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Leading Opinion

Smart biomaterials design for tissue engineering and regenerative medicine ☆

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Abstract

As a prominent tool in regenerative medicine, tissue engineering (TE) has been an active field of scientific research for nearly three decades. Clinical application of TE technologies has been relatively restricted, however, owing in part to the limited number of biomaterials that are approved for human use. While many excellent biomaterials have been developed in recent years, their translation into clinical practice has been slow. As a consequence, many investigators still employ biodegradable polymers that were first approved for use in humans over 30 years ago.

During normal development tissue morphogenesis is heavily influenced by the interaction of cells with the extracellular matrix (ECM). Yet simple polymers, while providing architectural support for neo-tissue development, do not adequately mimic the complex interactions between adult stem and progenitor cells and the ECM that promote functional tissue regeneration. Future advances in TE and regenerative medicine will depend on the development of "smart" biomaterials that actively participate in the formation of functional tissue. Clinical translation of these new classes of biomaterials will be supported by many of the same evaluation tools as those developed and described by Professor David F. Williams and colleagues over the past 30 years.

Keywords: Smart; Intelligent biomaterials; Clinical; Human use; Extracellular matrix

1. Smart biomaterials

In its simplest form a tissue engineering (TE) scaffold provides mechanical support, shape, and cell-scale architecture for neo-tissue construction *in vitro* or *in vivo* as seeded cells expand and organize. Most degradable biomaterials used to date comprise a class of synthetic polyesters such as poly(L-lactic acid) (PLLA) and poly (L-glycolic acid) (PLGA), and/or natural biological polymers such as alginate, chitosan, collagen, and fibrin [1]. A multitude of fabrication techniques have been devised and afford an abundance of potential shapes, sizes, porosities, and architectures [2,3]. Composites of these synthetic and natural polymers, alone or with bioactive ceramics such as hydroxyapatite or certain glasses, can be designed to yield materials with a range of strengths and porosities, particularly for the engineering of hard tissues [4].

It has become increasingly apparent that for many TE applications biomaterial scaffolds should provide more than temporary architectural structure to a developing tissue construct. As cell and molecular biology converge with materials science and biomedical engineering, new applications in regenerative medicine will benefit from interactive biomaterials that serve to orchestrate cell attachment and growth, as well as tissue morphogenesis. However, many of the same tools developed for evaluating the biocompatibility of traditional biodegradable polymers are still used to investigate the fundamental interactions between new classes of biomaterials and their host [5–8]. Importantly, quantitative methods of assessing host tissue

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response to extracellular matrix (ECM) biomaterials such as collagen can also be employed [9].

1.1. Extracellular matrix

A scaffold used for TE can be considered a surrogate ECM [10]. The normal biological ECM, in addition to contributing to mechanical integrity, has important signaling and regulatory functions in the development, maintenance, and regeneration of tissues. ECM components, in synergy with soluble signals provided by growth factors and hormones, participate in the tissue-specific control of gene expression through a variety of transduction mechanisms [11–13]. Furthermore, the ECM is itself a dynamic structure that is actively remodeled by the cells with which it interacts [14]. An important area of TE is to develop improved scaffolds that more nearly recapitulate the biological properties of native ECM [15]. However, deconstructing mature ECM and understanding its complex functions in mature or regenerating tissues is a formidable task. The ECM is a dynamic matrix that is constantly changing in composition and structure as tissues develop, remodel, repair, and age. Biomaterials scientists have sought to approximate its functions using several different approaches.

In the absence of methods for *de novo* construction of a true ECM mimic from purified components, decellularized tissues or organs can serve as sources of biological ECM for TE [16]. The relatively high degree of evolutionary conservation of many ECM components allows the use of xenogeneic materials. Various acellular matrices have been utilized successfully for TE in animal models and a limited number of xenogeneic products have received regulatory approval for clinical use. These include decellularized heart valves, small intestinal submucosa (SIS), and urinary bladder [17]. The use of decellularized matrices is likely to expand because they retain a complex set of molecules and the three-dimensional microarchitecture of native ECM. Indeed several decellularized xenogeneic medical products are now being introduced into the market. However, despite many advantages, there are concerns about the use of decellularized materials. These include the potential for immunogenicity, the possible presence of infectious agents, variability among preparations, and the inability to completely specify and characterize the bioactive components of the material.

1.2. Naturally derived biopolymers

Native ECM can also be approximated by the use of some components of ECM, either alone or in simple combinations. Structural proteins such as collagen, laminin, elastin, and fibronectin have been used as matrices for TE and as vehicles for cell delivery [18]. Collagen has found widespread use as a scaffold and carrier for cells in TE and regenerative medicine, particularly in soft tissue applications such as skin [19,20].

Carbohydrate polymers have been utilized in hydrogels for drug delivery but also in TE [21]. The linear glycosaminoglycan hyaluronic acid (HA), composed of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine, is widely distributed in the ECM and plays an important role in vertebrate tissue morphogenesis [22]. HA has been approved for use in human patients both as viscous fluid and sheet formulations, and is indicated for knee pain and surgical adhesions, respectively. Many large patient trials have confirmed HA's effectiveness for these applications [23–27]. The activity of HA, like that of other relatively simple carbohydrate matrix components, may be enhanced by modification to promote cell migration, spreading, and multiplication (see below).

Other carbohydrate polymers such as chitosan and alginate, derived from the exoskeleton of shellfish and brown algae, respectively, have been used in several biomedical applications. Chitosan is a polycationic material produced by the deacetylation of chitin. It readily forms hydrogels that have been used in a number of gene and drug delivery applications. Its application in regenerative medicine and TE has recently been reviewed [28,29]. Alginate has been used extensively in gel form for cell encapsulation and drug delivery [30] and in TE [31].

1.3. Proteins and mimetics

More broadly, the design of genetically modified proteins or of hybrid polymers incorporating peptide and protein domains may will enable the creation of a wealth of novel biomaterials that also can be designated as "smart" [32]. These include engineered mutant variants of existing proteins, semi-synthetic scaffold materials incorporating protein domains, scaffold materials linked to synthetic peptides, and engineered peptides capable of self-assembly into nanofibers.

Genetic engineering may improve on natural proteins for applications in TE. For example, a collagen-like protein was generated by using recombinant DNA technology to introduce tandem repeats of the domain of human collagen II most critically associated with the migration of chondrocytes [33]. When coated onto a PLGA scaffold and seeded with chondrocytes, the engineered collagen was superior to wild-type collagen II in promoting artificial cartilage formation. Incorporation of cysteine-tagged functional domains of fibronectin into thiol-modified HA gels, likewise, was found to stimulate spreading and proliferation of human fibroblasts *in vitro*, and to promote recruitment of dermal fibroblasts in an *in vivo* cutaneous wound model [34]. Similarly, recombinant technology has been employed to generate a series of elastin-mimetic protein triblock copolymers [35]. These varied broadly in their mechanical and viscoelastic properties, offering substantial choices for the production of novel materials for TE.

The incorporation of bioactive signals into scaffold materials of the types described above can be accomplished

by the chemical linkage of synthetic peptides as tethered ligands. Numerous studies have confirmed that incorporation of the integrin-binding motif arginine-glycine-aspartic acid (RGD), first identified in fibronectin, enhances the binding of many types of cells to a variety of synthetic scaffolds and surfaces [36]. The CS5 cell-binding domain of fibronectin has also been incorporated into scaffolds, and its activity shown to be subject to regulation by sequence context [37]. It is likely that greater selectivity and potency in cellular binding and enhancement of growth and function will be achieved in the future by taking advantage of the growing understanding of the role of additional binding motifs in addition to and/or in concert with RGD [38,39]. The integrin family comprises two dozen heterodimeric proteins, so there is great opportunity to expand the set of peptide binding motifs that could be utilized on TE scaffolds with the objective of achieving greater selectivity and control over cell behavior. A remaining challenge is to find the optimal balance between the greater information and, in many cases, biological activity provided by recombinant protein domains, and the simplicity and lower cost of small synthetic peptides [34].

The modification of matrices with bioactive peptides and proteins can extend well beyond binding motifs to promote cell adhesion [40]. Cells also need to migrate in order to form remodeled tissues. Thus, the rate of degradation of scaffolds used for TE is a crucial parameter affecting successful regeneration [41]. Control of the degradation rate can be achieved by varying physical and chemical parameters of the scaffold. For example, target sites for specific proteolytic degradation can be built into the scaffold [42,43]. These sequences are known to play an important role in cell invasion, and their use in a synthetic matrix could modulate tissue regeneration. Indeed, incorporation of matrix metalloproteases' target sequences into a cross-linked synthetic hydrogel was shown to enhance the migration of fibroblasts *in vitro* and the healing of bony defects *in vivo* [44].

1.4. Smart polymers

At the chemical level, a number of groups have begun to explore the synthesis of biomaterials that unite the advantages of smart synthetic polymers with the biological activities of proteins. The concept of smart polymers initially derived from the development of materials that show large conformational changes in response to small environmental stimuli such as temperature, ionic strength, pH, or light [45]. The responses of the polymer may include precipitation or gelation, reversible adsorption on a surface, collapse of a hydrogel or surface graft, and alternation between hydrophilic and hydrophobic states [46]. In many cases the change in the state of the polymer is reversible. Biological applications of this technology currently under development span diverse areas including bioseparation, drug delivery, reusable enzymatic catalysts, molecular switches, biosensors, regulated protein folding, microfluidics, and gene therapy [47]. Smart polymers may offer promise for revolutionary improvements in TE scaffolds. Beyond the physical properties of polymers, a major goal is to impart smart biomaterials with the specific properties of signaling proteins such as ECM components and growth factors.

One approach is to link smart polymers to proteins [48]. The proteins can be conjugated either randomly or in a site-specific manner, through engineering of the protein to introduce a reactive amino acid at a particular position. If a conjugation site is introduced near the ligand-binding domain of a protein, it has been shown that induction of a change in conformational state of the smart polymer can serve to regulate the protein's activity [49]. This may allow selective capture and recovery of specific cells, delivery of cells to a desired location, and modulation of enzymes such as matrix metalloproteases that influence tissue remodeling.

1.5. Discovery of new materials

A next stage of smart biomaterials development extends to the design, discovery, and evaluation of bioactive materials. At one level this may entail the relatively straightforward chemical synthesis of new materials, coupled with a search for novel activities and evaluation of their behavior in biological systems. By adapting the combinatorial library approach already well established for synthetic peptides and small molecule drugs, together with high throughput assays, thousands of candidate scaffold materials can be generated and tested. As one example of this approach, screening of a combinatorial library derived from commercially available monomers in the acrylate family revealed novel synthetic polymers that influenced the attachment, growth and differentiation of human embryonic stem cells in unexpected ways [50].

Potentially more revolutionary developments in biomaterials will continue to arise at the interface of TE with nanotechnology. Basic understanding of the three-dimensional structure of existing biological molecules is being applied to a 'bottom-up' approach to generate new, self-assembling supramolecular architectures [51]. In particular, self-assembling peptides offer promise because of the large variety of sequences that can be made easily by automated chemical synthesis. The potential for bioactivity, the ability to form nanofibers, and responsiveness to environmental cues are inherent in some of these materials [52]. Recent advances include the design of short peptides (e.g., heptamers) based on coiled-coil motifs that reversibly assemble into nanofilaments and nanoropes, without excessive aggregation [53]. These smart peptide amphiphiles can be induced to self-assemble by changes in concentration, pH, or the level of divalent cations [54]. Branched structures can be designed to present bioactive sequences such as RGD to cells via nanofiber gels or as coatings on conventional TE scaffolds [55]. In addition, assembly can occur under conditions that permit the

entrapment of viable cells in the resulting nanofiber matrix [56]. The entrapped cells retain motility and the ability to proliferate.

Peptide-based nanofibers may be designed to present bioactive sequences to cells at very high density, substantially exceeding that of corresponding peptide epitopes in biological ECM. For example, a pentapeptide epitope of laminin, isoleucine-lysine-valine-alanine-valine (IKVAV), known to promote neurite extension, was incorporated into peptide amphiphiles capable of self-assembly into nanofibers that form highly hydrated gels [57]. When neural progenitor cells capable of differentiating into neurons or glia were encapsulated during assembly of the nanofibers, they survived over several weeks in culture. Moreover, even without the addition of neurotrophic growth factors, they displayed neuronal differentiation as exemplified by the extension of large neurites, already obvious after 1 day, and by expression of βIII-tubulin. The production of neuron-like cells from neural progenitors, whether dissociated or grown as clustered neurospheres, was more rapid and robust in the IKVAV-PA gels than on laminin-coated substrates or with soluble IKVAV. By contrast, the production of cells expressing glial fibrillary acidic protein (GFAP), a marker of astrocytic differentiation, was suppressed significantly in the IKVAV-PA gels even when compared to growth on laminin, which favors neuronal differentiation. The ability to direct stem or progenitor cell differentiation via a chemically synthesized biomaterial, without the need to incorporate growth factors, offers many potential advantages in regenerative medicine.

The discovery of new classes of biomaterials may provide yet another opportunity to address clinical needs. Our laboratory has recently been investigating the utility of keratin biomaterials for regenerative medicine applications. Keratins are a large family of structural proteins found in the cytoskeleton and in the protective tissues of vertebrates. The hard keratins, as the name implies, form the more resilient structures such as hair, horn, and hooves. Those from hair and wool have been investigated as a source of biomaterials since the early 1900s. Early applications included wound healing and drug delivery [58]. More traditional biomaterials applications were developed beginning in 1982 [59–61]. Wound healing, drug delivery, TE, and medical devices have been the subject of continued keratin-based research over the past 20 years. Wound healing in particular has been the subject of patents granted to an international collection of inventors [62–66].

Keratin biomaterials also contain intrinsic sites of cellular recognition that mimic the ECM. It has been shown that in addition to the widely known RGD motif, the "X"-Aspartic Acid-"Y" motif on fibronectin (where X equals glycine, leucine, or glutamic acid and Y equals serine or valine) is also recognized by the integrin $\alpha4\beta1$. Keratin biomaterials derived from human hair contain these same binding motifs. A recent search of the NCBI protein database revealed sequences for 71 discrete, unique human hair keratin proteins. Of these, 78% contain at least one fibronectin-like integrin receptor-binding motif and 25% contain at lease two or more. Two recent papers have highlighted the fact that these binding sites are likely present on the surface of keratin biomaterials by demonstrating excellent cell adhesion onto processed keratin foams [67,68].

We have developed keratin-based biomaterials that demonstrate cell instructive capabilities. Certain keratin biomaterials have been shown to be mitogenic and chemotactic for a variety of cell types, and to mediate changes in gene expression consistent with the promotion of wound healing. We are working to elucidate the mechanistic basis for these activities.

2. Summary

It is evident from the foregoing examples that the diversity in biomaterials is immense. Many clever approaches to mimicking the structure, and more importantly, the function of the ECM have been devised. It is imperative that these important technologies continue to be investigated for their ability to interact in biological systems. An essential toolset has previously been described that will enable comprehensive evaluation of novel biomaterials in their host environment. Successful regulatory approval of new categories of regenerative technologies entering human clinical trials will continue to be based on these fundamental principles of biocompatibility and biological interaction.

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