BIOMATERIALS

Designing Regenerative Biomaterial Therapies for the Clinic

E. Thomas Pashuck[1,2] and Molly M. Stevens[1,2,3]*

The ability to regenerate damaged tissue is one of the great challenges in biomaterials and medicine. Successful treatments will require advances in areas ranging from basic cell biology to materials synthesis, but there have been major hurdles in translating the biomedical advances, such as scaffolds that direct stem cell differentiation, into marketed products. Careful consideration of the challenges going from bench to bedside is paramount in maximizing the chances that a good idea becomes a good treatment. We look at a variety of material-based platforms that have made it into the clinic, from biodegradable polymers for wound healing to organs grown ex vivo, and how they have been able to navigate the scientific, regulatory, and business hurdles into the market place.

THE ROLE OF REGULATION: FROM THE LAB TO THE CLINIC

Society recognizes the importance of medical advances and has invested heavily in biomedical research. Babies born today will live almost 3 decades longer than those born in 1900 (*1*), and from 1960 to 2000, the life expectancy of a newborn increased by almost 7 years, at least half of which is due to medical advances (*2*). In addition, chronic disability among the elderly has decreased by almost one-third, showing that medicine has improved not only the length of a person's life but also the quality. To further these advances, pharmaceutical, biotechnology, and medical device companies spend more than $60 billion per year on research and development worldwide, and more than $100 billion per year is spent on biomedical research by the U.S. government, private industry, and charities (*3*). Translating research into a product is increasingly expensive, with pharmaceutical companies currently spending more than $850 million on research and development for each successful new drug or biologic, which goes up to almost $1.8 billion after capitalization (*4*).

Given that these costs are rising at an unsustainable rate, the future structure of the pharmaceutical industry is uncertain (*5*). Within the biotech industry, regenerative medicine products have seen rapid expansion and have been used in more than 300,000 patients (*6*). There have also been commercially profitable devices, such as INFUSE from Medtronic, a spinal fusion device that has registered $750 million annually in sales (*7*). This is a change from the doldrums of the early 2000s, when exuberance about the potential of tissue engineering led to a surge in funding high-risk biotech start-ups, in which investors subsequently over a billion dollars (*8*). With added experience and more realistic aims, regenerative medicine products have entered the marketplace, and promising research continues with better focus.

The field of regenerative medicine presents a challenge for large pharmaceutical companies who have spent decades putting small-molecule drugs through a well-established U.S. Food and Drug Administration (FDA) regulatory process and then manufacturing large quantities of successful drugs at enormous profit margins. However, research in biomaterials, molecular biology, and stem cell biology is advancing rapidly, generating novel therapeutic approaches that fall outside the standard pharmaceutical model and, increasingly, outside well-developed regulatory pathways (*9*). Despite these hurdles, the future of regenerative medicine seems likely to continue to move away from simple small-molecule drugs toward increasingly complex biologic and cell-based therapies.

In most countries, recognized regulatory bodies must approve biomedical devices and drugs before they can be used to treat patients. There are a variety of organizations that provide accreditation in different parts of the world, but this review will be focused on the U.S. FDA, given that the United States accounts for 81% of research and development investment in tissue engineering and stem cell products (*7*). The FDA assigns prospective therapies to one of three regulatory bodies: the Center for Drug Evaluation and Research (CDER), the Center for Biological Evaluation and Research (CBER), and the Center for Device and Radiological Health (CDRH) (Fig. 1A). The CDER handles small-molecule drugs as well as therapeutic proteins, antibodies, and immunomodulators. "Biological" products consist mostly of viruses, toxins, vaccines, and blood components, but within the CBER, there is an Office of Cellular, Tissue and Gene Therapy (OCTGT), which has purview over all cells, tissues, gene vectors, and tissue-engineered products. Medical devices are regulated by the CDRH and are subdivided into three different categories: class I, which includes things like medical examination gloves that are used in an ancillary way and are lightly regulated; class II, which are defined as having "moderate risk"; and class III, which are more heavily regulated and include devices that improve or sustain life. Therapies that have separate components from more than one category are called combination products and are assigned to one of the three main centers, but also interact with the Office of Combination Products (OCP). As synthetic biomaterials become increasingly bioactive and small molecules can be designed to self-assemble into biomimetic scaffolds, the lines between drugs, biologics, and devices have become increasingly blurred.

The choice of which FDA center to regulate products in the gray area of biomaterials and tissue engineering has important financial consequences because the time and costs associated with approval via different centers can vary considerably. The FDA defines a device as something that does not "achieve its primary intended purposes through chemical action within or on the body of man or other animals

[1]Department of Materials, Imperial College London, London SW7 2AZ, UK. [2]Department of Bioengineering, Imperial College London, London SW7 2AZ, UK. [3]Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, UK.
*To whom correspondence should be addressed. E-mail: m.stevens@imperial.ac.uk

and which is not dependent upon being metabolized for the achievement of its primary intended purposes," with chemical action being defined as "chemical reaction or intermolecular forces or both" (*10*). So, although a drug-eluting stent would be classified as a combination device, a degradable scaffold incorporating a bioactive molecule could also fall under this classification.

This has major implications in scaffold design because the difference between having a product regulated as a biologic instead of a device adds to the regulatory process and cost (Fig. 1, B and C). Getting premarket approval (PMA) for a new medical device can cost between $45 million and $150 million and typically takes less than 5 years (*11*) (Fig. 1C), whereas getting a new drug or biologic through their respective centers can cost hundreds of millions of dollars and typically takes 5 to 10 years (*12*) (Fig. 1B). Thus, the regulatory pathway is extremely important in translating biomaterials into the clinic and should be considered at the earliest stages of system design. Most advanced therapies in regenerative medicine will have to go through the CDER or CBER, which will require interest from investors who aim to earn a profit. Aspects of the biomaterial design that substantially increase the cost of clinical trials or the final product will reduce the desirability of the technology to investors, making it less likely to attract the funding needed for translation from bench to bedside.

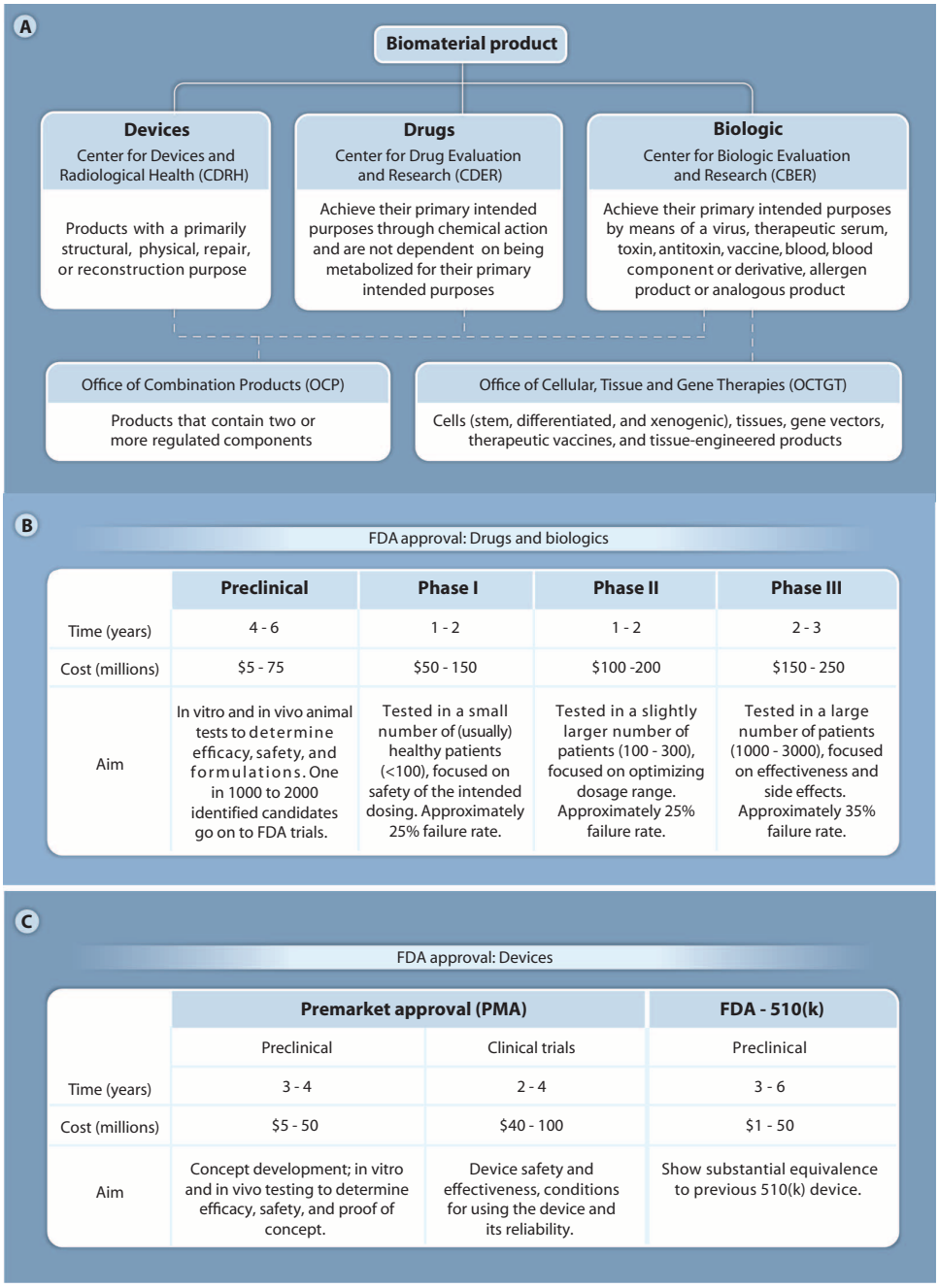

Fig. 1. Biomedical regulatory framework in the United States. (**A**) Organization of the centers and offices of the FDA. (**B**) Path to the clinic for drugs and biologics. (**C**) Path to the clinic for devices follows either PMA or the 510(k) process.

CREDIT: Y. HAMMOND/*SCIENCE TRANSLATIONAL MEDICINE*

DESIGNING MATERIALS FOR CLINICAL SUCCESS

In biomedical research, potential therapies are typically first screened in vitro for biological activity and toxicity, and then promising candidates are further tested in vivo. Efforts are under way to increase the fidelity of these steps (*13*), and there is reason to believe that biomaterials can be optimized to reduce the chances of unwanted side effects. Bioactive materials, in which either the material itself or a released factor elicits an effect, increase the chances of local regeneration while decreasing the likelihood of negative effects elsewhere. Devices typically go through a less extensive testing process to achieve FDA approval because the likelihood of unwanted systemic side effects is decreased in a device with local, mechanical effects as opposed to systemic drugs or biologics. Any negative reaction is typically confined to the area around the device, which is more easily detected in preclinical development. In vivo studies are performed in the animal model that most closely resembles the human condition, but these species may differ from humans in other organ systems, which can lead to unexpected toxicities during clinical trials. Some side effects are also difficult to diagnose in animal models, even when present or not present at all (*14*). These advantages and disadvantages emerge from the very earliest stages of scientific development and should be considered when moving forward in the design of acellular and cell-based biomaterials.

REGULATION OF ACELLULAR BIOMATERIALS

Cell-free biomaterials can be manufactured from polymers to ceramics to metals and vary from simple collagen gels to complex scaffolds that actively respond to their environments. Because they do not contain cells, they can often be used "off the shelf" and are generally less expensive than cell-based therapies. For regulatory purposes, the classification of cell-free biomaterials depends on the primary mechanism of action through which the treatment works. An injectable colloid composed of peptides that inhibit angiogenesis around a tumor might be classified as a drug, whereas a comparable treatment consisting of injected microspheres that physically occlude tumor vasculature would be considered a medical device (*15*). Biomaterials can also exploit physical phenomenon, such as mechanical properties, pore size, and roughness, to influence cell behavior and, in turn, the bioactivity of proteins and how biomolecules are tethered to the matrix (*16*). The physical properties are especially important in devices because they are not "chemical" in nature but can still be used to influence cell behavior without being classified as a drug.

Biomaterials that are regulated as devices can take three general paths to the clinic. High-risk devices typically require PMA, which involves controlled clinical testing to demonstrate the safety and efficacy of the device (*17*), the cost of which can range from $45 million to $150 million (*18*) (Fig. 1C). Only 1% of medical devices enter the market through the PMA process. The second, increasingly common route in which new devices enter the U.S. market (accounting for 31% of new devices) is through premarket notification (PMN). These devices are commonly referred to by its section in the Safe Food and Drug Act as "510(k) devices" (*17*). Costing between $1 million and $50 million to develop (*11*), 510(k) devices must be substantially equivalent to an already marketed device called the predicate, which are generally other 510(k) devices (most PMA devices do not qualify as predicates) (Fig. 1C). The FDA has adopted a broad interpretation of "substantial equivalence," and certain changes, such as switching a wound dressing from collagen to extracellular matrix (ECM), have been approved through the 510(k) process without the requirement for additional clinical data. Because of the limited requirement for premarket clinical data, the 510(k) process is faster and less expensive than the PMA pathway (*17*), and as the number of predicate devices increases, the range of technologies that will be brought to the market through this path is expected to grow. Most medical devices (67%), including items such as tongue depressors, are considered low-risk, meaning they are not life-supporting or life-sustaining, and generally do not require either PMA or PMN.

MATERIAL EFFECTS ON CELLS

There are many ways in which cell behavior is influenced by the extracellular environment (Fig. 2A), and acellular biomaterials have been developed to exploit these signaling mechanisms. The most common approach to improving the bioactivity of a material is by increasing cellular adhesion. This is most often done by adding an Arg-Gly-Asp (RGD) peptide sequence to mimic the integrin-signaling domain of fibronectin, but can also include other proteins that bind the cell adhesion integrin receptors, or targeting other cell adhesion molecules, such as syndecans (*19*) (Fig. 2B). Recent advances in engineered biomaterials have increased bioactivity through multivalent interactions (*20*) or recombinant fibronectin fragments that include both adhesion and growth factor binding motifs (*21*). Another popular method to influence cell behavior is to include proteins that bind growth factor receptors, such as vascular endothelial growth factor (VEGF) or fibroblast growth factors (FGFs) (*22*). Other surface receptors, such as the heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptor, which typically binds small nonprotein molecules like acetylcholine, have also been targeted for applications such as neural repair (*23*).

Cell-cell interactions are an important part of many tissues, and although acellular biomaterials lack the native tissue signals to promote cell-cell interaction, these can be mimicked by functionalizing a scaffold with surface molecules such as cadherins (*24*) or eph-ephrins (*25*) (Fig. 2A). The ability to influence cell behavior extends below the cell surface, to the nucleus. The process of transcription is tightly controlled in cells, and although few materials have targeted histone modifications or transcription factors directly, these processes may prove to be important in opening up native cells to significant phenotypic changes (*26*). For instance, mesenchymal stem cells (MSCs) in aged mice show increased histone methylation and decreased osteogenesis, suggesting that biomaterial therapies for bone repair that include MSCs can target histone demethylases, especially in older patients (*26*). Finally, a complex web of coding and noncoding RNA exists (Fig. 2A) and is a promising target with a single RNA sequence capable of affecting the expression levels of hundreds of mRNA. RNA delivery is challenging because it must be protected from enzymatic degradation and delivered inside the desired cell population, but combinatorial synthesis has led to the discovery of delivery materials that allowed for silencing target genes in nonhuman primates (*27*).

In the past decade, biomaterial design has advanced in complexity and in its ability to modulate cell behavior through a variety of different mechanisms, many of which are depicted in Fig. 2B. Materials can present cells with biological cues to encourage certain synergistic signaling mechanisms for therapeutic applications. Because these cues are spatially localized in the biomaterial, they will be less likely to interact with cells in other parts of the body, which reduces the chance of off-target effects. Using physical characteristics to influence cell behavior, such as mechanical stiffness (*28*), geometry (*29*), substrate alignment (*30*), or hydrophobicity (*31*), rather than chemical cues [as defined by the FDA (*32*)], can help improve the bioactivity of a device and its clinical use while retaining the regulatory path to the market as a traditional medical device. This offers the possibility of providing complex signaling to cells in a local environment without necessitating the expensive testing to assess the potential systemic toxicity of a drug or biologic.

BIODEGRADABLE POLYMERS: LONG HISTORY, BRIGHT FUTURE

Polymeric biomaterials, ranging from synthetic polymer scaffolds to naturally derived biopolymers, are the most commonly used class of material in regenerative medicine (*33*). Poly(α-hydroxy acids), including poly(glycolic acid) (PGA), different isomeric forms of poly(lactic acid) (PLLA and PDLA), and poly(lactic-*co*-glycolic acid) (PLGA) copolymers, have been used clinically since PGA sutures were approved by the FDA in 1969 (*34*). They are biocompatible, although PGA can cause local inflammation. These polymers have been fabricated into a variety of microstructures, including porous polymer matrices, nonwoven

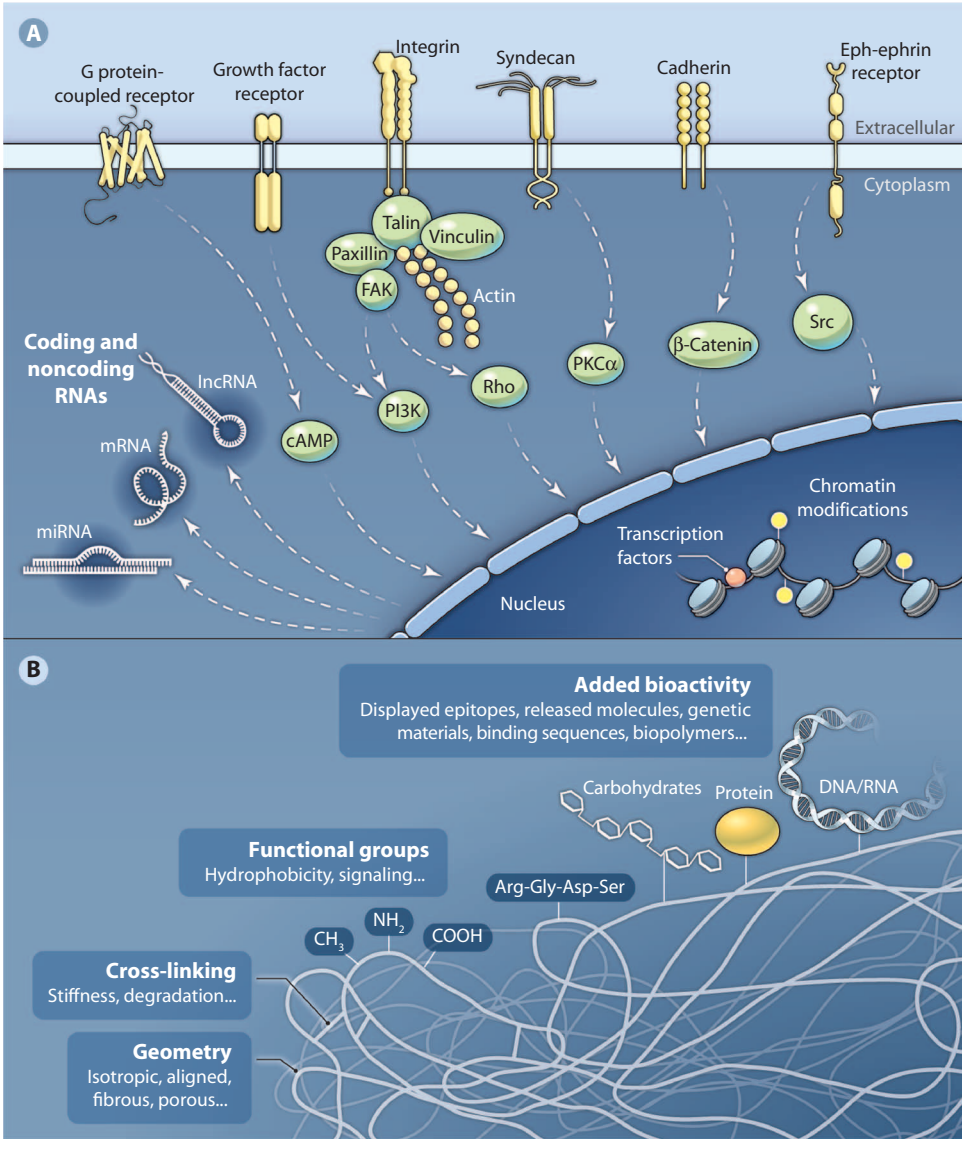

Fig. 2. Mechanisms regulating cell behavior and modifiable aspects of biomaterials. (**A**) Cell behavior is influenced by a variety of extracellular signals and then tightly regulated inside the cell. Different classes of surface receptors are used to bind diffusible molecules (for example, G protein–coupled receptor and growth factor receptors), for cell adhesion (for example, integrins and syndecans), or to bind receptors on other cells (for example, cadherins and eph-ephrins). Once these surface receptors are activated, the signal is propagated through intracellular pathways and into the nucleus. Transcription is controlled in a variety of ways, including transcription factors and chromatin modifications, and several noncoding RNAs regulate gene expression. (**B**) Scaffold functionalities that can be altered to improve bioactivity. Some of these, such as geometry, mechanical properties, signaling displayed functional groups, and degradation rate, are inherent properties of materials and are less likely to achieve their effect through chemical means. Incorporating proteins, nucleic acids, biopolymers, or aptamers can also increase the bioactivity of the scaffold.

fabrics, and electrospun scaffolds (Fig. 3A) (*35*, *36*). The versatility and biocompatibility of poly(α-hydroxy acids) have led to a variety of clinical applications over the past 40 years, including as a bone void filler (InQu, ISTO Technologies) and as woven surgical meshes (X-Repair, Synthasome Inc.)—both of which were cleared through the 510(k) process. Synthetic polymers typically lack cell adhesion sites, although there is surface chemistry–dependent adsorption of proteins and biopolymers to the implant surface. Functionalization with peptide sequences can target a single cell–integrin pair (*37*), or can bind many of them, because the RGD sequence binds 8 of the 24 integrin dimers (*38*). Poly(α-hydroxy acids) do not have functional groups along their chain, so adding cell adhesion sequences requires either surface treatments or incorporation of other monomers with functional groups (*33*). The inclusion of other monomers often changes the well-studied properties of the polymer, and the presence of new degradation products (and bioactive sequences) will increase regulatory scrutiny.

As our understanding of the complex nature of ECM increases, efforts have been made to mimic it using three-dimensional (3D) hydrogels. Polymeric hydrogels benefit from minimally invasive delivery and can be injected into the desired site and gelled in situ. This way, they are able to fill in complex geometries, and the easy delivery reduces the chances of local injury associated with surgical implantation. Hydrogels generally lack the mechanical properties needed for locations that are load bearing or require significant resistance to strain and are most often studied in soft tissue applications. The first class of materials studied for these applications are synthetic polymers, which have well-defined chemistries and are easily modified. An example of this is poly(ethylene glycol) (PEG), a food additive that has been used pharmaceutically to increase the half-life and reduce the immunogenicity of drugs. Most polymers, including PEG, are gelled through cross-linking. The amount and types of these cross-links control the mechanical properties of the gel, as well as the degradation mechanism and rate (*39*) (Fig. 3B). In vitro efforts have created gels that recapitulate many aspects of the native milieu, through physical means and the incorporation of a wide variety of bioactive signals, making it possible that these could exert a significant influence on both delivered cells and those in the local environment (*40*). Cross-linked PEG hydrogels are getting closer to the clinic; for example, GelrinC (Regentis Biomaterials), a PEG diacrylate with fibrinogen (*41*), which allows for enzymatic degradation, is currently in FDA trials to assess safety and performance (NCT00989794). In a recent pilot study in humans, a PEG–hyaluronic acid hybrid was developed for soft tissue reconstruction that was photocross-linked in situ and degraded enzymatically (*42*).

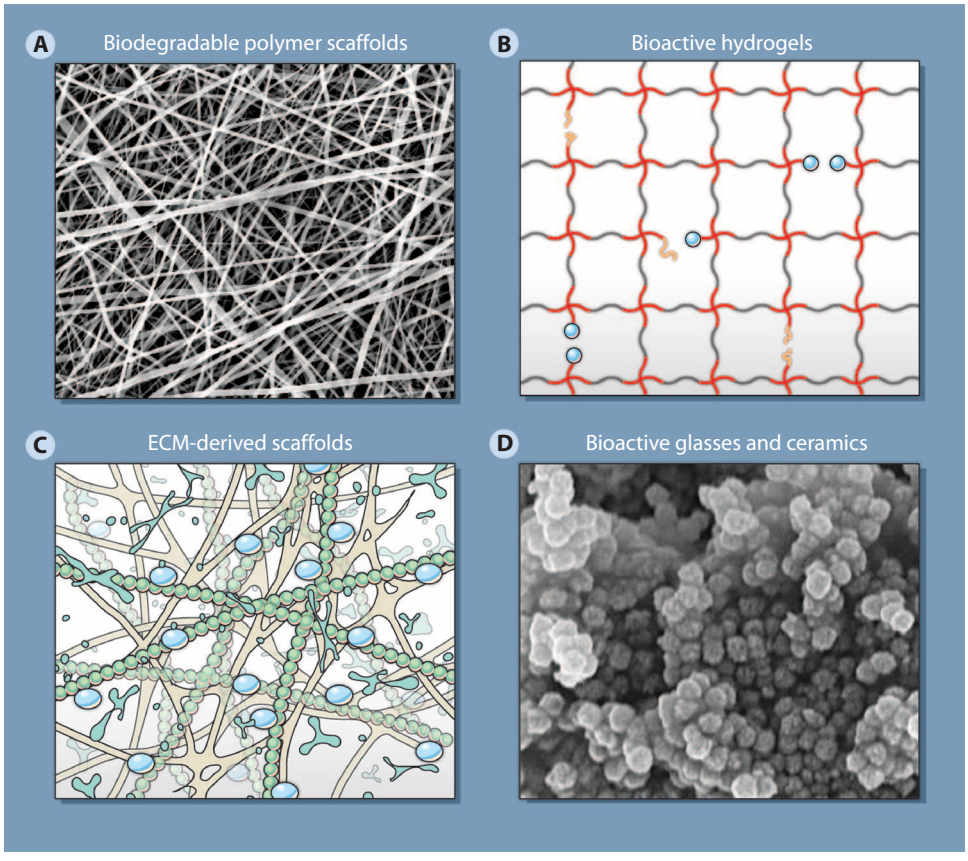

Fig. 3. Materials common in regenerative medicine. (**A**) Polymer scaffolds can use materials with controlled degradation over the course of months and that have previous FDA approval in different device presentations. (**B**) Hydrogels can benefit from minimally invasive delivery and easy modifications to include bioactive cues and enzymatic degradation. (**C**) ECM-derived scaffolds can include a variety of scaffold proteins, like collagen, GAGs, and growth factors, which have been used extensively in the clinic for wound repair. (**D**) Bioactive glasses and ceramics are designed to stimulate bone growth in vivo.

Most materials induce gelation through covalent cross-linking. However, there is a growing class of biomaterials that uses noncovalent interactions to create a self-assembled, physically cross-linked hydrogel (*43*). These hydrogels tend to be completely synthetic, injectable, and often gel under physiological conditions in the body, which reduces the need for chemical cross-linkers or other initiators. Self-assembling peptides constitute the largest class of supramolecular biomaterials, with facile addition of bioactive peptide epitopes or binding sequences, and have been used preclinically for applications such as wound repair (*44*), cardiac repair (*45*), and spinal cord injury (*46*). They are mostly composed of amino acids (thus having natural degradation products) and are generally nonimmunogenic (*47*). The regulatory status of these molecules will likely depend on whether they achieve their intended effect through physical means, which would make them a device, or through displayed ligands or released growth factors, which would likely lead to a drug classification. Knowing the classification is key to designing preclinical studies and thus reducing regulatory headaches in later stages (*9*).

One approach to creating bioactive hydrogels without having to specifically modify the material is to use natural biopolymers. Some of these, such as hyaluronic acid, have been used clinically for over 30 years for applications including osteoarthritic knee pain (Table 1) (*48*). Natural biopolymers have the advantage that they can recapitulate some of the functions these molecules have in the body because many integrins bind collagen and growth factors commonly have heparin-binding domains. Because many are present in the body, the unmodified versions do not elicit an immune response, and they can be removed through intrinsic biodegradation pathways. However, most of these require some form of cross-linking to form stable hydrogels. These products are used in the clinic as cross-linked collagen for diabetic foot ulcers (Excellagen, Cardium Therapeutics) and bone putties (Integra Mozaik, Integra LifeSciences), which also include tricalcium phosphates (TCPs). Biopolymers are typically used as cross-linked hydrogels but can also be combined with synthetic polymers (*49*) or modified into a processable material, such as Hyalomatrix (Anika Therapeutics), a benzyl-esterified version of hyaluronic acid. A list of representative regenerative products in the clinic and clinical trials can be found in Table 1.

From self-assembled peptides to synthetic hydrogels, polymers are the broadest class of biomaterials and are likely to be the one that will find the most applications in the clinic. Extensive modifications of the chemistry and molecular architecture allow for substantial control over the physical and mechanical properties of the biomaterial, which can be made responsive to temperature, pH, or presence of enzymes (Figs. 2B and 3B). These polymers can fill unmet needs in regenerative medicine by providing a more controlled environment within the body that can guide local tissue regeneration, responding to local events while suppressing unwanted signals.

ECM-DERIVED BIOMATERIALS

Although efforts are under way to create hydrogels that mimic the cells' native milieu, a different approach is to remove the cellular components from tissue, leaving the protein and glycosaminoglycan (GAG)–rich ECM scaffold, in a process known as "decellularization" (Fig. 3C). ECM was initially thought to serve primarily as a physical scaffold for cells (*50*), and acellular ECM-based regenerative products have accordingly been classified as devices by the FDA for applications in dermal repair. Current understanding of the cell-ECM relationship reveals a more complex and dynamic process, with the ECM displaying bioactive epitopes, cryptic binding sites, and the ability to bind, display, and potentiate the activity of growth factors (*51*). Cells remodel ECM extensively throughout development (*52*) and homeostasis (*53*).

Table 1. Representative commercial biomaterial products in the clinic and in preclinical development. Data were obtained from Place *et al.* (*83*), Jaklenec *et al.* (*7*), http://www.fda.gov, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm, and http://www.clinicaltrials.gov.

Company	Product	Application	FDA center	Regulatory pathway	Product description
Acellular biomaterial therapies					
ACell	MatriStem	Wound repair	CDRH	510(k)	Porcine ECM
Anika Therapeutics	Hyalomatrix	Wound repair	CDRH	510(k)	Benzyl ester of hyaluronic acid and a semipermeable silicone membrane
Baxter	Actifuse	Bone void filler	CDRH	510(k)	Silicon substituted hydroxyapatite mixed with a resorbable polymer
BioMimetic Therapeutics	Augment bone graft	Hind foot and ankle fusions	CDRH	PMA submitted	β-TCP particles and recombinant human platelet-derived growth factor-BB
BioTissue Technologies	Prokera	Ophthalmic applications	CDRH	510(k)	Amniotic membrane sheet
Cardium Therapeutics	Excellagen	Diabetic foot ulcers	CDRH	510(k)	Bovine collagen gel
Cook Biotech	Oasis Wound Matrix	Wound repair	CDRH	510(k)	Porcine small intestinal submucosa
Cordis	Cypher	Vascular stent	CDRH	PMA	Steel stent coated in rapamycin-eluting polymer
CryoLife	CryoValve SG	Pulmonary heart valve replacement	CDRH	510(k)	Decellularized allogeneic donor valve
Cytomedix	Autologel	Wound repair	CDRH	510(k)	Platelet-rich plasma with ascorbic acid and calcified thrombin
Exactech Inc.	Optefil	Orthopedics	CDRH	510(k)	Demineralized bone matrix with gelatin
Genzyme	Synvisc-One	Osteoarthritic knee pain	CDRH	PMA	Cross-linked hyaluronan
Integra LifeSciences	Integral Dermal Regeneration Template	Full-thickness or deep, partial-thickness thermal injuries	CDRH	PMA	Bilayer scaffold with a collagen-GAG inner later and a silicone outer layer
Integra LifeSciences	Integra Flowable Wound Matrix	Tunneled and difficult to access wound sites	CDRH	510(k)	Granulated, cross-linked bovine tendon collagen and GAGs
Integra LifeSciences	Mozaik	Bone void filler	CDRH	510(k)	Collagen with β-TCP
ISTO Technologies	InQu	Bone void filler	CDRH	510(k)	PLGA with hyaluronic acid particles
Mesynthes	Endoform	Wound dressing	CDRH	510(k)	Ovine collagen matrix
Medtronic	INFUSE Bone Graft	Spinal fusion	CDRH	PMA	Metal cage with bovine type I collagen sponge containing rhBMP-2
MiMedx	HydroFix	Vessel cover during anterior vertebral surgery	CDRH	510(k)	Poly(vinyl alcohol) sheet
Neomend	Progel	Surgical sealant	CDRH	PMA	Succinate-modified PEG and human serum albumin surgical sealant
NovaBone Products	PerioGlas	Fill oral, dental intraosseous, and craniofacial defects	CDRH	510(k)	Calcium phospho-silicate bioactive glass
Olympus Biotech	OP-1 Putty	Lumbar spinal fusion	CDRH	Humanitarian Device Exemption	Graft material containing bovine collagen and rhBMP-7
Q-Med	Deflux	Vesicoureteral reflux	CDRH	PMA	Dextranomer and hyaluronic acid hydrogel
Regentis Biomaterials	GelrinC	Cartilage repair	CDRH	Phase 1/2	PEG diacrylate and denatured fibrinogen implant
Synovis	Vascu-Guard	Peripheral vascular patch	CDRH	510(k)	Decellularized bovine pericardium
Synthasome	X-Repair	Soft tissue reinforcement during surgery	CDRH	510(k)	Woven PLLA surgical mesh

Table 1 continued on page 7

Company	Product	Application	FDA center	Regulatory pathway	Product description
TEI Biosciences	PriMatrix AG	Wound repair	CDRH	510(k)	Decellularized fetal bovine dermis scaffold with ionic silver
Tornier	Conexa	Rotator cuff repair	CDRH	510(k)	Porcine dermal matrix
Wright Medical Technology	GraftJacket	Tendon and ligament repair		Unregulated	Decellularized human dermis
		Cell and material therapies			
Forticell	OrCel	Burn injuries	CDRH	PMA	Allogeneic keratinocytes and fibroblasts on bovine collagen
Genzyme	MACI	Symptomatic cartilage defects of the femoral condyle	CBER	Phase 3	Autologous chondrocytes cultured on a collagen membrane
Organogenesis	Apligraf	Diabetic foot ulcers	CDRH	PMA	Bilayer scaffold with human foreskin–derived neonatal fibroblast/bovine type I collagen matrix layer and a foreskin-derived neonatal epidermal keratinocyte layer
Organogenesis	Gintuit	Mucogingival conditions	CBER	BLA	Bilayer scaffold with human foreskin–derived neonatal fibroblast/bovine type I collagen matrix layer and a foreskin-derived neonatal epidermal keratinocyte layer
Regenicin Inc.	PermaDerm	Catastrophic burns	CBER	Orphan Status Approval	Type I bovine collagen and chondroitin sulfate populated with autologous epidermal keratinocyte and dermal fibroblasts
Shire	Dermagraft	Full-thickness diabetic foot ulcers	CDRH	PMA	Cryopreserved human fibroblasts on a PGA mesh
Shire	TransCyte	Full-thickness or deep, partial-thickness thermal injuries	CDRH	PMA	Nylon mesh coated with porcine collagen, containing nonviable human fibroblasts and an upper layer of silicone
Shire	Vascugel	Arteriovenous access for hemodialysis	CBER	Phase 2	Allogeneic aortic endothelial cells in gelatin
Tengion	Neo-Urinary Conduit	Conduit from kidneys to external device	CBER	Phase 1	PGA mesh coated with poly-D,L-lactide-*co*-glycolide seeded with autologous smooth muscle cells, peripheral blood, and bladder tissue
TETEC AG	NOVOCART 3D	Herniated disk repair	CBER	Phase 1	Autologous chondrocytes seeded on a bilayered type 1 collagen sponge containing chondroitin sulfate

Decellularized ECM-based devices have been taken from a variety of sources, including dermis from human, porcine, and bovine sources, as well as the small intestinal submucosa and urinary bladder matrix of pigs. All xenogenic ECM must gain FDA approval before being marketed. However, some decellularized cadaveric dermis is considered a transplant and is regulated as human cells, tissues, and cellular and tissue-based products (HCT/Ps), for which safety or effectiveness do not need to be demonstrated before being marketed. Removing the cellular components that initiate an immune response without stripping the scaffold of growth factors and other bioactive cues is difficult and is an area of active research (*54*). ECM products are naturally degraded in the body and are often cross-linked to increase their mechanical properties and decrease their degradation rate, although this can also increase the inflammatory foreign body response (*55*). These products have made their largest impact in the area of wound healing, where they are used mainly for chronic wounds, diabetic foot ulcers, and venous leg ulcers. GraftJacket (Wright Medical Technology) (Table 1), a scaffold composed of processed human dermis, which retains collagen, elastin, and proteoglycans, currently has sales of $340 million per year (*7*).

The efficacy of ECM-based biomaterials will continue to increase as our understanding of ECM biology and scaffold processing evolves. Current donor sites are limited by the mechanical properties of the decellularized tissue, and improving the mechanical properties of the ECM scaffold in a biocompatible manner will allow for the selection of tissues for their specific combination of growth factors and regenerative properties. There are large variations in ECM across tissue sites (*56*), and between developing and adult tissues (*57*), and careful study of these differences will aid in development of materials that more actively promote regeneration. Decellularized matrices contain growth factors at physiological concentrations (although these can vary by species), and a scaffold that mimics the developing tissue would be an important step in enhancing the body's natural regenerative capabilities. Selecting new tissue sites that have optimal growth factor combinations

or improved processing methods would likely enter the clinic through the 510(k) pathway. Processing can also selectively remove some growth factors or GAGs, while leaving others (*54*), opening up the possibility that unwanted signals in the ECM could be selectively removed.

BIOACTIVE INORGANICS

Bioactive glasses and ceramics were the first class of biomedical materials designed to mimic the body. Bone has an extensive capacity to regenerate, but large defects cannot completely repair themselves naturally, so bioinorganics, which have chemical compositions similar to that of bone, are commonly implanted and bind strongly to native bone (*58*). Since first gaining FDA approval, bioactive ceramics and glasses have been steadily improved and modified into a series of products, such as particulates (Fig. 3D), foams, and putties, with the goal of making a material that is easy to administer and more mechanically similar to bone. Bioactive glasses have been approved for applications including periodontal disease, a bone filler, and ossicular reconstruction (Table 1). However, these materials, especially when porous, do not have the necessary fracture toughness for load-bearing applications and have mostly been used as bone fillers or coatings (*59*).

Inorganic-polymer composites are currently being studied preclinically to improve the mechanical properties and tissue integration of the bioactive glass scaffolds (*60*). Soluble ions released from bioactive glasses can affect gene expression in osteoblasts (*61*), and therefore, recent work has involved changing the elemental composition of bioactive glasses (*62*). Strontium, which is a clinical treatment for osteoporosis (*63*), has also been included (*64*). Bioceramics have generally inferior bioactivity to bioactive glasses, although they can also induce bone formation (*65*). They are modeled on the hydroxyapatite inorganic phase found in bone, and TCP bioceramics are most commonly used in the clinic owing to their desirable resorption rates compared to hydroxyapatite (*66*). A clinical example is Actifuse (Baxter), which is a malleable silicate–substituted calcium phosphate intended for maxillofacial bone defects (Table 1). Bioactive inorganics are an example of steady iterative improvement and innovation in the clinic and have entered the market through the 510(k) process. They are widely used in the clinic and, in 2011, had sales of $2.2 billion (*67*).

THERAPEUTIC-RELEASING MATERIALS

Apart from modifying the physical properties of biomaterials, research has also been focused on using materials to control the release of bioactive molecules, ranging from small-molecule drugs to proteins to nucleic acids (*51*) (Fig. 2B). Because high-throughput studies are more difficult to perform with biomaterials, most systems have incorporated a relatively small number of well-studied proteins. These include the growth factors like VEGF and FGF, which have relatively well-understood signaling mechanisms and are commercially available in sufficient quantities for clinical testing. Using larger proteins rather than small molecules allows for slower diffusion and often facile covalent attachment without drastically affecting bioactivity. However, a consideration with these well-studied proteins is that they often play multiple disparate roles in the body, which can be undesirable therapeutically. High-throughput systems to assess a larger number of molecules in different combinations are important in optimizing scaffold conditions, and progress is being made combining these systems with biomaterials (*40*).

Recombinant growth factors play a major role in tissue regeneration and have been particularly well studied for the regeneration of bone. The most commonly used factors are from the bone morphogenetic protein (BMP) family, especially BMP-2 and BMP-7. A clinical example of this is the product INFUSE (Medtronic), which was approved in 2004 through the PMA process as a combination product for a narrow range of spinal fusions. INFUSE consists of a metal cage with recombinant human BMP-2 (rhBMP-2) adsorbed onto a collagen sponge. This rhBMP-2 loading costs thousands of dollars and leads to a concentration that is a million times higher than physiological levels and many times higher than needed in nonhuman primates and mice in vivo (*68*). These supraphysiological BMP-2 concentrations have led to adverse events in people, including bone overgrowth, epidermal hematoma, and cervical swelling (*69*). Another system from Medtronic, called AMPLIFY, which contained an even larger dose of rhBMP-2, also showed significant side effects (*69*) and was denied approval by the FDA in 2011.

The lesson from the INFUSE/AMPLIFY studies is that using proteins and growth factors far above physiological levels greatly increases the chances of systemic side effects, as well as the cost of production. There are two main routes through which the amount of recombinant growth factor needed in a scaffold can be lowered and yet still achieve the intended therapeutic effect. The first is to alter the kinetic release profile to maximize the effect of the factor on the intended cell population. Many growth factor–loaded systems undergo a large bolus release when the scaffold is first implanted, followed by a slower release profile. This is generally undesirable, and biomaterials have been developed to release one or more growth factors at controlled rates (*51*, *70*).

Systems that mimic the developmental processes and actively respond to biological events are even more promising. When, exactly, a therapeutic treatment is needed will vary depending on the stage of an injury or illness, and progression through these phases can vary in different people, so timing the release to biological events improves the efficiency of growth factor delivery. Also, a scaffold that gives tailored responses to the different cell types present in tissue can guide multiple processes in a spatiotemporal manner. Enzymes, including the 18 secreted matrix metalloproteinases, have been the most heavily studied class of molecules that can modulate the release of soluble factors from scaffolds (*71*). The second route is to "sensitize" the desired cell population to the released molecule. Heparan sulfate has been shown to potentiate the effects of BMP-2 (*72*), and other cues, such as integrin activation, can act synergistically to increase signaling (*73*). This route is particularly effective in biomaterials because GAGs and ligands for integrin signaling can be easily incorporated into the scaffold or even included in a hydrogel that is administered to the desired area. PEG-heparin hydrogels have been used in vivo (*74*) and have the benefit that heparin binds and activates many growth factors naturally, and can be used to deliver exogenous growth factors or simply bind and present factors produced by cells in the area. This encourages regeneration locally without delivering large doses of growth factors.

DESIGNING SYNTHETIC MATERIALS

Most injury and disease processes involve the interactions of a variety of biological mechanisms. In spinal cord injury, for instance, a

combination of apoptosis, immune response, scar formation, proteoglycans, and inhibitory proteins all work together to prevent recovery. Not surprisingly, functional regeneration of the spinal cord has proven difficult, despite decades of active research. In such complicated regenerative targets, it is unlikely that a single growth factor or drug will be sufficient for regeneration. Therefore, the necessary therapy will require several different factors released with temporal and spatial control. As a result, there is a natural tendency to try and include a variety of components to address each of the impediments to the desired outcome in a system.

This creates several challenges, the first of which, as seen with rhBMP-2, is that the dosing levels needed for a therapeutic effect in humans can be substantially different than the species in which preclinical tests are performed. In one scenario, a biomaterial is designed with one growth factor and introduced into the market, and then updated in later iterations of the device with additional biological factors once the dosing levels of the first factor are well understood. Although the FDA reviews applications on a case-by-case basis, modifications are less likely to require multiphase clinical trials if they are not chemical in nature and do not constitute the primary mechanism of action for the therapy. Combination therapies are increasingly being used in the clinic, such as Cypher (Cordis), a stent that elutes rapamycin to prevent restenosis, which gained FDA approval in 2003 (Table 1).

Another obstacle is combination products; as more therapies are incorporated into a single product, the greater the likelihood that some will need to be licensed by one competitor to another. Although not a technological obstacle to the performance of a biomaterial, this is certainly a barrier to commercialization. This is less of an issue with drugs and biologics because each component is often sold separately, and thus, the value of the separate drugs in the combined product is easily determined. However, this licensing issue can be highly complicated in a tissue scaffold or regenerative medicine product where the function of the combined device cannot be mimicked by using either component separately. Despite these problems, the ability of materials to release multiple bioactive molecules with controlled or physiological release makes them ideally situated to harness our increased understanding of the complex mechanisms that govern cell and tissue behavior.

Cell-free biomaterials have been the most commercially successful products in regenerative medicine, compared with cell-laden materials, owing to the relative simplicity of the scaffolds and their simpler regulatory path to the clinic. Although the addition of cells offers several regenerative advantages, which will be discussed briefly in the next section, for many acute injuries and particularly for applications in developing countries, the requirements for the administration of a cell therapy will likely prove difficult to overcome. For these targets, acellular biomaterials are not a stepping stone to more complex therapies but the end product. Biomaterial development will continue to undergo a transition from tissue-derived scaffolds and simple synthetic polymers to complex scaffolds engineered to interact with cells and guide tissue regeneration. The FDA has steadily evolved to address the challenges presented by new biomedical therapies and created the OCTGT and the OCP (Fig. 1A) to bring together the appropriate regulatory experience and develop a regulatory framework to fit the changing landscape.

Dramatic advances in cell biology have yet to be fully realized in the biomaterials field, and nucleic acid delivery (*75*), local cell recruitment (*76*), epigenetic modifications (*77*), immune modulation (*78*), and even the in vivo reprogramming of cells (*79*) are all powerful tools that will push the field forward to address unmet needs in regenerative medicine, such as spinal cord injury repair, neurodegenerative diseases, and myocardial infarction.

CELL-MATERIAL COMBINATION DEVICES

Many disease targets in regenerative medicine feature conditions where a necessary population of cells is either impaired or missing. Recapitulating the physiological role of these cells using an acellular biomaterial construct is generally beyond the limits of the current materials technology, and the most realistic therapy is the delivery of a new cell population. Cell-based therapies can both functionally replace the diseased or damaged cells directly, but they can also deliver a range of therapeutic molecules even if the cells themselves do not become incorporated into the regenerated tissue.

Cell-based therapies in the context of biomaterials (not including organ or bone marrow transplantations) have two general routes to the clinic in the United States. The first is described in section 361 under the Public Health Services Act and is referred to as the "361 pathway," and does not require premarket review. It is primarily aimed at safety and preventing the introduction and transmission of communicable disease and is typically a shorter and less expensive route to the market. HCT/P therapies must be "minimally manipulated," which means that a product must be intended for homologous use; not be processed in a way that "[alters] the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement" (*80*); not have a systemic effect or be dependent on metabolic effects for its primary function (except in homologous use or from close relatives); and not be combined with other drugs or devices. Homologous use means that the cells and tissues have a similar function in the therapy and is meant to be defined broadly. Homologous use includes allografts, and an example of nonhomologous use would be implanting cartilage in the bladder (*80*). Minimal manipulation covers sterilization and techniques that are aimed to purify out a specific cell population, like density gradient centrifugation, but does not cover any explicit modifications to the cells, including proliferation (*80*). These considerations are important when designing cell-material combination products. However, the general area of cell therapy is beyond the scope of this review, and the interested reader is referred to other recent literature on the topic (*81*, *82*).

The human body, especially in disease or injury, can be a difficult and hostile place for cell engraftment and survival. One way to predictably control the cell microenvironment is to use a biomaterial that acts as a physical scaffold and can also contain specific adhesion sites or release bioactive factors (*83*). Materials have the added benefit of helping cell localization and engraftment during administration of the therapy, which is a major problem for most free-cell injections (*84*). As an example, Genzyme has developed a new product called MACI (matrix-induced chondrocyte implantation) (Table 1) that features autologous chondrocytes cultured on a collagen membrane whose primary benefit is a less invasive, simpler operation for cartilage repair than their previous, cell-only Carticel treatment, which required extra surgical steps to keep the implanted chondrocytes localized.

Cell-material hybrids have thus far found their largest clinical use in wound healing (Table 1), with Dermagraft (Shire Regenerative Medicine) and Apligraf (Organogenesis), each boasting more than $100 million per year in sales (*7*). Dermagraft consists of human neonatal

dermal fibroblasts cultured on a PGA mesh scaffold in vitro, which is then cryopreserved until use. Apligraf is a slightly more complex system, in which human foreskin–derived neonatal fibroblasts are cultured on a bovine type I collagen matrix, upon which foreskin-derived neonatal epidermal keratinocytes are then cultured. The cells in these systems express various growth factors that encourage angiogenesis and re-epithelialization and have been used successfully in humans in a variety of applications from diabetic foot ulcers to burns (*85*). Unfortunately, allogeneic cell grafts for wound healing do not contain hair or pigment (*86*). Research into epidermal stem cells could lead to a therapy that both protects the wound during healing and is more physiologically similar to the native skin (*85*). The FDA has treated cell-based wound dressings as devices, and both Apligraf and Dermagraft entered the clinic through the PMA process. Organogenesis brought an identical sister product called Gintuit to the market to treat mucogingival conditions, but this product had to attain a biological license application (BLA) from the CBER (Table 1), indicating a change in the regulatory environment at the FDA.

TISSUE-ENGINEERED ORGANS

Soon after scientists combined cells and scaffolds in vitro, it was postulated that tissue engineering could eventually lead to laboratory-grown organs—a crucial need, as hundreds of thousands of people die each year with end-stage organ failure in the United States alone (*87*). Tissue-engineered organs pose a major challenge owing to their size and complexity (*88*). All organs are composed of many cell types, with precise arrangements and vascularization that are difficult to replicate in vitro. The adult human heart, for instance, contains roughly 5×10^9 cells, which is orders of magnitude more than most in vitro studies, and undergoes rapid cell death when circulation is disrupted. Attempts to recreate tissues have typically used cells seeded on synthetic polymers, such as PLGA, natural polymers like collagen, or decellularized organ ECM (*89*).

The early successes in the field have been in respiratory and urogenital engineering, which are tissues that have predominantly structural functions (*86*). An example of this is the Neo-Urinary Conduit from Tengion (Table 1), which diverts urine from the ureters to an external bag after bladder removal. This device is similar to a previous device from Tengion, the Neo-Bladder, which was a tissue-engineered bladder replacement. Although it was unsuccessful in FDA trials, it did work in a majority of the patients (*90*). These devices consist of a PGA mesh coated with poly-D,L-lactide-*co*-glycolide seeded with autologous smooth muscle cells, peripheral blood, and bladder tissue. The Neo-Urinary Conduit is currently enrolling in a phase 1 clinical trial (ClinicalTrials.gov identifier: NCT01087697).

To engineer whole organs, others have used decellularized ECM scaffolds, which retain the organ microstructure and localized adhesive signals that synthetic polymer scaffolds lack (*87*). An example of this is decellularized human trachea, which was seeded with autologous epithelial cells and human MSCs (hMSCs) (*91*) or hMSC-derived chondrocytes (*92*) and surgically implanted in patients. These scaffolds are quickly revascularized in vivo and were functional after 2 years without immunosuppressive drugs (*87*, *91*). In vivo animal studies have been performed on more ambitious targets, such as the heart (*93*) or lungs (*94*), which also use decellularized organs as a scaffold. A cell-scaffold combination device will typically receive more regulatory scrutiny than a scaffold-only therapy, but this difference can vary depending on the application. For conditions that are not life-threatening, such as articular cartilage repair, unwanted side effects are a more substantial impediment to regulatory approval than for a tissue-engineered construct treating end-stage organ failure. Printing synthetic polymers or biopolymers in 3D for organ regeneration is an area of active research, which could offer an alternative to the problems with variability associated with decellularization (*95*, *96*).

Current studies and clinical trials of tissue-engineered organs have used only a small number of patients and are focused on the safety of the implanted device. These clinical studies offer important feedback about the problems arising when implanting the combination devices in humans and highlight the areas that need to be improved upon and more thoroughly tested in vitro and in vivo. It takes weeks of scaffold preparation before it can be implanted clinically, and every step can be improved. Optimization of the decellularization process (*97*), cell expansion (*98*), and bioreactors for cell seeding (*90*, *99*) will be important. Current cell seeding techniques typically rely on bolus injections of either a mixed population of the cells found in the target organ or stem cells. As our understanding of the regenerative capacity of cell types within tissues and the effects of microenvironment increases, the parameters of the seeding process can be improved to better recapitulate physiological structures in the engineered tissue. In some cases, it is possible for single cells to form physiological structures in vitro (*100*), and increasingly complex devices have been developed that incorporate several cell types and mimic organ function (*101*). Furthermore, materials can be developed to interact with cells and guide them through these regenerative processes to create a 3D environment that mimics development. Creating an organ ex vivo is an enormous challenge, but as advances in cell and systems biology coalesce with improved decellularization, tissue-engineered structures will steadily improve in physiological function until they will one day be a realistic alternative to transplants.

LOOKING FORWARD

Researchers from fields ranging from cell biology to materials science to chemistry to clinical medicine have all come together to develop the tools necessary to regenerate damaged tissue in the body. What begins as a concept faces a steep and difficult path into the clinic, beginning with efficacy in cell culture, relevant animal models, and then work in clinical trials, all without significant side effects or safety issues. Furthermore, the material must be able to pass through the relevant regulatory bodies and be attractive enough to investors to warrant the investment of tens of millions to more than a billion dollars. Fortunately, there have been successful products to come out of the field of regenerative medicine from a range of platforms, as seen in Table 1, such as decellularized tissue, synthetic polymers, and bioinorganics. Most of these have resisted complicated and expensive therapeutic designs and are usually modifications of existing therapies, which allows for an easier regulatory path. These have had success in areas like bone, wound repair, and tissue-engineered organs with a primarily mechanical function, all of which have some amount of natural regeneration.

However, there are still areas, such as the cardiovascular and central nervous systems, where regenerative biomaterials have yet to make a large clinical impact. The human body is incredibly complex, and as our understanding of cell biology untangles the layers of regulatory

control of gene and protein expression, the burden falls on biomaterial scientists to weave several mechanisms of bioactivity into tangible therapies targeted to specific populations of cells. Careful evaluation of the necessary parameters for success can help minimize the chances of unexpected negative off-target effects in clinical trials, reduce the regulatory pathway, and increase the commercial desirability and profitability of the therapy as it transitions from laboratory to clinic. Translating these biomedical advances to medical successes will help fulfill the long-standing promise of regenerative medicine to patients, clinicians, investors, and society.

REFERENCES AND NOTES

1. National Institutes of Health, http://www.nih.gov/about/impact/
2. D. M. Cutler, A. B. Rosen, S. Vijan, The value of medical spending in the United States, 1960–2000. *N. Engl. J. Med.* **355**, 920–927 (2006).
3. E. R. Dorsey, J. de Roulet, J. P. Thompson, J. I. Reminick, A. Thai, Z. White-Stellato, C. A. Beck, B. P. George, H. Moses III, Funding of US biomedical research, 2003-2008. *JAMA* **303**, 137–143 (2010).
4. S. M. Paul, D. S. Mytelka, C. T. Dunwiddie, C. C. Persinger, B. H. Munos, S. R. Lindborg, A. L. Schacht, How to improve R&D productivity: The pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* **9**, 203–214 (2010).
5. B. Munos, Lessons from 60 years of pharmaceutical innovation. *Nat. Rev. Drug Discov.* **8**, 959–968 (2009).
6. C. Mason, E. Manzotti, Regenerative medicine cell therapies: Numbers of units manufactured and patients treated between 1988 and 2010. *Regen. Med.* **5**, 307–313 (2010).
7. A. Jaklenec, A. Stamp, E. Deweerd, A. Sherwin, R. Langer, Progress in the tissue engineering and stem cell industry "are we there yet?" *Tissue Eng. Part B Rev.* **18**, 155–166 (2012).
8. M. J. Lysaght, A. L. Hazlehurst, Tissue engineering: The end of the beginning. *Tissue Eng.* **10**, 309–320 (2004).
9. G. Prestwich, S. Bhatia, C. K. Breuer, S. Dahl, C. Mason, R. McFarland, D. J. McQuillan, J. Sackner-Bernstein, J. Schox, A. Trounson, W. E. Tente, K. LaMarco, M. L. Frisk, What is the greatest regulatory challenge in the translation of biomaterials into the clinic? *Sci. Transl. Med.* **4**, 160cm14 (2012).
10. Guidance for Industry and FDA Staff: Classification of Products as Drugs and Devices & Additional Product Classification Issues; http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM258957.pdf
11. *Medical Technology Innovation Scorecard* (PricewaterhouseCoopers LLP, New York, 2011).
12. S. Keyhani, M. Diener-West, N. Powe, Are development times for pharmaceuticals increasing or decreasing? *Health Aff.* **25**, 461–468 (2006).
13. A. Astashkina, B. Mann, D. W. Grainger, A critical evaluation of in vitro cell culture models for high-throughput drug screening and toxicity. *Pharmacol. Ther.* **134**, 82–106 (2012).
14. D. Krewski, D. Acosta Jr., M. Andersen, H. Anderson, J. C. Bailar III, K. Boekelheide, R. Brent, G. Charnley, V. G. Cheung, S. Green Jr., K. T. Kelsey, N. I. Kerkvliet, A. A. Li, L. McCray, O. Meyer, R. D. Patterson, W. Pennie, R. A. Scala, G. M. Solomon, M. Stephens, J. Yager, L. Zeise, Toxicity testing in the 21st century: A vision and a strategy. *J. Toxicol. Environ. Health B Crit. Rev.* **13**, 51–138 (2010).
15. *Definition of Primary Mode of Action of a Combination Product* (Federal Register).
16. B. Trappmann, J. E. Gautrot, J. T. Connelly, D. G. T. Strange, Y. Li, M. L. Oyen, M. A. Cohen Stuart, H. Boehm, B. Li, V. Vogel, J. P. Spatz, F. M. Watt, W. T. S. Huck, Extracellular-matrix tethering regulates stem-cell fate. *Nat. Mater.* **11**, 642–649 (2012).
17. Institute of Medicine, *Medical Devices and the Public's Health: The FDA 510(k) Clearance Process at 35 Years* (The National Academies Press, Washington, DC, 2011).
18. J. Makower, A. Meer, L. Denend, *FDA Impact on U.S. Medical Technology Innovation* (PricewaterhouseCoopers LLP, New York, 2010).
19. K. Hozumi, D. Otagiri, Y. Yamada, A. Sasaki, C. Fujimori, Y. Wakai, T. Uchida, F. Katagiri, Y. Kikkawa, M. Nomizu, Cell surface receptor-specific scaffold requirements for adhesion to laminin-derived peptide–chitosan membranes. *Biomaterials* **31**, 3237–3243 (2010).
20. T. A. Petrie, J. E. Raynor, D. W. Dumbauld, T. T. Lee, S. Jagtap, K. L. Templeman, D. M. Collard, A. J. García, Multivalent integrin-specific ligands enhance tissue healing and biomaterial integration. *Sci. Transl. Med.* **2**, 45ra60 (2010).
21. M. M. Martino, F. Tortelli, M. Mochizuki, S. Traub, D. Ben-David, G. A. Kuhn, R. Müller, E. Livne, S. A. Eming, J. A. Hubbell, Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. *Sci. Transl. Med.* **3**, 100ra89 (2011).
22. R. Censi, P. Di Martino, T. Vermonden, W. E. Hennink, Hydrogels for protein delivery in tissue engineering. *J. Control. Release* **161**, 680–692 (2012).
23. Z. Zhou, P. Yu, H. M. Geller, C. K. Ober, The role of hydrogels with tethered acetylcholine functionality on the adhesion and viability of hippocampal neurons and glial cells. *Biomaterials* **33**, 2473–2481 (2012).
24. A. Haque, X. S. Yue, A. Motazedian, Y. Tagawa, T. Akaike, Characterization and neural differentiation of mouse embryonic and induced pluripotent stem cells on cadherin-based substrata. *Biomaterials* **33**, 5094–5106 (2012).
25. J. J. Moon, S. H. Lee, J. L. West, Synthetic biomimetic hydrogels incorporated with Ephrin-A1 for therapeutic angiogenesis. *Biomacromolecules* **8**, 42–49 (2007).
26. L. Ye, Z. Fan, B. Yu, J. Chang, K. Al Hezaimi, X. Zhou, N. H. Park, C. Y. Wang, Histone demethylases KDM4B and KDM6B promotes osteogenic differentiation of human MSCs. *Cell Stem Cell* **11**, 50–61 (2012).
27. K. T. Love, K. P. Mahon, C. G. Levins, K. A. Whitehead, W. Querbes, J. R. Dorkin, J. Qin, W. Cantley, L. L. Qin, T. Racie, M. Frank-Kamenetsky, K. N. Yip, R. Alvarez, D. W. Y. Sah, A. de Fougerolles, K. Fitzgerald, V. Koteliansky, A. Akinc, R. Langer, D. G. Anderson, Lipid-like materials for low-dose, in vivo gene silencing. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 1864–1869 (2010).
28. A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
29. R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, C. S. Chen, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483–495 (2004).
30. S. H. Lim, X. Y. Liu, H. Song, K. J. Yarema, H. Q. Mao, The effect of nanofiber-guided cell alignment on the preferential differentiation of neural stem cells. *Biomaterials* **31**, 9031–9039 (2010).
31. B. G. Keselowsky, D. M. Collard, A. J. García, Integrin binding specificity regulates biomaterial surface chemistry effects on cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5953–5957 (2005).
32. Guidance for Industry and FDA Staff: Interpretation of the Term "Chemical Action" in the Definition of Device under Section 201(h) of the Federal Food, Drug, and Cosmetic Act; http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM259068.pdf
33. X. Liu, J. M. Holzwarth, P. X. Ma, Functionalized synthetic biodegradable polymer scaffolds for tissue engineering. *Macromol. Biosci.* **12**, 911–919 (2012).
34. L. S. Nair, C. T. Laurencin, Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* **32**, 762–798 (2007).
35. S. Yang, K. F. Leong, Z. Du, C. K. Chua, The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Eng.* **7**, 679–689 (2001).
36. G. Wei, P. X. Ma, Nanostructured biomaterials for regeneration. *Adv. Funct. Mater.* **18**, 3566–3582 (2008).
37. K. G. Sreejalekshmi, P. D. Nair, Biomimeticity in tissue engineering scaffolds through synthetic peptide modifications-altering chemistry for enhanced biological response. *J. Biomed. Mater. Res. A* **96**, 477–491 (2011).
38. M. Barczyk, S. Carracedo, D. Gullberg, Integrins. *Cell Tissue Res.* **339**, 269–280 (2010).
39. A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* **324**, 59–63 (2009).
40. A. Ranga, M. P. Lutolf, High-throughput approaches for the analysis of extrinsic regulators of stem cell fate. *Curr. Opin. Cell Biol.* **24**, 236–244 (2012).
41. L. Almany, D. Seliktar, Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures. *Biomaterials* **26**, 2467–2477 (2005).
42. A. T. Hillel, S. Unterman, Z. Nahas, B. Reid, J. M. Coburn, J. Axelman, J. J. Chae, Q. Guo, R. Trow, A. Thomas, Z. Hou, S. Lichtsteiner, D. Sutton, C. Matheson, P. Walker, N. David, S. Mori, J. M. Taube, J. H. Elisseeff, Photoactivated composite biomaterial for soft tissue restoration in rodents and in humans. *Sci. Transl. Med.* **3**, 93ra67 (2011).
43. T. Aida, E. W. Meijer, S. I. Stupp, Functional supramolecular polymers. *Science* **335**, 813–817 (2012).
44. M. J. Webber, J. Tongers, C. J. Newcomb, K.-T. Marquardt, J. Bauersachs, D. W. Losordo, S. I. Stupp, Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13438–13443 (2011).
45. Y.-D. Lin, C.-Y. Luo, Y.-N. Hu, M.-L. Yeh, Y.-C. Hsueh, M.-Y. Chang, D.-C. Tsai, J.-N. Wang, M.-J. Tang, E. I. H. Wei, M. L. Springer, P. C. H. Hsieh, Instructive nanofiber scaffolds with VEGF create a microenvironment for arteriogenesis and cardiac repair. *Sci. Transl. Med.* **4**, 146ra109 (2012).
46. V. M. Tysseling-Mattiace, V. Sahni, K. L. Niece, D. Birch, C. Czeisler, M. G. Fehlings, S. I. Stupp, J. A. Kessler, Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. *J. Neurosci.* **28**, 3814–3823 (2008).
47. J. S. Rudra, T. Sun, K. C. Bird, M. D. Daniels, J. Z. Gasiorowski, A. S. Chong, J. H. Collier, Modulating adaptive immune responses to peptide self-assemblies. *ACS Nano* **6**, 1557–1564 (2012).
48. J. A. Burdick, G. D. Prestwich, Hyaluronic acid hydrogels for biomedical applications. *Adv. Mater.* **23**, H41–H56 (2011).
49. A. D. Baldwin, K. L. Kiick, Polysaccharide-modified synthetic polymeric biomaterials. *Biopolymers* **94**, 128–140 (2010).
50. W. P. Daley, S. B. Peters, M. Larsen, Extracellular matrix dynamics in development and regenerative medicine. *J. Cell Sci.* **121**, 255–264 (2008).

51. K. Lee, E. A. Silva, D. J. Mooney, Growth factor delivery-based tissue engineering: General approaches and a review of recent developments. *J. R. Soc. Interface* **8**, 153–170 (2011).
52. S. Thorsteinsdóttir, M. Deries, A. S. Cachaço, F. Bajanca, The extracellular matrix dimension of skeletal muscle development. *Dev. Biol.* **354**, 191–207 (2011).
53. T. R. Cox, J. T. Erler, Remodeling and homeostasis of the extracellular matrix: Implications for fibrotic diseases and cancer. *Dis. Model Mech.* **4**, 165–178 (2011).
54. J. E. Reing, B. N. Brown, K. A. Daly, J. M. Freund, T. W. Gilbert, S. X. Hsiong, A. Huber, K. E. Kullas, S. Tottey, M. T. Wolf, S. F. Badylak, The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. *Biomaterials* **31**, 8626–8633 (2010).
55. S. F. Badylak, D. O. Freytes, T. W. Gilbert, Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomater.* **5**, 1–13 (2009).
56. E. J. Vanderploeg, C. G. Wilson, S. M. Imler, C. H.-Y. Ling, M. E. Levenston, Regional variations in the distribution and colocalization of extracellular matrix proteins in the juvenile bovine meniscus. *J. Anat.* **221**, 174–186 (2012).
57. E. P. Brennan, X.-H. Tang, A. M. Stewart-Akers, L. J. Gudas, S. F. Badylak, Chemoattractant activity of degradation products of fetal and adult skin extracellular matrix for keratinocyte progenitor cells. *J. Tissue Eng. Regen. Med.* **2**, 491–498 (2008).
58. E. M. Valliant, J. R. Jones, Softening bioactive glass for bone regeneration: Sol–gel hybrid materials. *Soft Matter* **7**, 5083–5095 (2011).
59. C. Ohtsuki, M. Kamitakahara, T. Miyazaki, Bioactive ceramic-based materials with designed reactivity for bone tissue regeneration. *J. R. Soc. Interface* **6** (Suppl. 3), S349–S360 (2009).
60. K. Rezwan, Q. Z. Chen, J. J. Blaker, A. R. Boccaccini, Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* **27**, 3413–3431 (2006).
61. I. D. Xynos, A. J. Edgar, L. D. Buttery, L. L. Hench, J. M. Polak, Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. *J. Biomed. Mater. Res.* **55**, 151–157 (2001).
62. M. M. Azevedo, G. Jell, M. D. O'Donnell, R. V. Law, R. G. Hill, M. M. Stevens, Synthesis and characterization of hypoxia-mimicking bioactive glasses for skeletal regeneration. *J. Mater. Chem.* **20**, 8854–8864 (2010).
63. E. Boanini, M. Gazzano, A. Bigi, Ionic substitutions in calcium phosphates synthesized at low temperature. *Acta Biomater.* **6**, 1882–1894 (2010).
64. M. D. O'Donnell, P. L. Candarlioglu, C. A. Miller, E. Gentleman, M. M. Stevens, Materials characterisation and cytotoxic assessment of strontium-substituted bioactive glasses for bone regeneration. *J. Mater. Chem.* **20**, 8934–8941 (2010).
65. H. Yuan, H. Fernandes, P. Habibovic, J. de Boer, A. M. C. Barradas, A. de Ruiter, W. R. Walsh, C. A. van Blitterswijk, J. D. de Bruijn, Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13614–13619 (2010).
66. S. V. Dorozhkin, Bioceramics of calcium orthophosphates. *Biomaterials* **31**, 1465–1485 (2010).
67. BCC Research, *Technical and Advanced Structural Ceramics (AVM011D)*; http://www.bccresearch.com/pressroom/report/code/AVM011D
68. P. C. Bessa, M. Casal, R. L. Reis, Bone morphogenetic proteins in tissue engineering: The road from laboratory to clinic, part II (BMP delivery). *J. Tissue Eng. Regen. Med.* **2**, 81–96 (2008).
69. E. J. Carragee, E. L. Hurwitz, B. K. Weiner, A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: Emerging safety concerns and lessons learned. *Spine J.* **11**, 471–491 (2011).
70. R. G. Wylie, S. Ahsan, Y. Aizawa, K. L. Maxwell, C. M. Morshead, M. S. Shoichet, Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat. Mater.* **10**, 799–806 (2011).
71. M. P. Lutolf, F. E. Weber, H. G. Schmoekel, J. C. Schense, T. Kohler, R. Müller, J. A. Hubbell, Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nat. Biotechnol.* **21**, 513–518 (2003).
72. D. S. Bramono, S. Murali, B. Rai, L. Ling, W. T. Poh, Z. X. Lim, G. S. Stein, V. Nurcombe, A. J. van Wijnen, S. M. Cool, Bone marrow-derived heparan sulfate potentiates the osteogenic activity of bone morphogenetic protein-2 (BMP-2). *Bone* **50**, 954–964 (2012).
73. R. Tsou, F. F. Isik, Integrin activation is required for VEGF and FGF receptor protein presence on human microvascular endothelial cells. *Mol. Cell. Biochem.* **224**, 81–89 (2001).
74. M. Kim, S. E. Kim, S. S. Kang, Y. H. Kim, G. Tae, The use of de-differentiated chondrocytes delivered by a heparin-based hydrogel to regenerate cartilage in partial-thickness defects. *Biomaterials* **32**, 7883–7896 (2011).
75. H.-J. Park, F. Yang, S.-W. Cho, Nonviral delivery of genetic medicine for therapeutic angiogenesis. *Adv. Drug Deliv. Rev.* **64**, 40–52 (2012).
76. O. A. Ali, N. Huebsch, L. Cao, G. Dranoff, D. J. Mooney, Infection-mimicking materials to program dendritic cells in situ. *Nat. Mater.* **8**, 151–158 (2009).
77. C. H. Arrowsmith, C. Bountra, P. V. Fish, K. Lee, M. Schapira, Epigenetic protein families: A new frontier for drug discovery. *Nat. Rev. Drug Discov.* **11**, 384–400 (2012).
78. M. A. Swartz, S. Hirosue, J. A. Hubbell, Engineering approaches to immunotherapy. *Sci. Transl. Med.* **4**, 148rv9 (2012).
79. L. Qian, Y. Huang, C. I. Spencer, A. Foley, V. Vedantham, L. Liu, S. J. Conway, J.-D. Fu, D. Srivastava, In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* **485**, 593–598 (2012).
80. *Federal Register* (2001).
81. V. Sahni, J. A. Kessler, Stem cell therapies for spinal cord injury. *Nat. Rev. Neurol.* **6**, 363–372 (2010).
82. S. Wang, X. Qu, R. C. Zhao, Clinical applications of mesenchymal stem cells. *J. Hematol. Oncol.* **5**, 19 (2012).
83. E. S. Place, N. D. Evans, M. M. Stevens, Complexity in biomaterials for tissue engineering. *Nat. Mater.* **8**, 457–470 (2009).
84. J.-P. Karam, C. Muscari, C. N. Montero-Menei, Combining adult stem cells and polymeric devices for tissue engineering in infarcted myocardium. *Biomaterials* **33**, 5683–5695 (2012).
85. C. Dieckmann, R. Renner, L. Milkova, J. C. Simon, Regenerative medicine in dermatology: Biomaterials, tissue engineering, stem cells, gene transfer and beyond. *Exp. Dermatol.* **19**, 697–706 (2010).
86. G. Orlando, K. J. Wood, P. De Coppi, P. M. Baptista, K. W. Binder, K. N. Bitar, C. Breuer, L. Burnett, G. Christ, A. Farney, M. Figliuzzi, J. H. Holmes IV, K. Koch, P. Macchiarini, S.-H. Mirmalek Sani, E. Opara, A. Remuzzi, J. Rogers, J. M. Saul, D. Seliktar, K. Shapira-Schweitzer, T. Smith, D. Solomon, M. Van Dyke, J. J. Yoo, Y. Zhang, A. Atala, R. J. Stratta, S. Soker, Regenerative medicine as applied to general surgery. *Ann. Surg.* **255**, 867–880 (2012).
87. S. F. Badylak, D. J. Weiss, A. Caplan, P. Macchiarini, Engineered whole organs and complex tissues. *Lancet* **379**, 943–952 (2012).
88. A. Atala, F. K. Kasper, A. G. Mikos, Engineering complex tissues. *Sci. Transl. Med.* **4**, 160rv12 (2012).
89. A. Atala, Engineering organs. *Curr. Opin. Biotechnol.* **20**, 575–592 (2009).
90. J. J. Yoo, J. Olson, A. Atala, B. Kim, Regenerative medicine strategies for treating neurogenic bladder. *Int. Neurourol. J.* **15**, 109–119 (2011).
91. M. J. Elliott, P. De Coppi, S. Speggiorin, D. Roebuck, C. R. Butler, E. Samuel, C. Crowley, C. McLaren, A. Fierens, D. Vondrys, L. Cochrane, C. Jephson, S. Janes, N. J. Beaumont, T. Cogan, A. Bader, A. M. Seifalian, J. J. Hsuan, M. W. Lowdell, M. A. Birchall, Stem-cell-based, tissue engineered tracheal replacement in a child: A 2-year follow-up study. *Lancet* **380**, 994–1000 (2012).
92. P. Macchiarini, P. Jungebluth, T. Go, M. A. Asnaghi, L. E. Rees, T. A. Cogan, A. Dodson, J. Martorell, S. Bellini, P. P. Parnigotto, S. C. Dickinson, A. P. Hollander, S. Mantero, M. T. Conconi, M. A. Birchall, Clinical transplantation of a tissue-engineered airway. *Lancet* **372**, 2023–2030 (2008).
93. H. C. Ott, T. S. Matthiesen, S.-K. Goh, L. D. Black, S. M. Kren, T. I. Netoff, D. A. Taylor, Perfusion-decellularized matrix: Using nature's platform to engineer a bioartificial heart. *Nat. Med.* **14**, 213–221 (2008).
94. H. C. Ott, B. Clippinger, C. Conrad, C. Schuetz, I. Pomerantseva, L. Ikonomou, D. Kotton, J. P. Vacanti, Regeneration and orthotopic transplantation of a bioartificial lung. *Nat. Med.* **16**, 927–933 (2010).
95. J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, C. S. Chen, Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat. Mater.* **11**, 768–774 (2012).
96. B. Derby, Printing and prototyping of tissues and scaffolds. *Science* **338**, 921–926 (2012).
97. S. Baiguera, P. Jungebluth, A. Burns, C. Mavilia, J. Haag, P. De Coppi, P. Macchiarini, Tissue engineered human tracheas for in vivo implantation. *Biomaterials* **31**, 8931–8938 (2010).
98. S. Jung, K. M. Panchalingam, L. Rosenberg, L. A. Behie, Ex vivo expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells Int.* **2012**, 123030 (2012).
99. L. De Bartolo, A. Lendlein, D. Hofmann, A. Bader, A. de Grey, E. Curcio, E. Drioli, Bio-hybrid organs and tissues for patient therapy: A future vision for 2030. *Chem. Eng. Process.* **51**, 79–87 (2012).
100. T. Sato, R. G. Vries, H. J. Snippert, M. van de Wetering, N. Barker, D. E. Stange, J. H. van Es, A. Abo, P. Kujala, P. J. Peters, H. Clevers, Single Lgr5 stem cells build crypt–villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009).
101. D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin, D. E. Ingber, Reconstituting organ-level lung functions on a chip. *Science* **328**, 1662–1668 (2010).
102. E. S. Place, J. H. George, C. K. Williams, M. M. Stevens, Synthetic polymer scaffolds for tissue engineering. *Chem. Soc. Rev.* **38**, 1139–1151 (2009).

Acknowledgments: We thank L. Chow and K. Nitiputri, of the authors' laboratory, for the SEM images in Fig. 3 and J. Hulvat for useful suggestions. **Funding:** The European Commission under the 7th Framework Program for Research and Development Marie Curie International Incoming Fellowships program for E.T.P. M.M.S. is funded by ERC SIG grant "Naturale." **Competing interests:** The authors declare that they have no competing interests.

Submitted 27 May 2012
Accepted 25 October 2012
Published 14 November 2012
10.1126/scitranslmed.3002717

Citation: E. T. Pashuck, M. M. Stevens, Designing regenerative biomaterial therapies for the clinic. *Sci. Transl. Med.* **4**, 160sr4 (2012).