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Material strategies for creating artificial cell-instructive niches

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Abstract

There has been a tremendous growth in the use of biomaterials serving as cellular scaffolds for tissue engineering applications. Recently, advanced material strategies have been developed to incorporate structural, mechanical, and biochemical signals that can interact with the cell and the *in vivo* environment in a biologically-specific manner. In this article, these strategies including the use of composite materials and material processing methods to better mimic the extracellular matrix, as well as integration of mechanical and topographical properties of materials in scaffold design, and incorporation of biochemical cues such cytokines in tethered, soluble, or time-released form are presented. Finally, the replication of the dynamic forces and biochemical gradients of the *in vivo* cellular environment through the use of microfluidics is highlighted.

Introduction

In the engineering of tissues, often, a scaffold is required to provide an environment or *niche* that favors the natural behavior of cells. This scaffold must fulfill a wide range of requirements, ranging from physical and biochemical to cellular parameters [1,2]. These requirements have stemmed from the notion that mimicking the extracellular environment—its structure, mechanical and biochemical properties—in designing cellular scaffolds, will coax cells to behave in the same manner as their *in vivo* counterparts. Engineering of such scaffolds requires close attentiveness to several material design criteria: (i) the 3-dimensional (3D) micro-geometry within the scaffold including porosity, pore size, and interpore connectivity to satisfy adequate mass transfer of gases, nutrients, and waste as well as cell attachment and spreading, and tissue formation; (ii) mechanical parameters such as linearity or non-linearity, elasticity, viscoelasticity, or anisotropy that must be tailored to the specific tissue in mind; and (iii) successful delivery of biologics including cells, nucleic

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acids, and cytokines. In this review, these three material design criteria will be discussed, methods utilized to mimic the *in vivo* cell microenvironment will be highlighted, and recent research contributing to better bioactive scaffold fabrication using advanced material strategies will be presented (figure 1). Such materials can either directly alter the cellular differentiation pathways or be used as permissive environments for approaches in which the cell phenotype is altered using “pathway engineering” approaches. An example of the latter approach is to develop advanced materials that enable the generation of induced pluripotent stem cells [3].

Creating the cellular scaffold

In choosing the material to be used for a scaffold, a wide range of options exists—natural and synthetic materials, and composites of two or more from the same class or different classes of materials; the advantages and disadvantages of using that material must be known, in addition to its suitability for the desired application. Naturally-derived materials are often purified extracellular matrix (ECM) proteins (collagen, gelatin [4], laminin, hyaluronic acid) or a mixture (Matrigel®). Other sources may be from plant, and animal constituents (silk, agarose, chitosan). Alternatively, decellularized organs that retain the ECM and architecture of tissues have been used to engineer blood vessel [5], heart [6], lung [7], liver [8], and bone [9]. The advantages of natural materials are their biological activity and biocompatibility. Synthetic materials, on the other hand, overcome the disadvantages posed by their natural counterparts—mainly, their manufacturing and processing variability and inability to control their physico-chemical properties. Additionally, synthetic materials provide a blank slate with absence of biological activity that may be modified through biochemical means (discussed in “Biochemical modulation of materials” section) [10].

More often than expected, single-component materials do not meet the requirements needed for a cellular scaffold. For instance, they may lack the desirable mechanical properties, electrical activity, or cell-matrix interactions. Composite materials may be used to overcome these limitations. While mixing materials from the same class [11] will provide a degree of modulation, combining materials from different classes will generate a greater measure of control over its properties. For instance, bone is composed of collagen (a polymer) and hydroxyapatite nanocrystals (a ceramic); hence polymer/ceramic composites have been widely used in bone tissue engineering [12]. Hydrogels are another case in point; they are a cross-linked network of monomers, oligomers, or polymers that contain 90-95% water in volume and structurally resemble the ECM [13]. However, they often lack the mechanical strength needed for certain tissue engineering applications. In a work by Shin *et al.*, gelatin methacrylate hydrogels, which favor cell attachment and spreading but lack mechanical integrity, were reinforced with carbon nanotubes, which resulted in a composite with increased compressive modulus, while material pore size and cell adhesiveness remained the same [14]. Furthermore, carbon nanotube-based composites have been used to direct differentiation of mesenchymal stem cells to the osteogenic lineage [15], increase connexin 43 expression of cardiac constructs [16], and enhance the electrical activity of neural tissues [17], given their electrical conduction properties.

In designing a scaffold, it is ideal that the scaffold, over an intended period of time, should degrade and be replaced with naturally deposited ECM and the newly formed tissue. In this regard, linear aliphatic polyesters such as poly(lactic acid) and poly(glycolic acid) have been routinely used due to their biodegradability—given the susceptibility of their ester bonds to hydrolysis—and ability to fine-tune their degradation rate. Alternatively, non-biodegradable materials, such as poly(ethylene glycol) (PEG), can be incorporated with matrix metalloproteinase-sensitive peptides to make them physiologically degradable. The addition of such peptides has been shown to directly affect gene expression, as shown by the

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increased maturation of cardioprogenitors via increase in myosin heavy chain-positive cells when grown on MMP-sensitive gels [18].

Another important consideration in cellular scaffold fabrication is scaffold structure. In the past, emphasis was placed on macroporous structures to facilitate mass transfer of vital molecules. These scaffolds were often fabricated with microspheres, salt leaches, or gas foams [19]. However, the micronscale dimensions of these material structures do not recapitulate the nanometer-scale, fibrillar aspect of the structure of ECM. To generate these nanofibers, techniques such as electrospinning [20], molecular self-assembly [21,22], and phase separation [23] have been employed. Hydrogel, discussed previously, is another class of material structure that has proven to be particularly biomimetic and are now widely used in the biological and medical fields [24].

Mimicking the physical aspects of the cell's microenvironment

The physical aspects of the cell's microenvironment can be broken down into substrate mechanics and surface topography. Depending on their anatomical location, tissues have a wide range of mechanical properties. For instance, the elastic moduli of brain (0.5 kPa) is relatively soft compared to muscles and skin (about 10 kPa) and precalcified bone (>30 kPa) [25]. Another challenge in recreating the native cellular environment is that many tissues are viscoelastic with non-linear, anisotropic, and heterogeneous mechanical properties [1]. Cellular scaffolds used for the repair or replacement of diseased tissues must have adequate mechanical integrity to withstand physiological loading, as in the case of engineered blood vessel, heart, bone, and cartilage constructs. Hence, in designing a material, the mechanical properties of the tissue that the material will replace should be carefully considered, and ideally the two should match. The effects of substrate stiffness on cell cytoskeletal remodeling, cell proliferation, and stem cell differentiation[26,27] are well known [28]. For instance, fibroblast, endothelial and epithelial cell growth is enhanced on stiffer substrates, whereas as neurons prefer softer substrates [29]. Employing this knowledge, Gilbert *et al.* showed that the skeletal muscle stem cells grown *in vitro* on laminin-coated PEG with elasticity similar to that of muscle had enhanced self-renewal, as assessed via myogenin transcription factor expression, and increased *in vivo* regenerative capacity, when subsequently transplanted in mice [30]. Common methods of altering the Young's modulus of polymeric materials have involved varying the precursor molecular weight or concentration, or the chemistry or degree of cross-linking. However, this often leads to a simultaneous change in the binding sites of the material and the two parameters cannot be independently assessed. While new chemistry approaches, such as Diels-Alder [31] or Huisgen [32] cycloadditions, have overcome these obstacles, there is often a compromise between the mechanical strength of a material and its porous nature. In this regard, composite materials have overcome these disadvantages with the use of nanoscale fibers (*e.g.* non-woven, woven, or knitted polymers) , tubes (*e.g.* carbon nanotubes) [33], wires and belts (*e.g.* nanotitanates) [34], or particulates (*e.g.* gold or silicate) [35] to modulate the bulk mechanical properties of materials. For instance, in work by Moutos *et al.*, 3D woven poly(glycolic acid) structures were generated as reinforcing agents in agarose and fibrin hydrogels that reproduced the viscoelasticity, tension-compression non-linearity, and anisotropy of native cartilage tissue, and maintained the rounded phenotype of chondrocytes [36].

Another important physical parameter in the cell's microenvironment is surface topography. The ECM, through its 3D structure, presents topographical cues that influences cell processes such as cell adhesion, morphology, migration, and differentiation. Micro- and nanofabrication technologies such as photolithography, soft lithography, and electron beam lithography have been widely used to create topographies such as grooves, pillars, lattices,

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among other shapes. These topographies are often used to create anisotropic cell and tissue constructs or direct specific cellular processes [37] such as neurite extension. In many tissue types (*e.g.* myocardium, bone, cartilage), the anisotropy of cells and tissues is intricately connected with the function of that tissue. Hence, fabrication of topographically-containing biomaterials has been used in various tissue-engineering applications. For instance, microfluidic-generated grooved alginate microfibers were shown to produce a greater degree of neurite extension and orientation compared to smooth microfibers [38]. Finally, the fabrication of a poly(glycerol-sebacate) scaffold with an accordion-shaped honeycomb structure resulted in anisotropic and tensile mechanical properties similar to that of the ventricular myocardium, and resulted in a directionally-dependent electrical excitation thresholds [39]. While the use of fabricated topography has provided insight into various cellular processes, future work must be directed into uncovering the mechanism behind such effects.

Biochemical modulation of materials

To generate cell-instructive scaffolds, it is necessary to encode them with biological information. *In vivo*, this information is in the form of signaling molecules or cytokines, in tethered or freely-soluble forms. Currently, the material strategies for presenting cytokines within scaffolds include covalent attachment, adsorption, and use of controlled-release particles [40]. One of the initial steps after seeding cells on or into a scaffold is integrin-mediated cell attachment. Hence, covalent attachment of arginine-glycine-aspartate (RGD) sequences, a ligand for integrins, to inert synthetic materials such as PEG or polysaccharides such as hyaluronic acid is a common method to promote cell adhesion. Covalent attachment of these peptides and also protein fragments or full length proteins are possible through targeting the thiol, amino, or carboxylic groups of these molecules [41]. While covalent conjugation presents a method that prevents the diffusion of cytokines out of the scaffold, it is an atypical mode of molecular presentation. *In vivo*, growth factors and other types of signaling molecules are often ECM-bound, through non-covalent interactions with glycosaminoglycans (GAGs) including heparin, heparin- and chondroitin-sulfate. The interaction of GAGs with growth factors involves their sequestration, protection from hydrolytic enzymes, and presentation to cell surface receptors. Therefore, the incorporation of GAGs in scaffolds have been employed as a method of presenting and delivering growth factors [42]. While tethering does simulate the presentation of some cytokines [43], many other cytokines are secreted by cells in soluble form for intercellular communication. Hence, drug release strategies have been employed in tissue engineered scaffolds to have control over the temporal kinetics of signaling molecules. These include encapsulation within porous scaffolds for burst release, micro- and nanoparticulates [44] synthesized through double emulsion techniques for sustained or delayed release [45], and on demand release through the use of stimuli-responsive polymers [46]. For instance, fibroblast growth factor-2 (FGF-2)-containing polyvalent coacervates were used for the subcutaneous delivery of FGF-2 and resulted in enhanced angiogenesis as evidenced by increased recruitment of endothelial and mural cells and maturation of blood vessels [47]. The successes of these drug release strategies are evident from their wide use in current clinical trials and therapeutics.

Another aspect of biomaterial chemical modification that deserves to be mentioned is surface chemistry. The chemical moieties present at the surface of biomaterials are critical for cellular functions such as cell adhesion, migration, proliferation, and differentiation [48]. In addition, the surface of biomaterials plays a key role in the integration of tissue engineered constructs and implants. Using a surface chemistry approach, Wang *et al.* functionalized the surface of chondroitin sulfate scaffold with an adhesive containing methacrylate and aldehyde groups that promoted tissue integration via the formation of

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covalent bonds with the scaffold (through polymerization reaction) and native cartilage, respectively[49].

Moving from static to dynamic environments

In the *in vivo* microenvironment, a dynamic interplay exists between cells and biochemical and physical cues that currently cannot be controlled in standard *in vitro* models. Microfluidics, a field involving the manipulation of fluids at the micron-scale dimension, has made it possible to replicate the dynamic *in vivo* conditions in *in vitro* models [50,51]. Commonly used materials in microfluidic devices include poly(dimethylsiloxane) or polyesters which may be undesirable given their non-degradability or lack of robust mechanical properties [52]. One solution has been through the use of silk fibroin, amenable to soft lithographic methods, to fabricate biocompatible and biodegradable microfluidic devices with high mechanical modulus and toughness [53].

An advantage central to using microfluidic devices in tissue engineering is the ability to control the mechanical properties of the cell's microenvironment [54]. One way that this is achieved is through mimicking the stress and shear forces present *in vivo* such as those produced by blood flow [55], heart contractions, and lung movements. For instance, engineered human microvessels exposed to high flow resulted in vascular barrier functions that rivaled those of *in vivo* conditions [56]. In another work, replication of cyclical mechanical stimulation of cardiac tissues was accomplished with a pulsatile pressure-actuated microfluidic device that induced the preload and afterload effects on embryonic cardiomyoblasts and resulted in the establishment of an *in vivo* phenotype [57]. Lastly, the breathing movement of the lungs was replicated by a vacuum-assisted microfluidic device, cyclically applying mechanical strain to an alveolar-capillary interface that reproduced pulmonary inflammation responses [58].

Microfluidics also allows for precise control over the chemistry and geometry of the environment. Methods to control cytokine concentration gradients [59], important in embryonic development and tissue formation [60] have been developed. For instance, Choi *et al*. developed a dual hydrogel membrane within microchannels to produce a stable concentration gradients that could be applied to chemotaxis studies [61]. Geometrically, besides being able to control channel dimensions or employ a layer-by-layer approach, recently, self-assembling microfluidic devices with curved patterns have been produced [62].

Disadvantages to incorporating microfluidics with tissue engineering include small sample sizes and methods that are not yet fully developed or scalable. However, the ability for microfluidics to generate mechanical, chemical, and geometric constraints similar to those found in physiological conditions are unparalleled by current methods, thus rendering it valuable to the field of tissue engineering as demonstrated by microfabricated models of brain, blood vessels, skeletal muscles, heart, lung, liver, intestines[63], liver, and tumors [64].

Future outlooks

Advanced material strategies stemming from materials science, physics, chemistry, and biology have heralded a new era in the design of tissue engineering scaffolds whereby the biochemical, mechanical, and structural details of a cell's microenvironment or *niche* can be replicated to influence cell behaviors such as gene expression, adhesion, migration, and differentiation. However, more work needs to be done to understand the properties of native tissues, to define proper mechanical characterization of biomaterials, and to detail out the mechanisms behind the regenerative processes that are necessary for a successful tissue

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replacement. Furthermore, a topic of importance not discussed here is the biomaterials' interaction with the body's immune system, which will also dictate how well an engineered implant is integrated within host tissue. Overall, the materials-driven tissue-engineering discoveries discussed hold great promises in the near future for the replacement of damaged or diseased tissues.

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Highlights

- Biomaterial selection based on origin, biodegradability, and microstructure
- Bulk and surface biochemical modification of scaffolds for presentation of cytokines
- Designing scaffolds with *in vivo*-like mechanical properties and topography
- Use of microfluidics to replicate dynamic mechanical and biochemical parameters

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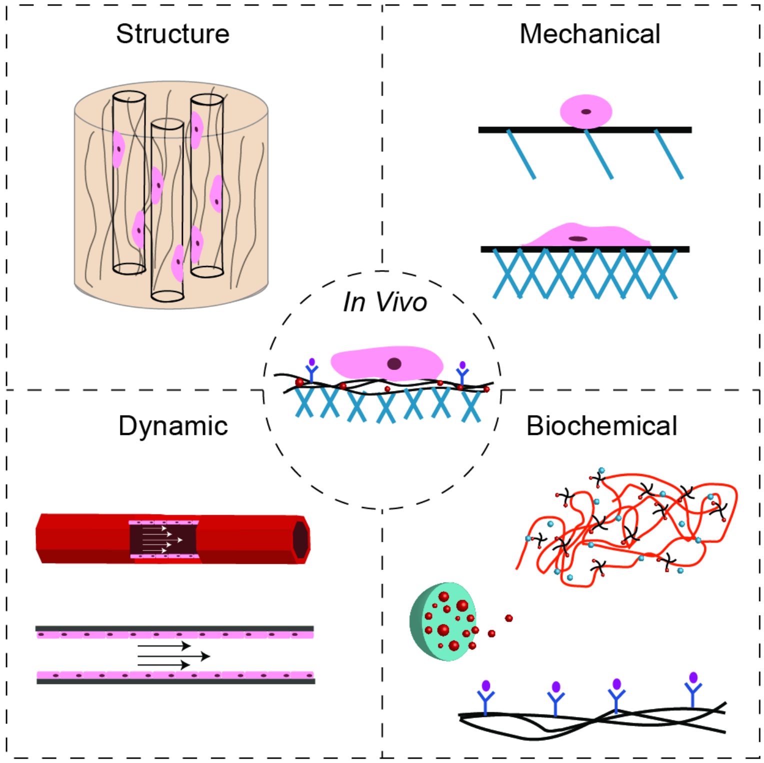

Figure 1.
Schematic representation of the material strategies (structural, mechanical, biochemical, and dynamic) utilized to encode tissue-engineering scaffolds with biological information to mimic the *in vivo* cellular microenvironment.