Polymeric Biomaterials in Tissue Engineering

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ABSTRACT: Polymeric biomaterials are one of the cornerstones of tissue engineering. A wide range of materials has been used. Approaches have shown increasing sophistication over recent years employing drug delivery functionality, micropatterning, microfluidics, and other technologies. Challenges such as producing three-dimensional matrixes and rendering them deliverable through minimally invasive techniques have been addressed. A major recent development is the design of biomaterials for tissue engineering matrices to achieve specific biologic effects on cells, and *vice versa*. Much remains to be achieved, particularly in integrating other new technologies into the field. (*Pediatr Res* **63: 487–491, 2008**)

The number of polymeric or other materials that are used in or as adjuncts to tissue engineering has increased enormously over the past decade. Furthermore, a host of previously unrelated technologies such as micromanufacturing, high-throughput screening, drug delivery, surface modification, and nanotechnology have become integral to the biomaterial aspects of tissue engineering, and many approaches have used more than one of these tool sets. Progress has been extensive. This review will cover selected aspects of that progress.

BIOMATERIALS FOR TISSUE ENGINEERING

The basic types of biomaterials used in tissue engineering can be broadly classified as synthetic polymers, which includes relatively hydrophobic materials such as the α-hydroxy acid [a family that includes poly(lactic-co-glycolic) acid, PLGA], polyanhydrides, and others; naturally occurring polymers, such as complex sugars (hyaluronan, chitosan); and inorganics (hydroxyapatite). There are also functional or structural classifications, such as whether they are hydrogels (1), injectable (2), surface modified (3,4), capable of drug delivery (5), by specific application, and so on. The breadth of materials used in tissue engineering arises from the multiplicity of anatomical locations, cell types, and special applications that apply. For example, relatively strong mechanical properties may be required in situations where the device may be subjected to weight-loading or strain, or where maintenance of a specific cyto-architecture is needed. In others, looser networks may be needed or even preferable. The type of mate-

rials used is also dependent on the anticipated mode of application (open implantation *vs.* injection or minimally invasive procedure), the nature of any bioactive molecules that might be released, the need for surface functionalization, the needs of the cell types of interest in terms of porosity, and other issues. Despite this broad spectrum of potential materials, there are certain generic properties that are desirable.

Biocompatibility is clearly important, although it is important to note that "biocompatibility" is not an intrinsic property of a material, but depends on the biologic environment and the leeway that exists with respect to tissue reaction. For example, a formulation that is biocompatible in subcutaneous tissue might not be so in nerve or in the peritoneum (6). Local tissue reaction to the biomaterial of a construct may be harmful to the host and/or the construct, even in the absence of immune-mediated reaction to nonautologous cellular material. For example, the inflammatory reaction to relatively benign polymers such as the α-hydroxy acids (7), together with the acidosis that results from their breakdown, can lead to bony destruction and development of draining fistulae (8); here, the absolute mass of the biomaterial may play a role. Conversely, inflammation can lead to invasion of the construct by host cells, with untoward consequences for the transplanted cells. Similarly, the material must be neither cytotoxic nor systemically toxic. Therefore, it is important to be aware of the potential toxicity of the materials' breakdown products, as well as of residual unreacted cross-linking agents (*e.g.*, glutaraldehyde), reactive groups on polymers (*e.g.*, aldehydes, amides, hydrazides), and similar issues. Even quite benign matrices, adequate for drug delivery in sensitive environments such as the peritoneum can be composed of relatively cytotoxic precursors (9,10). Of note, a material's apparent lack of cytotoxicity does not necessarily predict biocompatibility. For example, a cross-linked chitosan that was minimally toxic to mesothelial cells *in vitro* caused marked adhesions when placed in the peritoneum (11).

A basic concept in tissue engineering is that the scaffold performs a time-limited architectural or other function but that, being foreign to the natural environment, it will disappear once that function has been fulfilled, leaving behind a viable purely biologic system. Consequently, many materials used in tissue engineering are biodegradable. Biodegradable materials are particularly likely to be used if drug delivery functionality is intended. However, it is not necessary that the biomaterial have this property, in part or in whole.

As alluded to above, the mechanical properties for biomaterials in tissue engineering are determined by the target

environment and delivered cells. In general, the properties of the construct should match those of the surrounding tissue: *e.g.*, relatively tough in bone, softer in pliable tissues. The properties will also be defined by the delivered cells' need for porosity for in-growth, delivery of nutrients, or protection from the environment, perhaps especially in the case of non-autologous transplants.

Cell adhesion properties are obviously important, in that cells must attach to the matrix. However, there are circumstances, such as in micropatterning of engineered constructs (12,13), where materials with lower cell adhesion can be alternated with materials with better cell adhesion to form desired shapes.

Numerous material properties are useful for specific applications. For example, electrically conductive polymers have been developed that could be useful in the tissue engineering of excitable tissues (14). Further examples of specially tailored materials will be encountered below.

DRUG DELIVERY FUNCTIONALITY

Biologic systems develop in a rich milieu of biologic signals. The native signal (*e.g.*, a growth factor) may be lacking in the construct or present in insufficient quantity, or it may be desirable to add an exogenous drug. In this context, "drug" means essentially any bioactive molecule, from small molecules to proteins (15) and nucleic acids (16). There has been a natural emphasis on the delivery of growth factors. While the construct is incubated *in vitro*, it is often sufficient to add the drug to the ambient medium, provided that the compound is capable of diffusing throughout. However, it may be desirable to maintain exposure to that drug after the construct is implanted *in vivo*. Biomaterials play a key role in achieving this in tissue engineering, drawing on experiences in the broader field of drug delivery. Many of the examples below are drawn from that experience, rather than the narrower field of tissue engineering. A number of drug delivery approaches and polymers have been used. (These are related to, but distinct from drug immobilization and surface modification, which will be dealt with separately.) Many investigators have incorporated drugs into constructs; both polymeric (17) and hydrogel-based systems have been used (15,17), although the former are often better at controlling drug release kinetics. The drugs can be incorporated directly into the scaffolds, *i.e.*, distributed throughout the polymeric matrix during casting, as is the case with many micro- and nanoparticulate drug delivery systems (18) and bulk hydrogels (19,20). Drugs can also be reversibly conjugated to the matrix covalently (21) or by other means. However, other methods of providing drug delivery to scaffold-type systems have arisen that might provide greater flexibility in the types and quantities of drugs that can be delivered. For example, micro- (22) or nanoparticles (23) containing drugs can be dispersed throughout a scaffold (Fig. 1). All these approaches have pros and cons, particularly in terms of the stability of the drug payload during and after device manufacture.

There has been considerable interest in developing means of delivering multiple compounds. In the case of growth
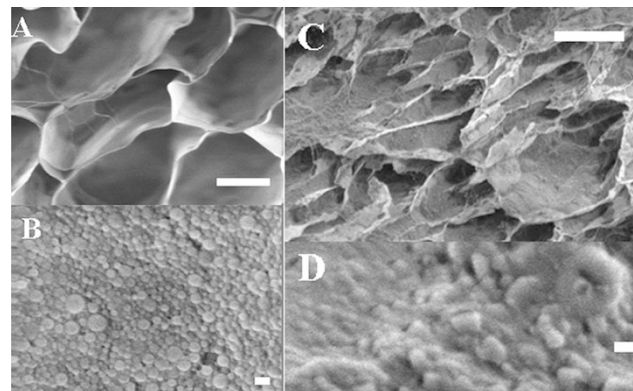

Figure 1. Scanning electron micrographs of a hydrogel, polymeric nanoparticles, and a composite. (*A*) Hydrogel of cross-linked hyaluronic acid (×2500). (*B*) PLGA nanoparticles (×33,000). (*C*) Hydrogel from *A* loaded with nanoparticles from *B* (×2500). Note the rougher surface. (*D*) Close-up of *C* (×33,000), revealing the nanoparticles. Scale bars are 10 μm (*A, C*), and 150 nm (*B, D*). (Courtesy of Dr. Yoon Yeo.)

factors, the rationale is particularly compelling in that most tissues are composed of more than one cell type, and sometimes, two factors work better than one. For example, polymeric scaffolds have been developed that simultaneously release vascular epithelial growth factor and platelet derived growth factor (24). Many of the methodologies described above could be suitable for achieving multiple drug release (*e.g.*, particles containing different drugs dispersed in a matrix). It is also possible to encapsulate many compounds within one particulate system (25).

Temporal control of drug release within constructs is important, either to deliver drugs in a pulsatile manner, or different drugs at different times. This could be achieved in a number of ways. For example, drugs could be entrapped within or beneath polymer layers of differing thicknesses or with differing degradation rates. They could be entrapped within separate populations of particles with differing release kinetics. Drugs could be contained within chip-like implantable devices that are programmed to release defined payloads in response to electrical stimuli (26) or polymer degradation (27). There is also an increasing literature on polymeric drug-releasing systems that respond to externally applied energy or forces, such as ultrasound (28), magnetism (29), and heat (30), or electricity (31). Polymers responsive to light and heat are discussed below.

POLYMERS FOR INJECTABLE TISSUE ENGINEERED SYSTEMS

Typically, tissue constructs are fully formed outside the body and then must be implanted surgically. There is, however, growing interest in being able to use minimally invasive methods such as laparoscopy, perhaps even simple injection (2). Ideally, for such applications, the entire construct should be of low viscosity (*i.e.*, easy to inject) when outside the body, but become more cohesive and gel-like once *in situ*. This attribute is particularly desirable if concurrent drug delivery functionality is intended, because the rate of drug release is related to viscosity.

Many approaches have been tried, in drug delivery and/or tissue engineering. One broad category includes polymers that cross-link physically, whether by hydrophobic, charge, or hydrogen bonding interactions, stereocomplexation, or supramolecular chemistry. One example within this category takes advantage of the difference in temperature between the inside and outside of the body, *i.e.*, thermogelling polymers. Triblock polymers of polypropylene oxide and polyethylene oxide (32) are among the most commonly used, relying on temperature-dependent hydrophobic interactions to change state; there are many other specific chemistries that achieve analogous goals. Polymers can also cross-link covalently. There are many potential chemistries. UV photopolymerization (33), and numerous small-molecule cross-linker mediated methods (34) have been used. However, it is worth noting that potential disadvantages for *in situ* use of UV photopolymerization are the need for additional equipment and perhaps physician squeamishness re UV irradiation, while with small molecule cross-linkers, the concern is the potential toxicity of residual unreacted reagents. The polymers themselves can be modified so that they cross-link covalently *in situ* (35). Here also there are numerous possible chemistries (36,37). Most commonly, the reaction schema involves two polymers (or two modifications of the same polymer) with complementary reactive functional groups that form a covalent bond when mixed, forming a gel. Aside from the relatively sophisticated chemistry involved, the principal potential disadvantages of these methods relate to the facts that the two prepolymers may have to be kept separated until use (*e.g.*, in a double-barreled syringe), and that the gelation process could occur within the delivery device (*e.g.*, needle). Injectability can also be provided by formulating the tissue construct as microspheres. For example, large porous biodegradable polymeric microspheres have been produced that could have potential for delivering cells (38). Also, polymeric microspheres have been surface-modified, allowing chondrocytes to adsorb to their surfaces (39) and subsequently delivered into the articular space.

One interesting type of polymer that could be of use for minimally invasive deployment of structured constructs is that with shape memory (40), *i.e.*, the capacity to revert to a predetermined shape in response to a defined cue. For example, there are thermoplastic biodegradable polymers that can change their shape upon heating. Potentially, this could reduce the aspect of a relatively bulky object, allowing it to be implanted *via* a small incision then expanding to its full geometry. Polymers have also been developed that can undergo similar shape changes in response to light (41), or that can undergo more than one shape change in response to graded temperature increases (42). In the latter case, one shape change is mediated by physical cross-links, the second by covalent links, or the rupture thereof.

SURFACE MODIFICATION OF POLYMERS FOR TISSUE ENGINEERING

Cells are sensitive to the environment in which they exist (43,44), responding to chemical cues, and morphologic aspects of the surfaces with which they are in contact. Although the common synthetic polymeric scaffolds have great advantages in terms of their manufacturing process and the reproducibility of their degradability and other properties, they suffer from a lack of those finer cues. One approach to providing them is by modifying their surfaces (3,4), by physical adsorption of compounds, or by chemical modification. Apart from immobilizing proteins or other compounds for specific biologic effects (*e.g.*, adhesion), this approach can also be used to increase polymer hydrophilicity to repel proteins and perhaps reduce the tendency toward adhesion (*e.g.*, in the peritoneum). Another common type of surface modification is micro- or nanopatterning to create structured cellular arrays (13) and influence cell behavior (45).

THREE-DIMENSIONAL POLYMERIC MATRICES

There has been a growing realization of the importance of three-dimensionality in engineered tissue constructs. One approach, which was largely driven by considerations relating to engineering large organs with, for example, complex issues of nutrient and oxygen delivery and waste removal (*i.e.*, need for a microvasculature), and by the need to pattern multiple cells types in precisely defined structures, relied on advanced fabrication tools to establish those matrices (46). A variety of polymeric materials have been patterned in 3-D using microfabrication and similar technologies, based on designs established by computational fluid dynamics. Early work using micropatterned silicon (47) evolved into micromolded polymer networks (48), which could be stacked into macroscopic 3-D networks (49,50). Such devices have been made with relatively hydrophobic polymers such as PLGA (49), as well as with hydrogels such as calcium alginate (51). These devices are amenable to other modifications that have been addressed here, such as drug delivery and surface modification.

Other approaches have focused less on tightly defined ultrastructures and more on nanoscale biologic interactions. Self-assembling nanofibrillar networks have been developed that incorporate biologic determinants (*e.g.*, peptide sequences) for molecular recognition or cell interactions (52,53) and have been shown to guide cell differentiation (53). The latter systems were composed of self-assembling amphiphilic peptides. Polymer systems have been used in a somewhat similar way, albeit with a different biologic approach. Synthetic hydrogels have been developed that degrade in response to the proteolytic activity of migrating cells (54), producing three-dimensional networks suitable for angiogenic in-growth. This approach is of interest in that here, the cells respond to the matrix, and the matrix responds to the cells.

One rationale for the need for a nanoscale level of architecture is that that is the size scale within which fibrils occur in the extracellular matrix. There is evidence that nanoscale topography affects cell behavior in many ways, including shape, proliferation, migration, and gene expression (55–58). A wide variety of polymers have been used for these applications (45), often made into nanofibers by electrospinning (59). The conditions required for this process may denature naturally occurring polymers.

BIOMATERIALS AS BIOACTIVE AGENTS

From the preceding, it is clear that in tissue engineering, the underlying scaffold or matrix is not an inert entity, providing only support, adhesion, and other mundane functions. (It bears mentioning, however, that the view that scaffold materials are "bio-inert" is still remarkably prevalent.) Although tissue-engineering matrices often have to be used with drug delivery and surface modification methods to attain specific biologic goals, it is still true that those biomaterials can be quite active, both indirectly and directly. Indirect actions can occur through inflammation and similar events, which recruit a range of inflammatory cells, which themselves can secrete molecules (cytokines and chemokines) with complex biologic sequelae. This might, for example, be one factor in the effectiveness of some particle-based vaccine delivery systems (60). Direct action of putatively inert materials is seen in the following examples relating to carbohydrate-based matrices. In the cross-linked chitosan mentioned above that caused severe peritoneal adhesions, it was found that the material itself caused marked increases in the expression of MIP-2 (a murine IL-8 analogue) and tumor necrosis factor alpha (TNF-α) in mesothelial cells (11). A cross-linked hyaluronic acid (35) used to prevent peritoneal adhesions (61) was shown to cause a small increase in tissue plasminogen activator in mesothelial cells, which might have contributed to the anti-adhesion efficacy.

Much research has imparted specific functionalities, such as encouraging proliferation or differentiation, to biomaterials by grafting, incorporating or delivering molecules with defined biologic functions. An alternative approach might have been to use rational design to construct biomaterials with those functions, but that is very difficult, although it is a goal of some aspects of glycomics (62)—the study of information encoded in the structure of carbohydrates—and structural biochemistry. Instead, som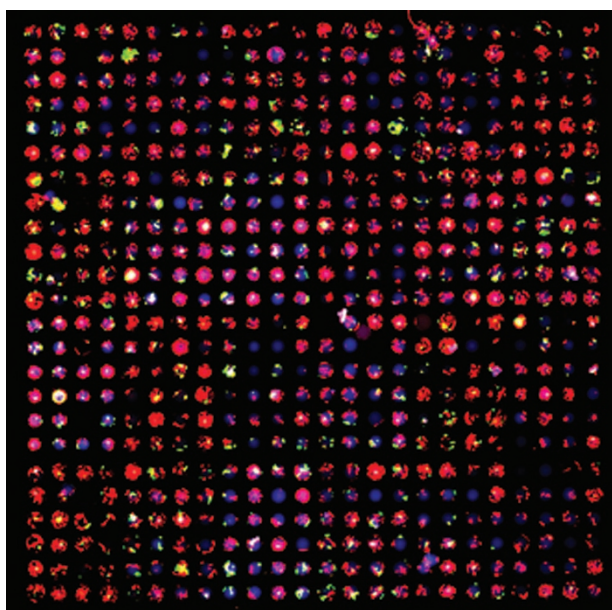e investigators have used high-throughput methods to perform rapid nanoliter-scale synthesis of thousands of potential biomaterials, followed by screening of the thousands of cell-polymer interactions on chip-like systems (63) (Fig. 2). This approach revealed that some polymers were markedly better than others in making human embryonic stem cells differentiate into epithelial cells. Furthermore, the various polymers had widely differing effects on the cellular response to a bioactive molecule, retinoic acid. It is possible, as has been suggested, that the effects of the biomaterials were actually mediated by adsorbed proteins (64) or other factors, but that does not alter the fact that the variable driving the differences in cellular response was the variety in polymeric structure. It is possible that approaches of this sort will allow for rapid identification of biomaterials—with or without addition of specific ligands—for specific tissue engineering applications.

LOOKING AHEAD

Although there has been impressive progress in the material science underlying tissue engineering in the past few years, enormous tasks remain. "Convergence" (65)—the melding of seemingly disparate scientific fields—is already an integral part of the field, and it is to be expected that that tendency will increase, incorporating features from electronics, computer science, bio-microelectromechanical systems (bio-MEMS) and similar fields. As constructs become more sophisticated, "smart" materials (66) such as switchable surfaces (67) may be use to create constructs with state-dependent cellular behaviors. Work will continue in the daunting task of producing multi-cell type constructs (68) and integrating them into an immunocompetent host. It will also be important to find means of preventing biofouling, both by adsorbed molecules and by inflammatory cells, which can suffocate the construct or prevent it from releasing its biologic products (*e.g.*, in the case of encapsulated pancreatic islet cells). On a related note, biomaterial permselectivity will have to be optimized, which will involve determining whether it is sufficient to keep the cellular components of the host immune response at bay, or whether humoral factors must also be kept from the donor cells. Important formulational issues remain to be dealt with, such as creating delivery systems with gradients across a matrix, but the seemingly pedestrian issues of drug stability are also likely to be challenging. A deep understanding of all aspects of the biology of tissues, including embryogenesis, will continue to be crucial. We are far from being able to create matrices that are rationally designed from the ground up to perform specific biologic task with the same specificity that is found in biomolecules. However, as is seen in some of the work described above, and in the rise of fields of study such as glycomics, that objective is perhaps on the horizon.

Figure 2. Human embryonic stem cell differentiation on a chip, in this case with 576 polymers. Green = cytokeratin, red/purple = vimentin, blue = polymer. (Courtesy of Dr. Daniel G. Anderson.)

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