive to plasmin or other proteases. The resulting gel achieved slow release of VEGF. Furthermore, the immobilized fibrin-conjugated VEGF variant remained an active mitogen. The incorporation of increasing amounts of a VEGF121 into the scaffold resulted in a dose-dependent enhancement of endothelial cell growth [145].

Surfaces can also be covalently functionalized with GFs by photo- [146] or microfluidic patterning [147] providing gradients of signals for cell adhesion and migration. For example, human epidermal keratinocytes cultured on EGF gradients immobilized on polystyrene plates by ultraviolet light preferentially migrated in the direction of higher EGF concentrations. Moreover, the cells exhibited unidirectional migration speeds and ranges that were more than fivefold greater than those observed on control surfaces [146]. These examples show that covalent modification of scaffolds can be a very useful tool to achieve continuous exposure to bioactive molecules.

3.2.2 *Controlled release from particles within the scaffold*

Bioactive molecules can also be encapsulated into microparticles contained within the matrix. This strategy is appealing, since bioactive molecules can be protected from chemical or enzymatic (proteolytic) degradation [148]. This approach may

also be simpler in some respects than, for example, covalent modification of scaffold and/or bioactives.

The rate of drug release from particles can be controlled by many factors such as particle size, the surface area-to-volume ratio and the composition of matter [149]. Particle size can also influence cellular uptake, tissue reaction and clearance from the injection site [150]. Microparticles can either be incorporated into an external matrix such as silk [151] or calcium phosphate cement [152], or be fused to build a porous scaffold [153].

Particles based on hydrophobic polymers such as PLA or PLGA have generally been formed by emulsion-based methods. If a hydrophilic agent is to be encapsulated, a double emulsion system is employed, as follows. Typically, an initial water–in–oil emulsion is generated containing the GF in the water phase and the polymer in the organic phase. A second emulsion (water–oil–water) is then formed by dispersion of the first emulsion in an aqueous phase [154]. Subsequently, the emulsion is stirred (perhaps at reduced pressure) to remove the organic solvent and form the microspheres, or a second solvent is used for the same purpose. The degradation rate and drug release of such particles are governed by the molecular weight, copolymer ratio, concentration, crystallinity, hydrophilicity and cross-link density of the polymer chains, as well as particles size, drug content, morphology and other parameters.

Alternatively, microparticles can be formed by cross-linking hydrophilic materials such as alginate or chitosan [155]. Alginate particles can be obtained by extrusion of a polymer solution into a solution of divalent cations (typically calcium) that diffuse inward rapidly forming a Ca-polysaccharide microgel [156]. Compared with hydrophobic particles, drug release from these particles is relatively rapid. For example, alginate microparticles containing VEGF and monocyte chemotactic protein-1 (MCP-1) integrated into a hydrogel matrix showed sustained GF release over a period of approximately 75 h and resulted in increased vessel formation when used to implant endothelial cells subcutaneously [157]. In a (hydrophobic) counterexample, a PLGA-based microparticulate delivery system for VEGF within a dextran scaffold released the bioactive drug for 10 days. The released GF enhanced vascular differentiation of human embryonic stem cells to a greater degree than extrinsic exposure to the same dose of GF [158]. Much longer release periods have been described from polymeric microspheres [159]. The reader is referred to a review for further details on this topic [160].

In general, nanometer-sized particulate delivery systems provide larger surface area-to-volume ratios and faster drug release compared with larger microparticles. Nanoparticulate delivery systems used in tissue engineering are often polymer-based (nanospheres, nanocapsules, nanotubes, nanowires, dendrimers, micelles) or lipid-based (liposomes, solid or liquid lipid nanoparticles) [161]. Nano-sized liposomes containing bFGF have promoted rapid collagen generation, dermal cell proliferation and wound regeneration [162]. Liposomes have also been used to deliver the Wnt3a protein to bone [163]; Wnt proteins are a group of secreted lipid-modified signaling proteins, which among other functions are involved in wound-healing processes [164]. Liposomal Wnt3a accelerated bone formation in peri-implant tissues and thus accelerated implant osseointegration [165]. Liposomes have also been used to enhance cell transplantation: liposomal thrombomodulin – an endothelial anticoagulant protein with anti-inflammatory properties – resulted in better glucose control over a 30-day period after transplantation into diabetic mice [166].

3.2.3 *Controlled release from integrated fibers*

The incorporated drug delivery system can be shaped to mimic the fibrous nano-topography of the cellular environment by techniques such as electrospinning. Nanofibers can incorporate bioactive molecules such as antibacterial agents [167] or GFs [168]. Micro- or nanofibers can be obtained by varying fabrication parameters such as polymer concentration, solvent system or other process parameters. Fiber alignment may also be biologically important: an aligned fiber structure has been shown to enhance skin cell migration during wound healing, compared with randomly oriented nanofibers [169]; fiber alignment also enhanced neurite outgrowth. Neurite outgrowth on aligned fibers was further enhanced by immobilized biochemical factors [169].

To fine-tune mechanical properties of electrospun fibers and to better mimic the physiological ECM, polymer blends can be used or nanoparticulate structures such as gold or hydroxyapatite nanoparticles can be incorporated [170,171]. The latter have been incorporated into silk nanofibers containing BMP-2, a protein that accelerates ossification. Human bone marrow-derived MSCs grown on silk nanofibers containing BMP-2 and hydroxyapatite resulted in the highest calcium deposition and upregulation of BMP-2 transcript levels in MSCs compared with controls [171]. Similarly, hydroxyapatite nanoparticles have also been integrated into nondegradable polyurethane nanofibers with potential applications in dentistry and bone tissue engineering [172].

Nanofibers can be designed to release a variety of cues such as peptides, enzymes, small-molecule drugs, DNA and RNA [173] and various release profiles can be engineered. Additional release systems such as drug-loaded nano- and microparticles have been integrated into or entrapped between fibers to prolong release of GFs such as EGF and bovine serum albumin (BSA) [174,175].

Alternative methods to modulate release of multiple GFs include the use of core-shell fibers, or a blank (no drug) outer shell to prevent burst release. For example, poly(p-xylylene) coatings slowed the release of two model proteins BSA and luciferase from poly(vinyl alcohol) nanofibers. This reduction in release was dependent on the coating thickness [176]. For more details on electrospun fibers in drug delivery and tissue engineering, please see the following article [177].

A graphic summary of the various hydrogel-based or solid scaffolds is given in **Figure 3**. Some of them contain an additional integrated drug delivery system.

3.3 Triggerable systems

Stimulus-responsive drug delivery systems can be designed to release drugs in response to a multitude of physical stimuli (e.g., light, heat, magnetic or electric fields, ultrasound [83]) or chemical triggers (e.g., pH, drug binding [178]; reviewed in [179]). **Figure 4** depicts light-, enzyme- or drug-activated hydrogel systems. Several of these approaches have been implemented into the design of materials for tissue engineering, which affect cell differentiation [180-182], and have the ultimate goal to develop smart scaffolds with the ability to sense specific tissue conditions and release compensating factors in response.

Protease-sensitive synthetic hydrogel materials have been produced that mimic the degradability of the ECM [183]. These PEG-based hydrogels incorporate small peptide sequences that can be degraded by cell-secreted matrix metalloproteinases during cellular invasion and migration, allowing cell penetration into the scaffold matrix. Depending on the k_{cat} (catalytic constant of the enzyme) and k_m (substrate concentration at which enzymatic reaction occurs with half-maximal velocity), the degradation kinetics of the different types of protease-sensitive 3D hydrogels can be fine-tuned [184]. Applications of such systems range from bone defect repair by mimicking collagenous extracellular matrices [183] to vascular engineering [185,186] or cardiac tissue engineering [187]. The protease substrate (the amino acid sequence in the peptidic cross-linker of the hydrogel) can be tailored to the specific enzyme activity in the target tissue, cell type and biological process of interest [184]. Protease-sensitive linkers have been covalently modified with biodegradable polymers such as hyaluronic acid [188], self-assembled peptide scaffolds [189] or nanofiber precursors [190]. These systems combine self-assembly and cell-responsive biodegradability.

Spatiotemporal control of scaffold properties by light was obtained by introducing photolabile nitrobenzyl ether-derived moieties into poly(ethylene glycol)-based hydrogels [191]. The *o*-nitrobenzylether-based moiety was acrylated to form a photodegradable cross-linker and photoreleasable tether, which readily polymerized to form light-degradable materials. Irradiation allowed post-gelation control of viscoelastic properties, creation of arbitrarily shaped features and on-demand release of pendant functionalities such as RGD-binding sites [192]. Channels could be formed by photodegradation within a hydrogel-containing cells and allowed cell migration; these changes in microenvironment elasticity were shown to direct cell phenotype by regulating α-smooth muscle actin expression and cytoskeletal reorganization [193]. Temporal variation of the gel composition and elasticity has been used to influence chondrogenic differentiation of encapsulated stem cells [191]. This concept can also provide spatiotemporal control of drug release.

Hydrogels can be designed to allow spatiotemporal control of compound release (or other hydrogel properties) in response to administration of a specific drug. One example is an antibiotic-sensing hydrogel that released VEGF, allowing remote control over the induction of neovascularization in engineered tissues [178]. The polymer component of the hydrogel was a polyacrylamide modified with genetically engineered bacterial gyrase subunit B (GyrB). Those GyrB units were dimerized by the antibiotic coumermycin, forming a hydrogel. The antibiotic novobiocin caused the GyrB subunits to dissociate, thereby dissociating the hydrogel. The resulting dose- and time-dependent release of entrapped VEGF allowed control of endothelial cell proliferation.

Another approach used calmodulin, a calcium-binding protein, as the biological recognition element. This protein underwent conformational changes upon binding calcium, certain peptides and phenothiazine drugs, resulting in fine-tuned control of swelling. Altered material properties in the presence of these specific ligands could be used for sensing (e.g., detecting the presence of specific ligands) or gating and transport of biomolecules across the polymeric network (e.g., controlling biomolecule flux by tuning ligand concentration) [194].

Hydrogels have also been made to sense protein–protein interactions. For example, an antigen-responsive hydrogel was prepared containing antigen–antibody cross-links between the polymeric hydrogel backbones. Competitive binding with free antigen broke those non-covalent cross-links, causing a reversible change in gel volume. Stepwise changes in antigen concentration induced pulsatile permeation of the network by a protein, allowing drug delivery in response to the presence of a specific antigen [195].

Similarly, hydrogels responsive to the presence of DNA have been developed. These hybrid hydrogels, cross-linked by rationally designed single-stranded (ss) DNA, were capable of shrinking or swelling in response to ssDNA with very specific structures [196].

4. Expert opinion

4.1 Bioactive molecules are essential for engineered tissues

Recreation of tissues from dissociated cells requires mimicry of the spatial and temporal microenvironments provided to native tissues by the ECM. Biomolecules in the ECM provide stimulation for many physiological processes such as cell survival, migration, vascularization, proliferation and differentiation. A decade ago 3D scaffolds for tissue engineering were thought of only for mechanical support of cells. Recently, more focus has been given to the biology underlying these scaffolds. Tissue engineers are now incorporating instructive cues inside the matrices to regulate the phenotype of the cells and trigger faster and more accurate organization to functional tissues.

That trend is likely to continue and accelerate, with increasing emphasis on regimens involving multiple GFs and/or specific temporal sequences of GF release. This is likely to become more important given the tendency toward cell constructs involving multiple cell types for a range of purposes

including prevascularization and the formation of complex tissues. These requirements will themselves necessitate the development of more sophisticated and tunable drug delivery systems.

The nature of the bioactives to be delivered is also likely to change. As we have seen, small molecules are now starting to be used in tissue engineering. Their use may present advantages in terms of stability within drug delivery systems, biological potency and other factors. RNA interference (RNAi) is starting to be applied in tissue engineering [197] and could be used to modulate expression of endogenous GFs, cytokines and so on. Controlled release, triggered release and patterning of such factors will greatly enhance the control of tissue-engineered systems.

4.2 Materials for drug delivery in tissue engineering

As described above, there is a broad range of biomaterials available for use in drug delivery as it pertains to tissue engineering. A crucial prerequisite from these materials is biocompatibility. However, 'biocompatibility' is not an inherent property of a biomaterial, but depends on the type of the engineered tissue and site of implantation [198], as well as the desired function [199]. For example, a biomaterial that is biocompatible in subcutaneous tissue might not be so in the heart or liver. Another requirement is that the materials' mechanical properties be compatible with those of the surrounding tissues. Material degradation rate has to be fine-tuned to maintain the desired mechanical properties and to guide the release kinetics of drugs. This can be done either by relatively conventional synthetic approaches (modulating cross-linking density, introducing cleavable linkers, etc.), by disruption by external energy sources [83], or by interaction with specific biological events. The latter is a particularly promising approach in that it would limit drug release to a predetermined biological context. In the future, there will be an increasing tendency – already existent – to develop scaffold biomaterials that have inherent effects on cell proliferation and differentiation, either by their composition of matter [200] or by their surface topography; the alteration of surface micro- or nanotopography is well known to affect many aspects of cell behavior [201]. It is likely that there will be increasing investigation and utilization of synergistic effects on cell behavior between released drugs and intrinsic properties of support materials. The range of materials to be considered is constantly expanding, not just by virtue of the creativity of synthetic chemists as has been the case to date, but by the integration of inorganic materials into the drug delivery field, usually in the context of triggerable drug delivery [83], but also in the context of affecting tissue conductivity or gene expression [202,203].

4.3 Controlled-release systems in tissue engineering

GFs, cytokines and other biomolecules have very short half-lives because of proteolytic activity in the intercellular environment and other factors such as simple diffusion from the site of release. It is important to shield these factors from that environment and/or to extend their effect on developing tissues. These goals can be achieved, to varying degrees, by chemical conjugation or admixture of the factors to the scaffolds or by physical encapsulation within drug delivery devices that can be added to the scaffold. The selection of approach is dependent on the specific release kinetics desired, the specific attributes of the tissue to be engineered (e.g., mechanical strength), chemical entities to be delivered and other parameters.

Controlled-release formulations have been available for decades; their application in tissue engineering has tended to lag somewhat, perhaps because in the past there were so many issues to be worked out relating to the *in vitro* phase of engineering tissues. Arguably, drug delivery systems are most useful *in vivo* – after all, all one has to do to provide drugs *in vitro* is to add them to the media. Drug delivery systems have the potential to allow a predetermined program of factor release – continuous, or with temporally distinct events – to occur once a construct is placed into the body. In the future, more sophisticated on demand or triggered drug delivery systems will allow fine control over the deployment of bioactive molecules; optimal use of such systems may require the development of methods imaging tissue development in real time. Furthermore, drug delivery systems that respond to their environment – whether it is the presence of an analyte or the ingress of a cell type – will allow the timing of the release of biological cues to be intrinsically timed to specific biological events.

One of the challenges in tissue engineering is achieving tight spatiotemporal control over the delivery of the molecules. Recently, there has been increasing interest in developing methods where drug release can be controlled directly, that is, triggered either by an operator or by an interaction between a 'smart' material and changes in its environment. Ideally, a smart controlled-release system for tissue engineering could sense the condition of the developing tissue and release compensating factors accordingly. Such systems could determine the time, duration, dosage and even location of release. It is likely that nanotechnology will have much to offer in the development of those approaches [83]. Tight control of the spatial distribution of GFs and other biological cues will allow the formation of well-defined patterning of areas where particular developmental outcomes are desired [144].

As in many areas of biotechnology, a key concept in the future development of drug delivery systems as they pertain to tissue engineering is likely to be 'convergence' [198,204], where combination products are created by the merger of scientific fields that are traditionally distinct. One such area of convergence that has enormous potential but that has barely entered the field of tissue engineering is nanoelectronics. There, nanowired arrays can be made to interface with cells and tissues [205]. Apart from their direct effects in modifying

the structure and composition of matter of the ECM, they could be connected to electrically driven drug delivery systems [206].

Also as in many areas of biotechnology, some of the greatest advances will come from basic biological research. Greater understanding of the biology will allow the development of pharmacological agents with which to load drug delivery systems.

Declaration of interest

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