

Regenerative Medicine as Applied to General Surgery

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The present review illustrates the state of the art of regenerative medicine (RM) as applied to surgical diseases and demonstrates that this field has the potential to address some of the unmet needs in surgery. RM is a multidisciplinary field whose purpose is to regenerate in vivo or ex vivo human cells, tissues, or organs to restore or establish normal function through exploitation of the potential to regenerate, which is intrinsic to human cells, tissues, and organs. RM uses cells and/or specially designed biomaterials to reach its goals and RM-based therapies are already in use in several clinical trials in most fields of surgery. The main challenges for investigators are threefold: Creation of an appropriate microenvironment ex vivo that is able to sustain cell physiology and function in order to generate the desired cells or body parts; identification and appropriate manipulation of cells that have the potential to generate parenchymal, stromal and vascular components on demand, both in vivo and ex vivo; and production of smart materials that are able to drive cell fate.

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*Never has there been a more exciting time to be involved
 in surgical science.*

—Hollander et al¹

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Regenerative medicine (RM) refers to a field in the health sciences that aims to replace or regenerate human cells, tissues, or organs to restore or establish normal function.^{2–4} The process of regenerating body parts can occur in vivo or ex vivo and may require cells, natural or synthetic cell-supporting scaffold materials, bioactive molecules, genetic manipulation, or combinations of all of these. As such, RM brings together multidisciplinary teams including physicians, scientists, veterinarians, engineers, chemists, physicists, statisticians, mathematicians, and industry in new and productive ways.^{5,6} Importantly, RM is not synonymous with tissue engineering, which refers to a field that is narrower in scope and strictly defined as engineering body parts ex vivo.³

Although the term RM was coined and appeared in the literature as recently as 1999,^{3,4} ante literam the field has existed for more than a century and its history is more closely intermingled with the history of surgery than with any other field in the health sciences.^{3,7,7} Recent reports on the fabrication and implantation in humans of bioengineered vessels,^{9–14} bladders,¹⁵ windpipe^{16,17} and urethras,¹⁸ and the production of heart,¹⁹ liver,^{20–23} and lung^{24,25} organoids, have provided glimpses of the immense intrinsic potential of RM as applied to surgical diseases.

Therefore, although we share the thought expressed by Hollander et al that “never has there been a more exciting time to be involved in surgical science,”¹ we believe that it is timely to illustrate the state of the art of RM applications as applied to different fields of general surgery.

SKIN

The main goal of currently available therapies is restoration of the cutaneous barrier to fluid loss and infection. This goal can be difficult to achieve in large acute wounds or nonhealing chronic wounds because patients need the epidermal barrier as quickly as possible. Treatments for large acute wounds focus mainly on coverage; however, treatments for chronic wounds must provide coverage and convert a nonhealing wound bed to an environment conducive to healing. Owing to the challenges in restoring skin, treatments for large acute wounds have not significantly changed in 30 years, and treatments for chronic wounds have only arisen in the past 10 to 15 years. The current “gold-standard” treatment is the split-thickness autograft,²⁶ which cannot be readily and completely performed in patients with limited skin for donor sites.²⁷ Other adjunctive therapies include Integra (Integra Life Sciences, Plainsboro, NJ) and cultured epithelial autografts, both of which have been commercially available since the 1990s but have not achieved widespread utilization. These currently available treatments can restore the epidermal barrier with clinically acceptable cosmetic outcomes. However, a clinically acceptable cosmetic outcome does not necessarily include adnexal skin structures such as hair and pigment, which are vital to the normal

functions of skin.²⁸ Therefore, the ultimate goal of RM for the integumentary system should be the restoration of fully functional skin that is physiologically and cosmetically equivalent to a patient's normal skin.

There are many promising regenerative therapies for skin restoration, and these can be divided into 2 broad categories, namely artificial skin substitutes and cell-based therapies. Artificial skin substitutes typically focus on a biomaterials approach to skin restoration, whereas cell-based therapies leverage the healing response of skin cells. New therapies range from novel formulations of naturally occurring extracellular matrix (ECM) to in situ delivery of stem cells (SCs). It is important to note that although many potential skin therapies show promise in rodent models, some therapies may not be applicable to humans because of significant differences in wound healing mechanisms between thin- and thick-skinned animals. The future of skin regeneration will likely include a combination of biomaterials and cell therapy.

Integra is the prototypical biomaterial approach to skin restoration. It is a bilayered construct consisting of type I bovine collagen combined with chondroitin-6-sulfate and an overlying silicone membrane.²⁹ The collagen/chondroitin layer guides the growth of a "neo-dermis," whereas the silicone membrane acts as a temporary epidermal barrier until the construct can be definitively covered with an autograft. Integra has been used most extensively in large burn wounds and is a frontline treatment at many burn centers.^{30–34} Many other treatments have mimicked its dermal and epidermal structure,³⁵ and any new treatment must compete with these existing technologies.

The future of skin regeneration using biomaterials lies in manipulating the healing properties of the natural ECM. ECM hydrogels such as collagen, fibrin, and hyaluronic acid are produced at specific intervals in the normal wound healing process to direct migration and proliferation of skin cells.^{36,37} Understanding the interactions between ECM and skin cells is the key to using ECM as a regenerative therapy for wounds. Control of ECM composition by fibroblasts and keratinocytes plays a significant role in scarring and wound contraction.^{38–42} Collagen is well known to facilitate cell migration and is the basis of many of the currently existing dermal substitutes such as Integra. Fibrin acts as the initial sealant and scaffolding in normal wound healing and is known to reduce wound contraction when used in combination with skin grafts.⁴³ As a result, fibrin is often used as a delivery vehicle for applying skin cells to a wound.^{44–46} Although collagen and fibrin have been most widely used in novel therapies for skin, hyaluronic acid will likely be a component of many future therapies. Hyaluronic acid is the major component of the ECM in fetal wounds that heal without scarring⁴⁷ and leveraging these properties may have implications for adult healing.^{48,49} Clearly, the choice of biomaterial for skin regeneration has a significant impact on the clinical outcomes. Future skin regeneration therapies will likely incorporate more natural scaffoldings to facilitate wound healing.

Although biomaterials have important wound healing properties, they lack the full wound healing potential of skin cells. Cells "close" a wound and create the skin structures that provide function. In the United States, cultured epithelial autografts (CEA) are the prototypical cell-based therapy, in which keratinocytes are grown in a sheet and applied to a wound.⁵⁰ CEA can be grown from autologous or allogeneic keratinocytes and can restore the epidermal barrier with clinically acceptable cosmetic outcomes.^{51–55} However, cell-based therapies are potentially limited by the survival of the cells and the diminished storage capabilities of living biological constructs.⁵⁶ Nonetheless, these limitations have not prevented certain cell-based therapies, such as Apligraf (Organogenesis Inc, Canton, MA) from being commercially viable treatments for chronic wounds.^{57–61}

CEAs are created from sheets of cells grown in culture and applied to wounds as sheets. These sheets can be extremely fragile

and difficult to handle. As a result, researchers and clinicians have examined the use of cell spraying to deliver the same skin cells without handling a fragile cell sheet.^{45,62–65} This technique allows the delivery of virtually any type of skin cell in a vehicle, typically a fibrin hydrogel. The use of cell sprays has been extended to noncultured autologous skin cells isolated in the operating room and applied directly to a wound.⁶⁶ This technology for burns, known as ReCell (Avita Medical, Woburn, MA), is currently in US clinical trials as part of the Department of Defense–funded Armed Forces Institute of Regenerative Medicine.

Cell therapies have shown great promise for the delivery of fibroblasts and keratinocytes as wound treatments. However, fibroblasts and keratinocytes do not fulfill all functions of skin, and it is likely that a source of SC will be required for full restoration of all integumentary structures in a major wound. The number of different cell types present in fully functional skin is too large to realistically deliver each different cell type in specified locations within a wound. Sources of skin SC have been identified in the epidermis and hair follicles.^{67–73} These highly proliferative cells have been recognized as major contributors to normal wound healing and show enhanced wound healing after culturing, likely due to selection of proliferative progenitors during the culturing process.⁶⁴ Furthermore, it is likely that nonscarring fetal wounds owe some of their unique properties to a high number of SC.^{74,75} A variety of SC types, including bone-marrow mesenchymal SC (MSC), adipose-derived SC, and embryonic SC (ESC), have been examined for treating cutaneous wounds.^{76,77} Although these cells have been able to generate dermis and epidermis, more research is necessary before SC therapy is a viable approach to the management of acute or chronic skin wounds.

Finally, as noted earlier, the ultimate future of skin regeneration may well lie in the successful development of a technology that embodies both a biomaterial and a cell-based therapy approach. The most promising option to date is Stratagraft (Stratatech Corporation, Madison, WI).^{78,79} It is a full-thickness skin substitute that contains a fully stratified epidermal layer composed of NIKS cells grown atop a dermal layer composed of human fibroblasts embedded in a collagen matrix. StrataGraft will enter a Phase I/II burn clinical trial in 2011.

BLOOD VESSELS

If one considers all patients requiring artery bypass grafting⁸⁰ or peripheral vascular bypass procedures,⁸¹ and the approximately 100,000 new individuals who will need hemodialysis access each year,⁸² more than 450,000 Americans per year potentially will require the implantation of a vascular conduit. Therefore, vessel bioengineering represents a burgeoning field of investigation for RM specialists.

Off the shelf, vascular tissue that is nonthrombogenic sustains physiologic blood pressure, resists infection and stenosis, grows, and is capable of remodeling represents the "holy grail" of vascular bioengineering. In 1986, Drs Weinberg and Bell cultured bovine smooth muscle cells (SMCs) in a collagen lattice reinforced with a Dacron mesh and cultured fibroblasts and seeded with endothelial cells.⁸³ These experiments are considered the birth of vascular tissue engineering. These early grafts, however, were limited by inadequate biophysical parameters. Although native arteries have rupture strengths higher than 2000 mm Hg, Weinberg and Bell's grafts failed at strengths lower than 180 mm Hg. Later, L'Heureux attempted to overcome the biophysical limitations of cultured tubes by innovating a method of sheet seeding, rolling concentric tubes of cultured SMC and fibroblasts to produce de novo grafts with burst pressures higher than 2500 mm Hg. Neo-vessels were successfully implanted in a canine model initially.⁸⁴

The adaptation of biodegradable polymers, especially polylactic acid (PGA), has allowed researchers to begin to overcome

the biophysical limitations of collagen based and de novo scaffolds. Niklason et al⁸⁵ utilized bovine SMCs cultured on PGA scaffolds over silicone tubing pulsed via peristaltic pump to produce a biomimetic microenvironment with the aim of stimulating physiologic organization of SMC and deposition of collagen. These constructs achieved burst pressures higher than 2100 mm Hg and were successfully implanted as arterial grafts in swine.

In vivo development and maturation of vascular scaffolds overcome the technical limitations of sheet-seeded and bioreactor-developed scaffolds that require several weeks to months of culture to produce a viable graft. Decellularized allogeneic vessels theoretically provide the optimal ECM to act as a scaffold for neovessel formation. However, xenograft experiments are complicated by antigenicity and rejection,⁸⁶ whereas allograft experiments have demonstrated high rates of early thrombosis.⁸⁷ The same group has recently developed novel scaffolds by utilizing a hybrid approach combining ex-vivo scaffold development of cell-seeded polymer-based scaffolds with in vivo scaffold maturation.⁸⁸ In this model, SMCs were seeded on biodegradable scaffolds and cultured in a bioreactor to allow maturation and then degradation of synthetic supporting scaffold, which was eventually replaced by natural ECM produced by SMC. Thereafter, the constructs were decellularized with detergent. The resultant grafts are nonimmunogenic, species-matched constructs with burst pressures between 2600 and 3300 mm Hg, which can be stored at 4°C and are available off-the-shelf. Animal models of baboon arteriovenous fistulae and canine coronary artery bypass have demonstrated short-term patency rates between 80% and 100%.

Shinoka et al combined in vivo scaffold development and degradable scaffolds by creating constructs of poly-L-lactic acid (PLLA) and poly-ε-caprolactone reinforced by woven PGA and seeded with bone marrow derived mononuclear cells before implantation as low-pressure venous grafts. These cells could be obtained from the patient on the day of surgery, and the technique did not require ex vivo cell culture.^{8,9} Later, Shinoka et al¹⁰ successfully implanted 25 patients with these conduits for total cavo-pulmonary connections, representing the first clinical application of tissue engineered vascular grafts (TEVG). In mid-term follow-up, there were no graft-related complications and 100% patency. At long-term follow-up (5.8 year mean follow-up), graft patency remained 100% and there was 1 (4%) partial graft thrombus and 6 (24%) cases of graft stenosis.¹¹ In another series, L'Heureux's sheet-seeded constructs have also begun human clinical trials. Ten patients requiring hemodialysis access were implanted with TEVGs as arteriovenous fistula grafts in hemodialysis patients with access failure and no suitable vein for a new arteriovenous fistula.^{12,13} At 3-year follow up, results were comparable to data from the Dialysis Outcomes Quality Initiative, whereas updated results that are currently under review show a considerable decrease in the overall complication rate relative to preoperative care (T. McAlister and N. L'Heureux, unpublished data, 2011).

Future research will have to answer the question of whether the seeded or cultured cells of TEVGs are integral to the biophysical and homeostatic properties of the mature graft and whether or not they induce a paracrine response from host tissue. For example, targeted drug delivery of vascular endothelial growth factor and monocyte chemoattractant protein-1 utilizing microsphere technology has been shown to improve endothelial cell survival and neovessel formation in endothelial cell transplant models.⁸⁹ In addition, induced pluripotent SCs (iPS) hold the potential to generate large amounts of autologous building blocks for both in vitro and in vivo neovessel developments.

CARDIAC RESTORATION

The limited ability for heart muscle to regenerate after a myocardial infarction remains the primary cause of progressive heart failure.⁹⁰ Important advances in SC research have afforded new treat-

ment options that may reinvigorate cardiac cell therapy and cardiac tissue engineering.⁹¹ Both these approaches aim to improve the function of damaged myocardium by promoting the formation of a new contractile cardiac tissue. The successful implementation of such approaches requires an appropriate choice of cells and an effective engraftment technique.^{92,93} Early work in cardiac restoration focused on direct bolus injection of cardiac cells in a saline suspension into the infarcted region of the heart.⁹⁴ A variety of different cell types have been used with this approach,⁹⁵ including skeletal myoblasts, neonatal cardiomyocytes, fibroblasts, smooth muscle cells, ESC, and adult SC (bone marrow progenitors). Although improved cardiac function was demonstrated in some clinical studies after cell injection or infusion,^{96,97} evidence from experimental models indicates that the percentage of grafted cell survival in the infarcted myocardium is generally very low.^{98,99} One recent experimental study showed that 1 hour after intracoronary delivery of autologous bone marrow mononuclear cells by saline injection, fewer than 7% of the cells appeared in the myocardium whereas more than 90% accumulated in the liver and spleen.⁹⁹

The limited efficacy of cardiac cell therapy using bolus injections is also linked to the poor contractility of certain engrafted cells. Improved clinical efficacy will depend on the availability of an optimal source of contractile human cardiac myocytes that can integrate with the host tissue to create a functional cardiac syncytium.^{91,100} However, the integration of engrafted myocytes—and the efficacy of the therapy itself—cannot be properly verified without addressing the confounding effects of poor cell survival and retention associated with the shortcomings of the bolus saline injection strategy. Most investigators have addressed these problems by introducing better cell immobilization techniques that employed either a tissue engineering approach¹⁰¹ or a simple biomaterial augmentation to direct cell injections.¹⁰² Whereas a tissue engineering strategy requires the ex vivo formation of a tissue analog from contractile cells and a polymer scaffold that is eventually sutured onto the infarcted myocardium, a biomaterial cell carrier can be delivered with cells in situ to increase their survival, retention, and the longevity of the graft without the need for ex vivo culture. The feasibility of surgically implanting a robust engineered cardiac patch comprised a biomaterial scaffold and cultivated cardiomyocytes has been demonstrated recently¹⁰³; yet such an approach may be more difficult to effectively implement on a clinically relevant scale. Moreover, minimally invasive surgical techniques favor a biomaterial cell carrier approach, even though there are fewer biomaterials that are suitable for in situ delivery of transplanted cells to the heart.

Whether one chooses a tissue engineering or biomaterial cell carrier approach, transplanted cardiac myocytes most certainly can benefit from the physical support of a biomaterial scaffold to maintain their placement in the infarct region, protect the cells from host inflammation, and facilitate functional integration within the injured myocardium. Identifying or designing an appropriate biomaterial for cardiomyocyte transplantation is one of the important focal points in the field of cardiac regeneration. In a cell carrier system, for example, an injectable biomaterial must undergo in situ liquid-to-solid transition (gelation) with cardiomyocytes in suspension without harming the cells or the surrounding host myocardium.¹⁰⁴ After in situ gelation, the cells should be able to readily migrate through the material and remodel the polymer so that true engraftment is possible by natural cell-mediated pathways. A biomaterial possessing susceptibility to tissue remodeling enzymes would be advantageous in this regard.¹⁰⁵ At the same time, the injectable polymer must not obstruct cellular remodeling,^{106,107} nor distort the myocardial geometry.¹⁰⁸ For this reason, it is important to consider the impact that material compliance has on cardiomyocyte phenotype^{109,110} and muscle mechanics.¹¹¹ Finally, the biomaterial needs to be a suitable growth environment

for myocardial cells to survive and express a contracting cardiac phenotype so that they can functionally integrate upon implantation.¹⁰⁷ Recently, Ott et al¹⁸ produced acellular ECM scaffolds from rat hearts that were repopulated with neonatal rat cardiomyocytes. Constructs were able to provide up to 2% of the normal contractile function.¹⁸

A number of recent experimental cardiomyocyte transplantation studies using injectable polymers such as fibrin glue,¹¹² Pegylated fibrin biomatrix,¹¹³ Pegylated fibrinogen hydrogels,¹¹⁴ matrigel,¹⁰⁸ and self-assembling peptide hydrogels¹¹⁵ have demonstrated improvements in cell transplant survival, vasculogenesis, and even cardiac function. Despite encouraging results, there are still unanswered questions about how composition and structure of a biomaterial may adversely affect cardiomyocyte remodeling and functional integration of the cell graft. To this end, a variety of biomaterials are currently being developed and tested in the context of cardiac cell therapy research. Despite having interesting *in vitro* results using novel biomaterials and cardiac myocytes, the ability to cultivate cardiac cell grafts and sustain cardiac function within a biomaterial has proven much more challenging than originally anticipated. Therefore, few biomaterials are currently being applied in clinical trials in cardiac cell therapy.⁹⁴ Nevertheless, continued advances in biomaterial design will provide a robust platform for improving the efficacy of cardiac cell delivery and thereby provide alternatives in myocardial restoration.

RESPIRATORY TRACT

Whole organ transplantation remains the only curable and definitive option for patients suffering from a wide variety of end-stage respiratory disease. However, this procedure is limited by high costs, the organ shortage, a relatively high incidence of complications, and an overall low 10-year survival rate (<20%).¹¹⁶ However, airways—and in particular the upper airways—appear to be an ideal arena for RM investigations.

Being a relatively simple and hollow organ, the trachea was the ideal starting point for evaluating the possibility to obtain clinical relevant respiratory organ engineering. Several synthetic degradable polymers or biomaterials, evaluated for their ability to support airway reconstruction, have not resulted in successful clinical applications.^{117,118} Recently, a decellularized human airway construct, repopulated with epithelial cells and chondrocytes of MSC origin, was used to restore lung function in a patient with end-stage airway disease and represents the first successful human tracheobronchial replacement.^{17,18} At present, the patient is well, active with normal lung function and, more importantly, does not require immunosuppressive drugs. The seminal procedure mentioned earlier has been improved, shortening the time to obtain tracheal decellularization and using an alternative cell technological approach.¹¹⁹ The improved approach involves the decellularized human tracheal graft being reseeded intraoperatively with autologous cells (bone marrow MSC and respiratory cells) and conditioned with growing, differentiation, and “boosting” factors. This *in vivo* tissue engineered approach was used in 5 patients with benign tracheal diseases and in 2 patients with primary tracheal cancers involving the entire trachea.¹²⁰ The findings suggest that autologous cells combined with appropriate biomaterials might provide successful treatment for patients with serious clinical tracheal disorders. Moreover, by comparison with classic surgery, the recovery from the *in vivo* tissue engineered airway replacement is more rapid and durable. Within 4 months of transplantation, lung function parameters return toward normal, patients can completely rejoin normal life (without any immunosuppressive therapy) and perform regular physical activity, and their quality of life returns to normal. These early clinical results demonstrate that a strategy based on optimally bioengineered materials combined with autologous cells and pharmacological intervention (to boost progenitor cell recruitment and commitment and thereby promote tissue

regeneration) can provide a therapeutic option and eventually a cure for patients with serious clinical tracheal disorders.

When the larynx is considered, it has been demonstrated that most of the larynx can be removed with preservation of the airway and breathing functions provided that one side retains movement. Therefore, for partial replacement of the larynx with a new bioengineered construct, the latter does not need to exert any neuromuscular activity to achieve an acceptable functional result from an airway and breathing perspective.^{121,122} However, voice and swallowing functions remain suboptimal because of the lack of a complete laryngeal architecture. It is not unrealistic to postulate, therefore, that the availability of substitutes displaying anatomical, physiological, and biomechanical properties equivalent to normal human larynxes would provide the appropriate complex architecture and dynamics for normal voice production and sphincter action.

The larynx is an order of magnitude more complicated than the trachea and its dynamic function makes it a more difficult organ to engineer. Recent studies suggest that ECM scaffolds could be promising templates also for construct remodeling of laryngeal tissue.^{123,124} After hemi-laryngectomy, human acellular laryngeal grafts may provide the precise anatomical reconstitution and native cartilaginous support necessary to retain airway, voice and swallowing functions in a manner superior to that provided by present techniques (S. Baiguera and P. Macchiarini, unpublished data, 2011).

Bioengineering of pulmonary tissue has been limited by the inability to generate a biodegradable, highly elastic lung scaffold that reproduces the lung's complex airway, alveolar, and vascular architecture that can support gas exchange over a large surface area. It has been reported that the ECM does not only define the lung's architecture and contain physical properties but also influences the direction of pulmonary cell differentiation.¹²⁵ Recently, animal cadaveric lungs were decellularized and whole lung scaffolds (with a perfusable vascular bed and preserved airway and alveolar geometry) were obtained,^{23,24} proving that the tracheal approach can be useful also for tissue of higher complexity and enlarged architecture. Although representing an initial step toward the ultimate goal of generating fully functional lungs *in vitro*, these results suggest that using decellularized lung matrix could be a viable strategy for lung regeneration.

KIDNEY

End-stage renal disease (ESRD) is treated with dialysis or transplantation. Dialysis treatments only partially replace kidney function and require intermittent connection with an artificial means of renal replacement, whereas organ transplantation cannot be extended as required to a large patient population because of the shortage of donor organs. As the number of ESRD patients continues to rise disproportionately to the number of available donor kidneys,¹²⁶ the identification of new sources of organs has become an urgent mandate.

RM has been proposed as a potential solution for patients with ESRD. Initially, the strategy was to induce regeneration of damaged kidney tissue on the basis of the use of progenitor/SCs. It has been shown that bone marrow SCs, such as MSCs, are recruited to the sites of injury and may contribute to kidney tissue regeneration.¹²⁷ In models of acute kidney injury, such as cisplatin or glycerol injections in mice, MSCs have been shown to accelerate organ structural and functional recovery.^{128,129} These studies demonstrated that systemically injected MSCs do not differentiate into renal cells, but they produce mediators that promote kidney tissue regeneration by resident cells. In the same experimental setting, the use of human cord blood MSC may limit leukocyte infiltration in the peritubular capillaries and reduce microvascular damage induced by cisplatin.¹³⁰

As the results obtained by cell therapy in acute kidney injury models have not been confirmed in chronic renal diseases, tissue

engineering decellularization–recellularization technology has been applied to the kidney. Ross et al¹³¹ successfully seeded rat renal ECM with murine ESC infused through the renal artery and the ureter and showed proliferation and differentiation of ESCs within the glomerular, vascular, and tubular compartments. More recently, Nakayama et al¹³² produced an acellular kidney scaffold from adult rhesus monkey with preserved expression patterns of native ECM proteins. Layering of fetal kidney cells on these scaffolds demonstrated the potential of the scaffold to support Pax2+/vimentin+ cell attachment and migration, important steps for scaffold recellularization.

URINARY TRACT

The overt architectural simplicity of hollow structures (such as the bladder) and tubes (such as the ureters and urethra), which are responsible for the collection, storage, and egress of urine from the body, make these organs particularly amenable to the application of RM technologies. The development and application of tissue engineering technologies for bladder and urethral repair have followed 2 basic approaches. The first is a cell-free, scaffold-only technology, and the second is a cell-based scaffold system. The first technique uses scaffolds/matrices without cells in which the overall concept is to create a microenvironment permissive and favorable to the regenerative process. In the second approach, cell-based scaffold systems are used for tissue regeneration. This latter strategy is currently centered on seeding urothelial and/or smooth muscle cells on scaffolds to recreate critical 3-dimensional aspects of the urinary tissue *in vitro*, resulting in a more fully differentiated and phenotypically mature construct at implantation. Furthermore, this approach reduces the inflammatory or immune component in response to the matrix and prevents graft contracture and shrinkage. Examples of each approach for bladder and urethral repair are provided below.

The necessity for developing tissue engineering technologies for bladder reconstruction is highlighted by the fact that detubularized bowel segments are still commonly used for this purpose. Besides the obvious physiological mismatch between the 2 tissue types, this technique has long been known to suffer from other limitations including infection, perforation, metabolic disturbances, urolithiasis (stone formation), and even malignancy.^{133–135} To this end, several distinct scaffolds/biomaterials have been evaluated for bladder augmentation procedures to replace the injured bladder wall in experimental studies. Biomaterials used to date have comprised those derived from both natural and synthetic materials, such as collagen, polyvinyl sponges, PGA, and Teflon, and decellularized scaffolds, such as small intestinal submucosa (SIS),^{136–139} and acellular bladder matrices.^{140–146} Although the use of cell-free scaffolds clearly has merit, experimental studies in general have revealed limitations intrinsic to the use of cell-free scaffolds, such as biocompatibility issues resulting in scarring and reduced reservoir volume, and graft contraction.^{147,148} Overall, the preclinical data favor the use of a cell-based scaffold system using bladder smooth muscle and urothelial cells as the critical components to promote the formation of normal bladder structure and function in the regenerated bladder, especially for larger defects in larger animal models.

The first human clinical study of cystoplasty was performed on 7 patients with a cell-seeded collagen scaffold either with or without omental coverage, or a combined PGA-collagen scaffold seeded with cells and covered with omentum was tested. The patients reconstructed with the engineered bladder tissue using the PGA-collagen cell-seeded scaffolds with omental coverage showed increased compliance, decreased end-filling pressures, increased capacities, and longer dry periods over time.¹⁴ This seminal study was the first to document the potential clinical utility of this approach. A recent search of the clinical trials database (www.clinicaltrials.gov, conducted on January 25, 2011) revealed 3 studies sponsored by Tension

Inc for tissue engineering approaches to bladder repair. Two of these involve the use of their proprietary autologous neo-bladder technology for augmentation cystoplasty of patients with spinal cord injuries or myelodysplasia. The third is an autologous neo-urinary conduit for incontinent urinary diversion in patients undergoing radical cystectomy for the treatment of bladder cancer.

Clinical data with respect to urethral tissue engineering has recently been reviewed.¹⁴⁹ Successful experimental studies of urethral replacement using decellularized porcine bladder submucosa¹⁵⁰ have also led to clinical trials in which some urethral defects were repaired using human bladder acellular collagen matrices.¹⁵¹ Neourethras that ranged from 5 to 15 cm were created by anastomosing the matrix in an onlay fashion to the urethral plate, and 3/4 patients had a successful outcome at the 3-year follow-up with respect to cosmetic appearance and function. Both pediatric and adult patients with primary urethral stricture disease showed successful results using an acellular collagen-based matrix.¹⁵² Another study in 30 patients with recurrent stricture disease showed that a healthy urethral bed (ie, 2 or fewer prior urethral surgeries) was needed for successful urethral reconstruction using the acellular collagen-based grafts.¹⁵³ Recently, Atala's group has reported 6-year follow-up of the successful implantation of artificial urethras bioengineered from autologous cells in 5 boys suffering from severe urethral stenosis.¹⁷

As with bladder reconstruction, although cell-free scaffolds were successfully applied to onlay urethral repairs experimentally and clinically, it has been shown that in cases in which a tubularized repair of the urethra is needed, cell-seeding is required because when cell-free tubular scaffolds are used, inadequate urethral tissue regeneration occurs, leading to graft contracture and stricture formation.^{154–156} Unlike the tubularized collagen matrices without cells, these cell-seeded matrices did not result in severe inflammation, fibrosis, or stricture formation. The aforementioned experimental findings were confirmed in a clinical trial using tubularized nonseeded SIS for urethral stricture repair that was performed in 8 patients. Two patients with short inflammatory strictures maintained urethral patency. Stricture recurrence developed in the other 6 patients within 3 months of surgery.¹⁵⁷ Finally, another review of the clinical trials database (see previously mentioned details) revealed the presence of 2 cell therapies (Cook, autologous muscle-derived cells) for the treatment of stress urinary incontinence.

In short, cell-seeded matrices are superior to nonseeded matrices for the reconstruction of large portions of the bladder or for tubularized urethral reconstruction. Although remarkable progress has been made with respect to engineering of both bladder and urethral tissues, there is no doubt that significant challenges persist. A global research effort is underway and focused on the development of alternative cell sources/programming and technologies for creating biologically active or “smart” biomaterials that may further improve the regenerative process *in vivo*. The use of bioreactors for imparting relevant biomechanical forces and improving cell maturation and tissue formation *in vitro* has also been proposed.^{158,159}

PANCREAS

Exogenous insulin administration for diabetes mellitus is unable to precisely match normal physiology, and the resulting chronic carbohydrate dysmetabolism is associated with progressive diabetic complications, poor quality of life, morbidity, and mortality. Whole organ pancreas transplantation maintains euglycemia for years and reverses some histopathologic and clinical changes associated with diabetes.^{160,161} However, the procedure is a major vascular operation and afterwards requires long-term immunosuppression. RM approaches, such as cell-based therapy, tissue engineering, and modulation or obstruction of the immune system, may provide treatment

alternatives for diabetes that are less invasive surgically and minimize or obviate the need for chronic immunosuppression.

Islet transplantation, a cell-based treatment, is a form of RM. Islets have the ability to engraft and function within many tissues including the liver.^{162–164} Patients who have received intraportal autologous islet transplants as an adjunct to total pancreatectomy for debilitating chronic pancreatitis have maintained euglycemia for more than 10 years.¹⁶⁵ In 2000, the Edmonton group described a series of 7 diabetic patients who received allogeneic islet transplants and remained insulin-independent 1 year after transplantation.¹⁶⁶ Success of the Edmonton protocol was in part attributed to transplantation of islets isolated from 2 to 3 deceased donor pancreases. Unfortunately, intermediate outcomes in an international multicenter trial using the Edmonton protocol showed a 2-year insulin-independence rate of only 31%.¹⁶⁷ The need to utilize multiple donor pancreases for clinical islet transplantation provides an impetus for employing newer RM techniques to provide an adequate mass of functional β -cells.

Used with “cocktails” of differentiation factors, ESC and other SC types can now be differentiated along the lineages of endoderm, then pancreatic cells, insulin-producing cells, and finally cells with partial phenotypic and functional characteristics of terminally differentiated β -cells.^{168–170} Chandra et al¹⁷¹ has reported that adipose-derived SCs differentiate into insulin-producing cells in culture and normalize blood glucose levels after transplantation into diabetic mice.¹⁷¹ Although there has been limited success in obtaining a full β -cell phenotype in vitro from current SC differentiation protocols, when transplanted in vivo, however, pre-differentiated human ESC and other SC types displayed the ability to respond to glucose and secrete insulin suggesting that the transplant “environment” provides the necessary signals to induce terminal differentiation.^{170–174} Indeed, it has been reported that insulin independence with significant levels of human C-peptide can be achieved after autologous hematopoietic SC therapy in newly diagnosed type 1 diabetic patients.¹⁷⁵

Nuclear reprogramming is another approach to β -cell engineering, based on the introduction of a genetic code and constitutive expression of transcription factors such as pancreatic and duodenal homeobox 1 and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA).^{176,177} Expression of these transcription factors increases the efficiency of derivation of β -cell surrogates from SC. It has been reported that constitutive expression of MafA facilitated the differentiation of placental-derived SC into insulin-producing cells that are capable of responding to high glucose levels in vitro and, after transplantation, restore euglycemia in diabetic mice.¹⁷⁶ Induced pluripotent and amniotic fluid SC may also differentiate into β -cells under the influence of appropriate genetic signaling and yet-to-be determined culture conditions. However, greater control over introduced transcription factors, including promoting transient gene expression, may be required to successfully guide SC to the desired cell phenotype.¹⁷⁷

Scaffolds or tissue constructs might provide the desired natural environment to enhance current cell-based approaches aimed at producing large quantities of functional pancreatic endocrine cells. One approach, cell sheet tissue engineering, has identified the basement membrane protein laminin-5 as a key ECM component, enabling the short-term culture of islets in vitro before in vivo implantation. Upon confluence, adherent islet cell populations could be placed into the subcutaneous space of rats in one single sheet, resulting in constant release of insulin over 7 days and demonstrating proof of concept.¹⁷⁸ An environment that allows 3-dimensional contacts would invariably recapitulate the natural medium in which cells are supported and may be necessary for appropriate terminal differentiation into functional phenotype in vitro and in vivo and increase the efficiency of cell-based approaches.^{179,180} To create the 3-dimensional environment like that of native tissue, natural or synthetic matrices can be used. Matrices or

scaffolds are acellular structures that are made either from artificial materials or prepared by removing cellular components from tissue using a specific detergent-based process called decellularization, which has been applied to a multitude of internal organs.^{1,2,21,181} The goal of decellularization techniques is to obtain a collagen-rich ECM scaffold that contains an intact vascular network, which provides cells with an environment conducive for cell growth. In early studies, rat pancreata, which were minced and decellularized, supported the survivability and functionality of whole rat islets, both in vitro and when encapsulated inside a polymeric tube. In these studies, insulin release was maintained for more than 42 days.¹⁸² Further work has created an intact pancreas via detergent perfusion, retaining an intact vascular network, which provides a conducive template for the differentiation of genetically modified amniotic fluid SC along a β -cell lineage. This technique is a potential platform for neo-pancreatic bioengineering.²¹

The host immune system may prove to be the final arbiter of the success of RM approaches to treat diabetes, especially autoimmune type 1 diabetes. Strategies to evade or tame the immune system include reduction of antigenicity, inducing tolerance, or physical immunoisolation. For example, efforts to breed pigs that lack expression of the galactose- α -1,3-galactose antigen, a target of naturally occurring antibody in humans, might make cell-based therapies using porcine donors possible.¹⁸³ Understanding mechanisms of immune recognition has led to the development of molecules that can specifically block key steps of the immune response but have not yet achieved tolerance.¹⁸⁴ A number of immunoisolation strategies have been used to protect islet tissue including implantable vascular devices, constructs impermeable to components of the immune system implanted into tissues, and microcapsules implanted into the peritoneal cavity.^{185–188} The ideal physical barrier would be easy to place (minimally invasive), easy to retrieve, biocompatible with host tissues, and friendly to the tissue it protects (allow diffusion of nutrients and wastes). No single device has yet met all these requirements, although microencapsulation of islets addresses many of them.¹⁸⁹

Considerable hurdles remain, namely development of consistent culture techniques to produce large numbers of functional β -cells, and prevention of xenogeneic, allogeneic, and autoimmune responses mounted by the immune system. Derivation and differentiation of autologous SC may address some of these challenges, but the autoimmune nature of type 1 diabetes could present a formidable obstacle that may require effective immunoisolation of the cells before they can be successfully transplanted in patients.

LIVER

Liver transplantation remains the definitive treatment for end-stage liver failure, and for fulminant liver failure, and some forms of inborn errors of metabolism. As the disparity between organ supply and demand continues to grow,¹⁹⁰ new strategies are being developed. Hepatocyte transplantation is certainly in the forefront of new therapeutic strategies. The first successful hepatocyte transplantation into a patient was carried out in June, 1992, to a French Canadian woman with familial hypercholesterolemia. After ex vivo transduction with a retrovirus encoding for the human low-density lipoprotein (LDL) receptor, the patient's hepatocytes were infused through the inferior mesenteric vein into the liver. LDL and high-density lipoprotein levels improved throughout the next 18 months, and transgene expression was detected in a liver biopsy.¹⁹¹ After this first success, other cases followed. However, not all of the patients treated had a clear benefit from the procedure.¹⁹² Subsequently, several other metabolic diseases have been treated with hepatocyte transplantation with different degrees of success.^{193–197} Hepatocyte transplantation has also been used as a support treatment to acute^{198–200} and chronic liver disease^{199–202} in bridging severely ill patients to transplant. Low efficacy and lack of long-term therapeutic effect have been common in all of these

procedures. These failures could be explained by the relatively small number of hepatocytes that engraft in the recipient liver because of poor quality, quantity and possibly toxicity of immunosuppression.²⁰³ However, transplantation of a number of hepatocytes corresponding to 1% to 5% of the total liver mass has been shown to exert a positive impact in transplanted patients, even if for a limited period of time.²⁰³

Because of the shortage of available human hepatocytes for transplantation, other cell sources have been used. Specifically, bone marrow–derived MSCs,²⁰⁴ hematopoietic SCs^{205,206} and fetal liver progenitor cells²⁰⁷ have shown to improve, to a certain extent, the condition of cirrhotic patients. The latter cell type holds enormous potential for RM therapies because of their high expansion capabilities and differentiation potential into hepatocytes and biliary epithelium.²⁰⁸ Several studies have established the required pathways to differentiate ESC or iPS into a hepatic fate by using defined soluble growth factor signals that mimic embryonic development.^{209,210} These cells, once transplanted into rodent livers, were able to engraft and express several normal hepatic functions.²¹¹

Apart from cellular therapies, other experimental approaches are not showing results that will indicate clinical translation in the near future. However, 2 experimental strategies that have high therapeutic potential may be successfully translated to the clinic soon. The first experimental approach is the cell sheet technology developed by Okano.²¹² Simple configuration and fabrication allows for the stacking of up to 4 hepatocyte cell sheets that can readily engraft and provide a defined metabolic relief to the recipient.²¹³ This technology has already been applied successfully to one patient with heart failure (Okano et al, unpublished data, 2011).

Decellularization-recellularization technology has been also implemented to manufacture liver organoids. Uygun et al decellularized rat livers and repopulated them with rat primary hepatocytes, showing promising hepatic function and the ability to heterotopically transplant these bioengineered livers into animals for up to 8 hours.¹⁹ Atala's group was able to take it one step further by bioengineering livers with human cells only. These livers express some of the functions displayed by the adult human liver.^{20,21} Similar results have been recently published by Badylak's group,²² providing further proof that this technology may one day deliver viable constructs for drug discovery and toxicology applications and for transplantation.

GASTROINTESTINAL TRACT

The gastrointestinal (GI) tract is made up of phasic neuromuscular segments (esophagus, stomach, small and large intestines) separated by tonic neuromuscular segments (sphincters). These segments are contiguous, both structurally and functionally. Phasic segments maximize absorption of nutrients and water from ingested food, whereas tonic segments create high-pressure barriers that facilitate unidirectional flow of luminal contents in the GI tract. Each functional segment is made up of multiple smooth muscle layers, intrinsic enteric neuronal plexuses, and interstitial cells. Functional regeneration of the intestine *in vitro* must recreate the subtle differences in patterns of innervation to facilitate a balance between the paucity of neurotransmitters required for physiological function and motility.

The primary hurdle to GI bioengineering is the functional regeneration of diverse motility patterns. Motility can range anywhere between continuous or at-will peristaltic motility (esophagus) to intermittent segmental contractions (stomach and intestines) to high-pressure tonic closure zones (sphincters). Motility is locally and globally coordinated and regulated by a tight synergy arising from myogenic and neuronal inputs.

The common route taken in bioengineering is to recreate GI architecture by seeding cells dispersed from a primary culture on to biocompatible scaffolds to promote remodeling. The use of biocompatible scaffolds can be dated back a couple of decades, with stepwise

increments made in the direction of optimal porosity, biocompatibility, and biodegradability. Commonly used biomaterials for intestinal tissue engineering have been collagen scaffolds, poly-lactic acid (PLA), PGA, composite PLA and PGA, and poly-ε-caprolactone, among others. Fibrin hydrogels have demonstrated an optimal mechanical rigidity to allow self-organization of circular sphincteric and intestinal smooth muscle, even in humans.^{214,215}

Survival of intestinal tissue grafts *in vivo* requires angiogenesis to promote efficient nutrient exchange. Delivery of basic fibroblast growth factor to intestinal smooth muscle in a mouse model demonstrated maintenance of viability of implanted sphincteric smooth muscle and small intestinal smooth muscle.^{216,217} A comparison of the delivery of various angiogenic growth factors demonstrated that the platelet-derived growth factor approved by Food and Drug Administration maintained viability and survival of implanted bioengineered internal anal sphincter (IAS) constructs.²¹⁸

Early attempts at esophageal tissue engineering with the use of bioinert prosthetic materials did not result in cellular in-growth or functionality. Rather, it promoted stricture formations and just served as a nonfunctional passive conduit.²¹⁹ Recently, Nakase et al²²⁰ demonstrated that portions of the esophagus can be replaced with smooth muscles on PGA sheets in combination with fibroblasts keratinocytes. Similarly, Hori et al²²¹ have repaired gastric wall defects in a canine model by using collagen sponge scaffolds. Limitations of the approaches mentioned earlier include no demonstration of physiological functionality or gut motility.

Grikscheit et al dispersed intestinal organoid units from the small intestine and remodeled them on biocompatible matrices.²²² Implantation of these constructs rescued morbidity associated with massive bowel resection in rats. Chen and Badylak²²³ have implanted scaffolds made of SIS to replace segments of short bowel in canine models. These approaches reported that the implantations met basic physiological demands but display limited or no enteric neuronal repopulation.

Gilmont et al²²⁴ demonstrated neovascularization and successful implantation of bioengineered IAS constructs in mice models. These constructs maintain key aspects of IAS physiology, such as generation of basal tone and relaxation to relevant neurotransmitters before and after implantation. This group has also recently cocultured progenitor enteric neuronal cells in combination with human intestinal smooth muscle cells and demonstrated the differentiation of progenitor neuronal cells into mature network-forming neurons associated with the smooth muscle. These intrinsically innervated constructs demonstrate physiology akin to innervated IAS tissues.^{224,225}

Ideally, the paradigm for functional GI tissue engineering for implantation should focus on manufacturing innervated smooth muscle replacement structures. Structurally sound compatible biomaterials have to be identified that do not significantly alter mechanotransduction but allow angiogenesis and neuronal in-growth. Physiological function of the GI smooth muscle is derived from the restoration of smooth muscle and the associated enteric neuronal plexus with adequate extrinsic innervation. A multitude of excitatory and inhibitory neurotransmitters pertinent to gut function are released by the intrinsic innervation of the enteric nervous system. This diversity of neurotransmitter release mediating cholinergic contraction or vasoactive intestinal peptide-ergic/nitric relaxation is essential for the diverse motility patterns arising in different segments of the GI tract.

Decellularization-recellularization technology has been used to engineer segments of esophagus²²⁶ in what could be referred to as semi-xenotransplantation.² A 10-cm segment of porcine jejunum was decellularized and repopulated with autologous cells. After maturation, the construct was implanted in the arm of a patient suffering from a major esophagotracheal defect and retrieved after 7 days. The purpose of the study was to assess whether the construct would sustain

implantation. The postoperative course was uneventful. Pathology showed viable cells and a patent vascular tree.

ORTHOPEDICS

RM may hold broad promise for trauma, and in particular orthopedic trauma, as many of these injuries result in a tissue deficit that can be addressed through tissue engineering or other replacement strategies. The key difference in trauma, however, is that immediate treatment is essential and any regenerative strategy must either take this into account, or be compatible with the initial treatment modality if the RM treatment is to be performed later. Much of the current RM research is directed toward the use of autologous somatic or stem/progenitor cells. Cell-based products complicate the trauma treatment landscape, even if the technology is built on the use of autologous cells. Several tissues including bone, muscle, tendon, ligament, cartilage, and nerve have been engineered using the straightforward approach of autologous cells and a biodegradable, implantable biomaterial scaffold.^{227–231}

These approaches often require obtaining a biopsy, dissociating the cells and expanding them in the laboratory, seeding the scaffold, and implanting the construct back into the patient. This modality is not compatible with acute trauma treatment, unless the patient is able to be stabilized and treated at a later time point, assuming the tissue biopsy is available. The use of autologous SC does not alleviate this constraint, as they typically require expansion under this same scenario. Off-the-shelf solutions have been proposed, including the use of SC banks comprising embryonic, fetal, adult, or iPS. However, these cells have been shown to act in a more indirect mode, rather than acting as the main tissue-building component.

Because of these and other constraints, many of the RM technologies in current clinical trials are not directed toward trauma unless the treatment can occur later. A recent search of the clinical trials online database (www.clinicaltrials.gov) using the search phrase “regenerative medicine” produced 49 hits. Of these, 29 are actively recruiting, 6 are active but not recruiting, 12 are complete, and 2 are enrolling by invitation. Several trials are aimed at testing drugs or other compounds that may stimulate tissue regeneration. Dental and cardiac applications are prevalent, especially those using stem or progenitor cells. None of the trials are truly trauma studies, and only a few are even directed toward orthopedics.

There appears to be a larger proportion of RM research at the preclinical stage relating to trauma and orthopedics, and the use of non-cell-based strategies is dominant, where biomaterials and biological signals are received by endogenous cells. One exception is in the area of muscle regeneration. The discovery of several different stem and progenitor cell phenotypes such as perivascular cells (pericytes), muscle progenitor cells (satellite cells), and muscle SC²³² within muscle tissue, and the capacity of bone marrow- and adipose-derived SC to differentiate into muscle cells,²³³ has led to a focus on the development of cell therapy-based regeneration strategies for muscle. Injection of cells in a carrier, or manipulation of endogenous stem or progenitor cell populations, is a less complicated alternative to classical tissue engineering. Cell therapies are typically less invasive and less technically demanding than growing muscle *ex vivo*. Muscle is also a highly demanding tissue metabolically, a characteristic that further complicates a classical tissue engineering approach. One group has performed implantation of muscle progenitors and investigated their potential as a cell therapy for skeletal muscle regeneration in a swine model.²³⁴ However, those stem/progenitor cell therapies closest to clinical practice have been and continue to be directed toward cardiac muscle.²³⁵

Non-cell-based RM products represent the low hanging fruit in trauma and orthopedics. Strategies for tendon and ligament, nerve, and especially bone are prevalent. These typically involve the use of

a scaffolding system that makes use of endogenous cells for tissue regeneration. Tendon and ligament allografts have been gaining popularity as an alternative to autograft.²³⁶ These connective tissues have the advantage that they are relatively acellular and do not require large numbers of endogenous cells or dense vascularization for remodeling. Peripheral nerve regeneration has been successfully achieved through nerve guidance conduits filled with saline.²³⁷ Bone represents more of a challenge because it is highly vascularized and innervated. Nevertheless, many acellular products dot the landscape of preclinical development and commercial use. Moreover, the commercialization of recombinant human bone morphogenetic protein 2 has facilitated the development of many new strategies for regenerating bone.²³⁸

BIOMATERIALS

Before recent advances in RM, the use of biomaterials was a cornerstone of surgery-based treatment of disease and injury in the cardiovascular and skeletal systems (eg, heart valve replacements and joint or bone fixation). First-generation materials such as bone cement, stainless steel, and Dacron were used extensively because of their mechanical stability and relatively inert nature that resulted in minimal foreign body responses. As the ability of the body to regenerate was recognized, more advanced materials were created to exhibit enhanced biodegradability and bio-integration.²³⁹ These materials included titanium (osseointegration), Bioglass (tissue integration), biodegradable synthetic polymers such as PLGA, and natural polymers such as bovine collagen (dermal fillers). Though many of these materials are still in widespread use, an increased understanding of the body's regenerative capacity and the ability to enhance this capacity through cellular treatments is pushing the field toward the use of bioactive, smart biomaterials.²⁴⁰ These advanced materials, tailored for specific diseases, injuries, or even individuals will likely play a major role in RM strategies for surgical medicine in the future.

A key challenge in designing the next generation of advanced biomaterials is that body tissues and organs are highly specialized in structure and function. Thus, a single biomaterial is unlikely to be suitable for all applications. However, we can define several criteria critical to future biomaterial for surgical modalities: suitable host response including minimal foreign body, inflammatory, and immune responses^{241,242}; tunable degradation profiles inversely proportional to rate of tissue regeneration²⁴³; appropriate presentation of intrinsic motifs or immobilized extrinsic factors to modulate cell attachment, proliferation, migration, and differentiation^{243,244}; controlled, efficient, and on-demand delivery of growth factors²⁴⁵; suitable material properties including porosity, stiffness, and strength²⁴⁶; provision of physical/topographic cues to guide cell migration²⁴⁷; and useful for both *in vitro* and *in vivo* approaches to achieving tissue formation.

The most promising materials achieving many of the ideal design criteria for RM strategies are the polymeric biomaterials. Polymeric biomaterials may be classified as synthetic or natural, and examples can be found in RM approaches for most tissue and organ systems. Taking into account the unique macro- and micro-environments of each tissue, we highlight the material approach to 3 tissue systems—namely, nerve, blood vessels, and bone—to show how the application of the biomaterial design criteria described earlier has been used to promote regeneration.

Nerve

Inducing axonal growth into areas damaged by injury, surgery, or degenerative disease is a clinical need in both the central and peripheral nervous systems. Existing collagen biomaterial nerve cuffs provide a physical bridge but have yet to replace surgical repair via autograft. New strategies utilizing natural polymer hydrogels including fibrin,²⁴⁸ keratin,²⁴⁹ and hyaluronic acid²⁵⁰ provide a favorable mechanical matrix to promote infiltration of Schwann cells and

regeneration of axons. Materials containing inherent binding sequences such as the amino acid motif RGD,²⁵¹ incorporation of covalently bound exogenous molecules,²⁵² or achieving sustained release of soluble factors²⁵³ all show promising results comparable to the current clinical standard of autograft.

Blood Vessels

The development of biomaterials for small-diameter vascular grafts highlights several important biomaterial characteristics and challenges. Electrospinning and other processing techniques provide a means to incorporate sufficient porosity into materials to allow smooth muscle cell infiltration and sufficient mechanical properties to withstand vascular pressure.²⁵⁴ The porosity of the electrospun scaffolds and the surface topography achieved have been demonstrated conceptually to promote smooth muscle cell infiltration²⁵⁴ and endothelial cell attachment,²⁵⁵ respectively. The use of elastomeric, biodegradable materials such as polyester urethane urea provides the ability to achieve sufficient compliance for vascular graft applications.²⁵⁶ However, incorporating porosity, surface topography, mechanical strength, and compliance into a single construct achieving long-term patency remains a challenge.

Bone

Bone regeneration for bridging of segmental defects, promoting healing in partial or nonunions, and promoting spinal disk fusion is an area of active research. Orthobiologics that release growth factors such as recombinant human bone morphogenetic protein 2 are widely used for bone repair but have been unable to completely eliminate autografting from the surgical repertoire. Research into materials for bone regeneration is focused on achieving better control over release of growth-promoting factors such as human bone morphogenetic protein 2, achieving mechanical properties that may allow for no fixation devices, and providing appropriate architecture to promote regeneration. The use of solid free-form fabrication and other three-dimensional architectural design methods have proven useful for providing complex architecture to promote bone regeneration with materials of mechanical and chemical properties comparable to native bone.²⁵⁷ The incorporation of controlled release of growth factors through gene delivery has also been demonstrated to promote regeneration, indicating the potential of bioactive scaffolds to achieve clinically relevant treatment modalities.²⁵⁸

THE IMMUNE RESPONSE TO BIOMATERIALS

As all bioengineered body parts implanted so far in humans were manufactured from autologous cells, information regarding the in vivo immune response to biomaterials relate to the implantation of synthetic or animal-derived acellular constructs.^{1,259–261} Moreover, basically no data are available in humans, whereas most evidence referring to the phenomenology of such response has been provided by experimental investigations in animal models, mainly rodents, pigs, and nonhuman primates.^{1,259–263}

Literature shows that despite the involvement of both innate and acquired immunity, the innate compartment of the immune system appears to play a key role. Regardless of the site of implantation, the first relevant event of the immune response is the contact between biomaterials and whole blood, which follows more or less the requisite hemorrhage caused by the incision. Thereafter, activation of the coagulation, contact, and complement systems follows in a domino effect, with the consequent release of myriads of molecules, and the recruitment of cellular elements that altogether will mount the initial acute inflammatory response.²⁶⁴ As implanted biomaterials are intended to remain in situ indefinitely or until degradation, they tend to generate response like a foreign body reaction.^{1,259,260} In fact, in the

presence of a persisting stimulus represented by permanent biomaterials, the acute inflammatory response generated by the initial injury to the vascularized connective tissue is destined to perpetuate and change, as different cells are recruited over time and the predominant cell type present in the inflammatory milieu varies with the age of the injury.²⁵⁹ There are 2 possible fates, depending on the biodegradability of the biomaterial. If the biomaterial is not degradable, acute inflammation becomes chronic, with formation of granulation tissue that will eventually lead to development of a hard fibrotic capsule, for example, the one that envelopes medical devices like port-a-cath months after implantation. On the other contrary, if the biomaterial is degradable, then inflammation progressively attenuates to ultimately extinguish with full *restitution ad integrum* on the site of implant. Ideally, biomaterials should be completely degraded and replaced by normal tissue, as their role is to support and enhance cell growth and proliferation, which would otherwise be inadequate.

Whereas investigators have focused their attention on the sequence of events involved in the inflammatory response, recent understanding of the mechanisms underlying the immune response to sterile stimuli has switched the attention to the pathways activated during the response to biomaterials.^{265,266} Robust data show that the key molecule in sterile inflammation is interleukin-1b, whose transcription is mediated by the inflammasome system. Inflammasomes are multiprotein complexes formed in the cell cytosol upon stimulation and whose activation is responsible for the initiation of inflammatory processes.

FINAL REMARKS

The present review demonstrates that RM has the potential to address some of the unmet needs in several surgical diseases through exploitation of the potential to regenerate, which is intrinsic to human cells, tissues, and organs. The main challenges for investigators are threefold: creation of an appropriate microenvironment *ex vivo* that is able to sustain cell physiology and function; identification and appropriate manipulation of cells that have the potential to generate parenchymal, stromal, and vascular components on demand, both *in vivo* and *ex vivo*; and production of smart materials that are able to drive cell fate *ad hoc*.

REFERENCES

- Hollander A, Macchiarini P, Gordijn B, Birchall M. The first stem cell-based tissue-engineered organ replacement: implications for regenerative medicine and society. *Regen Med*. 2009;4:147–148.
- Orlando G, Baptista P, Birchall M, et al. Regenerative medicine as applied to solid organ transplantation: current status and future challenges. *Transplant Int*. 2011;24:223–232.
- Orlando G, Wood KJ, Stratta RJ, et al. Regenerative medicine and organ transplantation: past, present, and future. *Transplantation*. 2011;91:1310–1317.
- Mason C, Dunnill P. A brief definition of regenerative medicine. *Regen Med*. 2008;3:1–5.
- Kemp P. History of regenerative medicine: looking backwards to move forwards. *Regen Med*. 2006;1:653–669.
- Atala A. Engineering organs. *Curr Opin Biotechnol*. 2009;20:575–592.
- Badyalak SF, Russell AJ, Santin M. Introduction: history of regenerative medicine. In: Santin M, ed. *Strategies in Regenerative Medicine. Integrating Biology With Material Design*. New York, NY: Springer Science-Business Media, LLC; 2009:1–13.
- Gomez PF, Morcuende JA. Early attempts at hip arthroplasty—1700s to 1950s. *Iowa Orthop J*. 2005;25:25–29.
- Shinoka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med*. 2001;344:532–533.
- Matsumura G, Hibino N, Ikada Y, et al. Successful application of tissue engineered vascular autografts: clinical experience. *Biomaterials*. 2003;24:2303–2308.

11. Shinoka T, Matsumura G, Hibino N, et al. Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. *J Thorac Cardiovasc Surg.* 2005;129:1330–1338.
12. Hibino N, McGillicuddy E, Matsumura G, et al. Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg.* 2010;139:431–6, 436.e1–e2.
13. McAllister TN, Maruszewski M, Garrido SA, et al. Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study. *Lancet.* 2009;373:1440–1446.
14. L'Heureux N, McAllister TN, de la Fuente LM. Tissue-engineered blood vessel for adult arterial revascularization. *N Engl J Med.* 2007;357:1451–1453.
15. Atala A, Bauer SB, Soker S, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet.* 2006;367:1241–1246.
16. Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. *Lancet.* 2008;372:2023–2030.
17. Baiguera S, Birchall MA, Macchiarini P. Tissue-engineered tracheal transplantation. *Transplantation.* 2010;89:485–491.
18. Raya-Rivera A, Esquiliano DR, Yoo JJ, et al. Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. *Lancet.* 2011;377:1175–1182.
19. Ott HC, Matthiesen TS, Goh SK, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med.* 2008;14:213–221.
20. Uygun BE, Soto-Gutierrez A, Yagi H, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med.* 2010;16:814–820.
21. Baptista PM, Siddiqui MM, Lozier G, et al. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology.* 2011;53:604–617.
22. Baptista PM, Orlando G, Mirmalek-Sani SH, et al. Whole organ decellularization—a tool for bioscaffold fabrication and organ bioengineering. *Conf Proc IEEE Eng Med Biol Soc.* 2009;2009:6526–6529.
23. Badylak SF, Zhang L, Medberry CJ, et al. A whole organ regenerative medicine approach for liver replacement. *Tissue Eng Part C Methods.* 2011;17:677–686.
24. Petersen TH, Calle EA, Zhao L, et al. Tissue-engineered lungs for in vivo implantation. *Science.* 2010;329:538–541.
25. Ott HC, Clippinger B, Conrad C, et al. Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med.* 2010;16:927–933.
26. Lineen E, Namias N. Biologic dressing in burns. *J Craniofac Surg.* 2008;19:923–928.
27. Atiyeh BS, Gunn SW, Hayek SN. State of the art in burn treatment. *World J Surg.* 2005;29:131–148.
28. Auger FA, Lacroix D, Germain L. Skin substitutes and wound healing. *Skin Pharmacol Physiol.* 2009;22:94–102.
29. Burke JF, Yannas IV, Quinby WC Jr, et al. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. *Ann Surg.* 1981;194:413–428.
30. Boyce ST, Kagan RJ, Meyer NA, et al. The 1999 clinical research award: cultured skin substitutes combined with Integra Artificial Skin to replace native skin autograft and allograft for the closure of excised full-thickness burns. *J Burn Care Rehabil.* 1999;20:453–461.
31. Branski LK, Herndon DN, Pereira C, et al. Longitudinal assessment of Integra in primary burn management: a randomized pediatric clinical trial. *Crit Care Med.* 2007;35:2615–2623.
32. Helgeson MD, Potter BK, Evans KN, et al. Bioartificial dermal substitute: a preliminary report on its use for the management of complex combat-related soft tissue wounds. *J Orthop Trauma.* 2007;21:394–399.
33. Jeng JC, Fidler PE, Sokolich JC, et al. Seven years' experience with Integra as a reconstructive tool. *J Burn Care Res.* 2007;28:120–126.
34. Pollard RL, Kennedy PJ, Maitz PK. The use of artificial dermis (Integra) and topical negative pressure to achieve limb salvage following soft-tissue loss caused by meningococcal septicaemia. *J Plast Reconstr Aesthet Surg.* 2008;61:319–322.
35. Pham C, Greenwood J, Cleland H, et al. Bioengineered skin substitutes for the management of burns: a systematic review. *Burns.* 2007;33:946–957.
36. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol.* 2005;23:47–55.
37. Broughton G, Janis JE, Attinger CE. The basic science of wound healing. *Plast Reconstr Surg.* 2006;117:12S–34S.
38. Souren JM, Ponc M, van WR. Contraction of collagen by human fibroblasts and keratinocytes. *In Vitro Cell Dev Biol.* 1989;25:1039–1045.
39. Chakrabarty KH, Heaton M, Dalley AJ, et al. Keratinocyte-driven contraction of reconstructed human skin. *Wound Repair Regen.* 2001;9:95–106.
40. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. *J Cell Biol.* 1994;124:401–404.
41. Harrison CA, MacNeil S. The mechanism of skin graft contraction: an update on current research and potential future therapies. *Burns.* 2008;34:153–163.
42. Ralston DR, Layton C, Dalley AJ, et al. Keratinocytes contract human dermal extracellular matrix and reduce soluble fibronectin production by fibroblasts in a skin composite model. *Br J Plast Surg.* 1997;50:408–415.
43. Brown DM, Barton BR, Young VL, et al. Decreased wound contraction with fibrin glue—treated skin grafts. *Arch Surg.* 1992;127:404–406.
44. Ahmed TA, Dare EV, Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Eng Part B Rev.* 2008;14:199–215.
45. Currie LJ, Martin R, Sharpe JR, et al. A comparison of keratinocyte cell sprays with and without fibrin glue. *Burns.* 2003;29:677–685.
46. Grant I, Warwick K, Marshall J, et al. The co-application of sprayed cultured autologous keratinocytes and autologous fibrin sealant in a porcine wound model. *Br J Plast Surg.* 2002;55:219–227.
47. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. *Plast Reconstr Surg.* 2010;126:1172–1180.
48. Bourguignon LY, Ramez M, Gilad E, et al. Hyaluronan-CD44 interaction stimulates keratinocyte differentiation, lamellar body formation/secretion, and permeability barrier homeostasis. *J Invest Dermatol.* 2006;126:1356–1365.
49. Scuderi N, Onesti MG, Bistoni G, et al. The clinical application of autologous bioengineered skin based on a hyaluronic acid scaffold. *Biomaterials.* 2008;29:1620–1629.
50. Thivolet J, Faure M, Demidem A, et al. Long-term survival and immunological tolerance of human epidermal allografts produced in culture. *Transplantation.* 1986;42:274–280.
51. Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. *Lancet.* 1986;1:1123–1124.
52. Gallico GG III, O'Connor NE, Compton CC, et al. Cultured epithelial autografts for giant congenital nevi. *Plast Reconstr Surg.* 1989;84:1–9.
53. Leigh IM, Purkis PE, Navsaria HA, et al. Treatment of chronic venous ulcers with sheets of cultured allogenic keratinocytes. *Br J Dermatol.* 1987;117:591–597.
54. Sood R, Balledux J, Koumanis DJ, et al. Coverage of large pediatric wounds with cultured epithelial autografts in congenital nevi and burns: results and technique. *J Burn Care Res.* 2009;30:576–586.
55. Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn injuries: a critical review of the literature. *Burns.* 2006;32:395–401.
56. Hernon CA, Dawson RA, Freedlander E, et al. Clinical experience using cultured epithelial autografts leads to an alternative methodology for transferring skin cells from the laboratory to the patient. *Regen Med.* 2006;1:809–821.
57. Brem H, Young J, Tomic-Canic M, et al. Clinical efficacy and mechanism of bilayered living human skin equivalent (HSE) in treatment of diabetic foot ulcers. *Surg Technol Int.* 2003;11:23–31.
58. Falanga V, Sabolinski M. A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen.* 1999;7:201–207.
59. Falanga V, Margolis D, Alvarez O, et al. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. *Arch Dermatol.* 1998;134:293–300.
60. Streit M, Braathen LR. Apligraf—a living human skin equivalent for the treatment of chronic wounds. *Int J Artif Organs.* 2000;23:831–833.
61. Zaulyanov L, Kirsner RS. A review of a bi-layered living cell treatment (Apligraf) in the treatment of venous leg ulcers and diabetic foot ulcers. *Clin Interv Aging.* 2007;2:93–98.
62. Grant I, Warwick K, Marshall J, et al. The co-application of sprayed cultured autologous keratinocytes and autologous fibrin sealant in a porcine wound model. *Br J Plast Surg.* 2002;55:219–227.
63. Horch RE, Bannasch H, Stark GB. Transplantation of cultured autologous keratinocytes in fibrin sealant biomatrix to resurface chronic wounds. *Transplant Proc.* 2001;33:642–644.
64. Svensjo T, Yao F, Pomahac B, et al. Autologous keratinocyte suspensions accelerate epidermal wound healing in pigs. *J Surg Res.* 2001;99:211–221.
65. Velander P, Theopold C, Bleiziffer O, et al. Cell suspensions of autologous keratinocytes or autologous fibroblasts accelerate the healing of full thickness

- skin wounds in a diabetic porcine wound healing model. *J Surg Res.* 2009;157:14–20.
66. Wood FM, Stoner ML, Fowler BV, et al. The use of a non-cultured autologous cell suspension and Integra dermal regeneration template to repair full-thickness skin wounds in a porcine model: a one-step process. *Burns.* 2007;33:693–700.
 67. Blanpain C, Lowry WE, Geoghegan A, et al. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell.* 2004;118:635–648.
 68. Fathke C, Wilson L, Shah K, et al. Wnt signaling induces epithelial differentiation during cutaneous wound healing. *BMC Cell Biol.* 2006;7:4–12.
 69. Ghazizadeh S, Taichman LB. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. *EMBO J.* 2001;20:1215–1222.
 70. Ito M, Cotsarelis G. Is the hair follicle necessary for normal wound healing? *J Invest Dermatol.* 2008;128:1059–1061.
 71. Kamimura J, Lee D, Baden HP, et al. Primary mouse keratinocyte cultures contain hair follicle progenitor cells with multiple differentiation potential. *J Invest Dermatol.* 1997;109:534–540.
 72. Levy V, Linton C, Harfe BD, et al. Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev Cell.* 2005;9:855–861.
 73. Yan X, Owens DM. The skin: a home to multiple classes of epithelial progenitor cells. *Stem Cell Rev.* 2008;4:113–118.
 74. Ferguson MW, O’Kane S. Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philos Trans R Soc Lond B Biol Sci.* 2004;359:839–850.
 75. Roh C, Lyle S. Cutaneous stem cells and wound healing. *Pediatr Res.* 2006;59:100R–103R.
 76. Guenou H, Nissan X, Larcher F, et al. Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. *Lancet.* 2009;374:1745–1753.
 77. Hanson SE, Bentz ML, Hematti P. Mesenchymal stem cell therapy for non-healing cutaneous wounds. *Plast Reconstr Surg.* 2010;125:510–516.
 78. Allen-Hoffmann BL, Schlosser SJ, Ivarie CA, et al. Normal growth and differentiation in a spontaneously immortalized near-diploid human keratinocyte cell line, NIKS. *J Invest Dermatol.* 2000;114:444–455.
 79. Schurr MJ, Foster KN, Centanni JM, et al. Phase I/II clinical evaluation of StrataGraft: a consistent, pathogen-free human skin substitute. *J Trauma.* 2009;66:866–873.
 80. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation.* 2010;121:e46–e215.
 81. Goodney PP, Beck AW, Nagle J, et al. National trends in lower extremity bypass surgery, endovascular interventions, and major amputations. *J Vasc Surg.* 2009;50:54–60.
 82. (CDC) CfDcAP. Incidence of end-stage renal disease attributed to diabetes among persons with diagnosed diabetes—United States and Puerto Rico, 1996–2007. *MMWR Morb Mortal Wkly Rep.* 2010;59:1361–1366.
 83. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science.* 1986;231:397–400.
 84. L’Heureux N, Pâquet S, Labbé R, et al. A completely biological tissue-engineered human blood vessel. *FASEB J.* 1998;12:47–56.
 85. Niklason LE, Abbott W, Gao J, et al. Morphologic and mechanical characteristics of engineered bovine arteries. *J Vasc Surg.* 2001;33:628–638.
 86. Allaire E, Guettier C, Bruneval P, et al. Cell-free arterial grafts: morphologic characteristics of aortic isografts, allografts, and xenografts in rats. *J Vasc Surg.* 1994;19:446–456.
 87. Malone JM, Brendel K, Duhamel RC, et al. Detergent-extracted small-diameter vascular prostheses. *J Vasc Surg.* 1984;1:181–191.
 88. Dahl SL, Kypon AP, Lawson JH, et al. Readily available tissue-engineered vascular grafts. *Sci Transl Med.* 2011;3:68–69.
 89. Jay SM, Shepherd BR, Andrejcek JW, et al. Dual delivery of VEGF and MCP-1 to support endothelial cell transplantation for therapeutic vascularization. *Biomaterials.* 2010;31:3054–3062.
 90. Sun Y, Weber KT. Infarct scar: a dynamic tissue. *Cardiovasc Res.* 2000;46:250–256.
 91. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest.* 2001;108:407–414.
 92. Christman KL, Lee RJ. Biomaterials for the treatment of myocardial infarction. *J Am Coll Cardiol.* 2006;48:907–913.
 93. Zimmermann WH, Melnychenko I, Wasmeier G, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med.* 2006;12:452–458.
 94. Fazel S, Tang GH, Angoulvant D, et al. Current status of cellular therapy for ischemic heart disease. *Ann Thorac Surg.* 2005;79:S2238–S2247.
 95. Murry CE, Field LJ, Menasche P. Cell-based cardiac repair: reflections at the 10-year point. *Circulation.* 2005;112:3174–3183.
 96. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation.* 2003;108:2212–2218.
 97. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol.* 2004;44:1690–1699.
 98. Hofmann M, Wollert KC, Meyer GP, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation.* 2005;111:2198–2202.
 99. Qian H, Yang Y, Huang J, et al. Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by 18F-fluoro-deoxy-glucose: tissue distribution and impact on post-infarct swine hearts. *J Cell Biochem.* 2007;102:64–74.
 100. Caspi O, Huber I, Kehat I, et al. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol.* 2007;50:1884–1893.
 101. Zimmermann WH, Schneiderbanger K, Schubert P, et al. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res.* 2002;90:223–230.
 102. Leor J, Landa N, Cohen S. Renovation of the injured heart with myocardial tissue engineering. *Expert Rev Cardiovasc Ther.* 2006;4:239–252.
 103. Zimmermann WH, Didie M, Doker S, et al. Heart muscle engineering: an update on cardiac muscle replacement therapy. *Cardiovasc Res.* 2006;71:419–429.
 104. Shapira K, Dikovsky D, Habib M, et al. Hydrogels for cardiac tissue regeneration. *Biomed Mater Eng.* 2008;18:309–314.
 105. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol.* 2005;23:47–55.
 106. Davis ME, Hsieh PC, Grodzinsky AJ, et al. Custom design of the cardiac microenvironment with biomaterials. *Circ Res.* 2005;97:8–15.
 107. Shapira-Schweitzer K, Habib M, Gepstein L, et al. An injectable hydrogel for 3-D culture of human embryonic stem cell-derived cardiomyocytes and rat neonatal cardiac cells. *JMCC.* 2009;46:213–224.
 108. Kofidis T, Lebl DR, Martinez EC, et al. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. *Circulation.* 2005;112:1173–1177.
 109. McDevitt TC, Woodhouse KA, Hauschka SD, Murry CE, Stayton PS. Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. *J Biomed Mater Res A.* 2003;66:586–595.
 110. Shapira-Schweitzer K, Seliktar D. Matrix stiffness affects spontaneous contraction of cardiomyocytes cultured within a PEGylated fibrinogen biomaterial. *Acta Biomaterialia.* 2007;3:33–41.
 111. Saha K, Pollock JF, Schaffer DV, et al. Designing synthetic materials to control stem cell phenotype. *Curr Opin Chem Biol.* 2007;11:381–387.
 112. Christman KL, Vardanian AJ, Fang Q, et al. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol.* 2004;44:654–660.
 113. Zhang G, Hu Q, Braunlin EA, et al. Enhancing efficacy of stem cell transplantation to the heart with a PEGylated fibrin biomatrix. *Tissue Eng Part A.* 2008;14:1025–1036.
 114. Habib M, Shapira-Schweitzer K, Caspi O, et al. A combined cell therapy and in-situ tissue-engineering approach for myocardial repair. *Biomaterials.* 2011;32:7514–7523.
 115. Davis ME, Motion JP, Narmoneva DA, et al. Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. *Circulation.* 2005;111:442–450.
 116. Orens JB, Garrity ER. General overview of lung transplantation and review of organ allocation. *Proc Am Thorac Soc.* 2009;6:13–19.
 117. Grillo HC. Tracheal replacement: a critical review. *Ann Thorac Surg.* 2002;73:1995–2004.
 118. Doss AE, Dunn SS, Kucera KA, et al. Tracheal replacements: part 2. *ASAIO J.* 2007;53:631–639.

119. Bader A, Macchiarini P. Moving towards in situ tracheal regeneration: the bionic tissue engineered transplantation approach. *J Cell Mol Med*. 2010;14:1877–1889.
120. Kalathur M, Baiguera S, Macchiarini P. Translating tissue-engineered tracheal replacement from bench to bedside. *Cell Mol Life Sci*. 2010;67:4185–4196.
121. Delaere P, Goeleven A, Poorten VV, et al. Organ preservation surgery for advanced unilateral glottic and subglottic cancer. *Laryngoscope*. 2007;117:1764–1769.
122. Omori K, Tada Y, Suzuki T, et al. Clinical application of in situ tissue engineering using a scaffolding technique for reconstruction of the larynx and trachea. *Ann Otol Rhinol Laryngol*. 2008;117:673–678.
123. Huber JE, Spievack A, Simmons-Byrd A, et al. Extracellular matrix as a scaffold for laryngeal reconstruction. *Ann Otol Rhinol Laryngol*. 2003;112:428–433.
124. Ringel RL, Kahane JC, Hillsamer PJ, et al. The application of tissue engineering procedures to repair the larynx. *J Speech Lang Hear Res*. 2006;49:194–208.
125. Lwebuga-Mukasa JS, Ingbar DH, Madri JA. Repopulation of a human alveolar matrix by adult rat type II pneumocytes in vitro: a novel system for type II pneumocyte culture. *Exp Cell Res*. 1986;162:423–435.
126. Meguid EI, Nahas A, Bello AK. Chronic kidney disease: the global challenge. *Lancet*. 2005;365:331–4.
127. Yen TH, Alison MR, Cook HT, et al. The cellular origin and proliferative status of regenerating renal parenchyma after mercuric chloride damage and erythropoietin treatment. *Cell Prolif*. 2007;40:143–156.
128. Herrera MB, Bussolati B, Bruno S, et al. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med*. 2004;14:1035–1041.
129. Morigi M, Introna M, Imberti B, et al. Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. *Stem Cells*. 2008;26:2075–2082.
130. Morigi M, Rota C, Montemurro T, et al. Life-sparing effect of human cord blood-mesenchymal stem cells in experimental acute kidney injury. *Stem Cells*. 2010;28:513–522.
131. Ross EA, Williams MJ, Hamazaki T, et al. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. *J Am Soc Nephrol*. 2009;20:2338–2347.
132. Nakayama KH, Batchelder CA, Lee CI, et al. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. *Tissue Eng Part A*. 2010;16:2207–2216.
133. McDougal WS. Metabolic complications of urinary intestinal diversion. *J Urol*. 1992;147:1199–1208.
134. Atala A, Bauer SB, Hendren WH, et al. The effect of gastric augmentation on bladder function. *J Urol*. 1993;149:1099–1102.
135. Kaefer M, Tobin MS, Hendren WH, et al. Continent urinary diversion: the Children's Hospital experience. *J Urol*. 1997;157:1394–1399.
136. Badylak SF, Lantz GC, Coffey A, et al. Small intestinal submucosa as a large diameter vascular graft in the dog. *J Surg Res*. 1989;47:74–80.
137. Kropp BP, Rippey MK, Badylak SF, et al. Regenerative urinary bladder augmentation using small intestinal submucosa: urodynamics and histopathologic assessment in long-term canine bladder augmentations. *J Urol*. 1996;155:2098–2104.
138. Kropp BP, Sawyer BD, Shannon HE, et al. Characterization of small intestinal submucosa regenerated canine detrusor: assessment of reinnervation, in vitro compliance and contractility. *J Urol*. 1996;156:599–607.
139. Vaught JD, Kropp BP, Sawyer BD, et al. Detrusor regeneration in the rat using porcine small intestinal submucosal grafts: functional innervation and receptor expression. *J Urol*. 1996;155:374–378.
140. Wefer J, Sievert KD, Schlote N, et al. Time dependent smooth muscle regeneration and maturation in a bladder acellular matrix graft: histological studies and in vivo functional evaluation. *J Urol*. 2001;165:1755–1759.
141. Sutherland RS, Baskin LS, Hayward SW, et al. Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix. *J Urol*. 1996;156:571–577.
142. Probst M, Dahiya R, Carrier S, et al. Reproduction of functional smooth muscle tissue and partial bladder replacement. *Br J Urol*. 1997;79:505–515.
143. Yoo JJ, Meng J, Oberpenning F, et al. Bladder augmentation using allogenic bladder submucosa seeded with cells. *Urology*. 1998;51:221–225.
144. Piechota HJ, Dahms SE, Nunes LS, et al. In vitro functional properties of the rat bladder regenerated by the bladder acellular matrix graft. *J Urol*. 1998;159:1717–1724.
145. Zhang Y, Frimberger D, Cheng EY, et al. Challenges in a larger bladder replacement with cell-seeded and unseeded small intestinal submucosa grafts in a subtotal cystectomy model. *BJU Int*. 2006;98:1100–1105.
146. Jayo MJ, Jain D, Wagner BJ, et al. Early cellular and stromal responses in regeneration versus repair of a mammalian bladder using autologous cell and biodegradable scaffold technologies. *J Urol*. 2008;180:392–397.
147. Atala A. Tissue engineering in urologic surgery. *Urol Clin N Am*. 1998;25:39–50.
148. Atala A. This month in investigative urology: commentary on the replacement of urologic associated mucosa [comment]. *J Urol*. 1996;156:338–339.
149. Mangera A, Chapple CR. Tissue engineering in urethral reconstruction. *F1000 Med Rep*. 2010;2:65.
150. Chen F, Yoo JJ, Atala A. Acellular collagen matrix as a possible “off the shelf” biomaterial for urethral repair. *Urology*. 1999;54:407–410.
151. Atala A, Guzman L, Retik AB. A novel inert collagen matrix for hypospadias repair. *J Urol*. 1999;162:1148–1151.
152. El-Kassaby AW, Retik AB, Yoo JJ, et al. Urethral stricture repair with an off-the-shelf collagen matrix. *J Urol*. 2003;169:170–173; discussion 173.
153. El-Kassaby A, AbouShwareb T, Atala A. Randomized comparative study between buccal mucosal and acellular bladder matrix grafts in complex anterior urethral strictures. *J Urol*. 2008;179:1432–1436.
154. De Filippo RE, Yoo JJ, Atala A. Urethral replacement using cell seeded tubularized collagen matrices. *J Urol*. 2002;168:1789–1792; discussion 1792–1783.
155. Kim BS, Atala A, Yoo JJ. A collagen matrix derived from bladder can be used to engineer smooth muscle tissue. *World J Urol*. 2008;26:307–314.
156. Dorin RP, Pohl HG, De Filippo RE, et al. Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration? *World J Urol*. 2008;26:323–326.
157. Le Roux PJ. Endoscopic urethroplasty with unseeded small intestinal submucosa collagen matrix grafts: a pilot study. *J Urol*. 2005;173:140–143.
158. Farhat WA, Yeager H. Does mechanical stimulation have any role in urinary bladder tissue engineering? *World J Urol*. 2008;26:301–305.
159. Devarapalli M, Lawrence BJ, Madhally SV. Modeling nutrient consumptions in large flow-through bioreactors for tissue engineering. *Biotechnol Bioeng*. 2009;103:1003–1015.
160. Fioretto P, Sutherland DE, Najafian B, Mauer M. Remodeling of renal interstitial and tubular lesions in pancreas transplant recipients. *Kidney Int*. 2006;69:907–912.
161. Sutherland DE, Gruessner AC. Long-term results after pancreas transplantation. *Transplant Proc*. 2007;39:2323–2325.
162. Korsgren O, Lundgren T, Felldin M, et al. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia*. 2008;51:227–232.
163. Mahmoud IM, Gabr MM, Refaie AF, et al. Purified murine islet allografts: islet engraftment as influenced by implantation site and glucotoxicity. *Transplant Proc*. 1998;30:369–372.
164. Watt PC, Mullen Y, Nomura Y, et al. Successful engraftment of autologous and allogeneic islets into the porcine thymus. *J Surg Res*. 1994;56:367–371.
165. Farney AC, Hering BJ, Nelson L, et al. No late failures of intraportal human islet autografts beyond 2 years. *Transplant Proc*. 1998;30:420.
166. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000;343:230–238.
167. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. 2006;355:1318–1330.
168. Aguayo-Mazzucato C, Bonner-Weir S. Stem cell therapy for type 1 diabetes mellitus. *Nat Rev Endocrinol*. 2010;6:139–148.
169. D'Amour KA, Bang AG, Eliazar S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol*. 2006;24:1392–1401.
170. Shi Y. Generation of functional insulin-producing cells from human embryonic stem cells in vitro. *Methods Mol Biol*. 2010;636:79–85.
171. Chandra VGS, Phadnis S, et al. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells*. 2009;27:1941–1953.
172. Ezquer FE, Ezquer ME, Parrau DB, et al. Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice. *Biol Blood Marrow Transplant*. 2008;14:631–640.
173. Gabr MM, Sobh MM, Zakaria MM, et al. Transplantation of insulin-producing clusters derived from adult bone marrow stem cells to treat diabetes in rats. *Exp Clin Transplant*. 2008;6:236–243.

174. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*. 2008;26:443–452.
175. Couri CE, Oliveira MC, Stracieri AB, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2009;301:1573–1579.
176. Chiou SH, Chen SJ, Chang YL, et al. MafA promotes the reprogramming of placenta-derived multipotent stem cells into pancreatic islets-like and insulin-positive cells. *J Cell Mol Med*. 2011;15:612–662.
177. Bernardo AS, Cho CH, Mason S, et al. Biphasic induction of Pdx1 in mouse and human embryonic stem cells can mimic development of pancreatic beta-cells. *Stem Cells*. 2009;27:341–351.
178. Shimizu H, Ohashi K, Utoh R, et al. Bioengineering of a functional sheet of islet cells for the treatment of diabetes mellitus. *Biomaterials*. 2009;30:5943–5949.
179. Mao GH, Chen GA, Bai HY, et al. The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds seeded with islet-like cells derived from human embryonic stem cells. *Biomaterials*. 2009;30:1706–1714.
180. Wang X, Ye K. Three-dimensional differentiation of embryonic stem cells into islet-like insulin-producing clusters. *Tissue Eng Part A*. 2009;15:1941–1952.
181. Badylak SF, Taylor D, Uygur K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng*. 2010 Jul 21. 2011;13:27–53.
182. De Carlo E, Baiguera S, Conconi MT, et al. Pancreatic acellular matrix supports islet survival and function in a synthetic tubular device: in vitro and in vivo studies. *Int J Mol Med*. 2010;25:195–202.
183. Cooper DK, Dorling A, Pierson RN III, et al. Alpha1,3-galactosyltransferase gene-knockout pigs for xenotransplantation: where do we go from here? *Transplantation*. 2007;84:1–7.
184. Posselt AM, Szot GL, Frassetto LA, et al. Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. *Transplantation*. 2010;90:1595–601.
185. Hoesli CA, Lu M, Piret JM. A novel alginate hollow fiber bioreactor process for cellular therapy applications. *Biotechnol Prog*. 2009;25:1740–1751.
186. Jain K, Yang H, Cai BR, et al. Retrievable, replaceable, macroencapsulated pancreatic islet xenografts: long-term engraftment without immunosuppression. *Transplantation*. 1995;59:319–324.
187. Cornolti R, Figliuzzi M, Remuzzi A. Effect of micro- and macroencapsulation on oxygen consumption by pancreatic islets. *Cell Transplant*. 2009;18:195–201.
188. Gimi B, Kwon J, Kuznetsov A, et al. A nanoporous, transparent microcontainer for encapsulated islet therapy. *J Diabetes Sci Technol*. 2009;3:297–303.
189. Opara EC, Mirmalek-Sani SH, Khanna O, Moya ML, Brey EM. Design of a bioartificial pancreas(+). *J Invest Med*. 2010;58:831–837.
190. Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation—how much of the promise has been realized? *Nat Med*. 2005;11:605–613.
191. Grossman M, Raper SE, Kozarsky K, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. *Nat Genet*. 1994;6:335–341.
192. Grossman M, Rader DJ, Muller DW, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. *Nat Med*. 1995;1:1148–1154.
193. Fox JJ, Chowdhury JR, Kaufman SS, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med*. 1998;338:1422–1426.
194. Horslen SP, McCowan TC, Goertzen TC, et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics*. 2003;111:1262–1267.
195. Ambrosino G, Varotto S, Strom SC, et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. *Cell Transplant*. 2005;14:151–157.
196. Muraca M, Gerunda G, Neri D, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet*. 2002;359:317–318.
197. Sokal EM, Smets F, Bourgois A, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation*. 2003;76:735–738.
198. Strom SC, Fisher RA, Thompson MT, et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation*. 1997;63:559–569.
199. Strom SC, Chowdhury JR, Fox JJ. Hepatocyte transplantation for the treatment of human disease. *Semin Liver Dis*. 1999;19:39–48.
200. Strom SC, Fisher RA, Rubinstein WS, et al. Transplantation of human hepatocytes. *Transplant Proc*. 1997;29:2103–2106.
201. Combs C, Brunt EM, Solomon H, et al. Rapid development of hepatic alpha1-antitrypsin globules after liver transplantation for chronic hepatitis C. *Gastroenterology*. 1997;112:1372–1375.
202. Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. *Transplant Proc*. 1992;24:3052–3053.
203. Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. *Transplantation*. 2006;82:441–449.
204. Kharazini P, Hellstrom PM, Noorinayer B, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. *Eur J Gastroenterol Hepatol*. 2009;21:1199–1205.
205. Salama H, Zekri AR, Zern M, et al. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. *Cell Transplant*. 2010;19:1475–1486.
206. Zacharoulis D, Milicevic MN, Helmy S, et al. Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients with alcoholic liver cirrhosis. *Am J Gastroenterol*. 2008;103:1952–1958.
207. Khan AA, Shaik MV, Parveen N, et al. Human fetal liver derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. *Cell Transplant*. 2010;19:409–418.
208. Schmelzer E, Zhang L, Bruce A, et al. Human hepatic stem cells from fetal and postnatal donors. *J Exp Med*. 2007;204:1973–1987.
209. Gouon-Evans V, Boussemart L, Gadue P, et al. BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. *Nat Biotechnol*. 2006;24:1402–1411.
210. Gadue P, Huber TL, Paddison PJ, Keller GM. Wnt and TGF-beta signaling are required for the induction of an in vitro model of primitive streak formation using embryonic stem cells. *Proc Natl Acad Sci USA*. 2006;103:16806–16811.
211. Basma H, Soto-Gutierrez A, Yannam GR, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterology*. 2009;136:990–999.
212. Yang J, Yamato M, Shimizu T, et al. Reconstruction of functional tissues with cell sheet engineering. *Biomaterials*. 2007;28:5033–5043.
213. Ohashi K, Yokoyama T, Yamato M, et al. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. *Nat Med*. 2007;13:880–885.
214. Hecker L, Baar K, Dennis RG, et al. Development of a three-dimensional physiological model of the internal anal sphincter bioengineered in vitro from isolated smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol*. 2005;289: G188–G196.
215. Somara S, Gilmont RR, Dennis RG, et al. Bioengineered internal anal sphincter derived from isolated human internal anal sphincter smooth muscle cells. *Gastroenterology*. 2009;137:53–61.
216. Raghavan S, Miyasaka EA, Hashish M, et al. Successful implantation of physiologically functional bioengineered mouse internal anal sphincter. *Am J Physiol Gastrointest Liver Physiol*. 2010;299:G430–G439.
217. Lee M, Wu BM, Stelzner M, et al. Intestinal smooth muscle cell maintenance by basic fibroblast growth factor. *Tissue Eng Part A*. 2008;14:1395–1402.
218. Miyasaka EA, Raghavan S, Gilmont RR, et al. In vivo growth of a bioengineered internal anal sphincter: comparison of growth factors for optimization of growth and survival. *Pediatr Surg Int*. 2011;27:137–143.
219. Freud E, Efrati I, Kidron D, et al. Comparative experimental study of esophageal wall regeneration after prosthetic replacement. *J Biomed Mater Res*. 1999;45:84–91.
220. Nakase Y, Nakamura T, Kin S, et al. Intrathoracic esophageal replacement by in situ tissue-engineered esophagus. *J Thorac Cardiovasc Surg*. 2008;136: 850–859.
221. Hori Y, Nakamura T, Kimura D, et al. Functional analysis of the tissue-engineered stomach wall. *Artif Organs*. 2002;26:868–872.
222. Grikscheit TC, Siddique A, Ochoa ER, et al. Tissue-engineered small intestine improves recovery after massive small bowel resection. *Ann Surg*. 2004;240:748–754.
223. Chen MK, Badylak SF. Small bowel tissue engineering using small intestinal submucosa as a scaffold. *J Surg Res*. 2001;99:352–358.
224. Gilmont RR, Somara S, Srinivasan S, Raghavan S, Bitar KN. IAS smooth muscle cells direct the differentiation of neuronal progenitor cells into mature neurons. *Gastroenterology*. 2010;138:S110.
225. Raghavan S, Gilmont RRS, Sita Srinivasan S, Bitar KN. Physiology of intrinsically innervated bioengineered construct from human internal anal sphincter smooth muscle cells. *Gastroenterology*. 2010;138:S31.

226. Mertsching H, Schanz J, Steger V, et al. Generation and transplantation of an autologous vascularized bioartificial human tissue. *Transplantation*. 2009;88:203–210.
227. Soucacos PN, Johnson EO, Babis G. An update on recent advances in bone regeneration. *Injury*. 2008;39:S1–S4.
228. Gates CB, Karthikeyan T, Fu F, Huard J. Regenerative medicine for the musculoskeletal system based on muscle-derived stem cells. *J Am Acad Orthop Surg*. 2008;16:68–76.
229. Lu HH, Subramony SD, Boushell MK, Zhang X. Tissue engineering strategies for the regeneration of orthopedic interfaces. *Ann Biomed Eng*. 2010;38:2142–2154.
230. Ahmed TA, Hincke MT. Strategies for articular cartilage lesion repair and functional restoration. *Tissue Eng Part B Rev*. 2010;16:305–329.
231. Battiston B, Raimondo S, Tos P, et al. Tissue engineering of peripheral nerves. *Int Rev Neurobiol*. 2009;87:227–249.
232. Peng H, Huard J. Muscle-derived stem cells for musculoskeletal tissue regeneration and repair. *Transpl Immunol*. 2004;12:311–319.
233. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol*. 2004;36:568–584.
234. Holzer N, Hogendoorn S, Zürcher L, et al. Autologous transplantation of porcine myogenic precursor cells in skeletal muscle. *Neuromuscul Disord*. 2005;15:237–244.
235. Burt RK, Loh Y, Pearce W, et al. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. *JAMA*. 2008;299:925–936.
236. Poehling GG, Curl WW, Lee CA, et al. Analysis of outcomes of anterior cruciate ligament repair with 5-year follow-up: allograft versus autograft. *Arthroscopy*. 2005;21:774–785.
237. Meek MF, Coert JH. US Food and Drug Administration/Conformit Europe-approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. *Ann Plast Surg*. 2008;60:466–472.
238. McKay WF, Peckham SM, Badura JM. A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). *Int Orthop*. 2007;31:729–734.
239. Langer R, Vacanti JP. Tissue engineering. *Science*. 1993;260:920–926.
240. Furth ME, Atala A, Van Dyke ME. Smart biomaterials design for tissue engineering and regenerative medicine. *Biomaterials*. 2007;28:5068–5073.
241. Cooper ML, Spielvogel RL. Artificial skin for wound healing. *Clin Dermatol*. 1994;12:183–191.
242. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Semin Immunol*. 2008;20:86–100.
243. Mieszawska AJ, Kaplan DL. Smart biomaterials—regulating cell behavior through signaling molecules. *BMC Biol*. 2010;8:59.
244. Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. *Nature*. 2009;462:433–441.
245. Biondi M, Ungaro F, Quaglia F, et al. Controlled drug delivery in tissue engineering. *Adv Drug Deliv Rev*. 2008;60:229–242.
246. Hollister SJ. Porous scaffold design for tissue engineering. *Nat Mater*. 2005;4:518–524.
247. Hoffman-Kim D, Mitchel JA, Bellamkonda RV. Topography, cell response, and nerve regeneration. *Annu Rev Biomed Eng*. 2010;12:203–231.
248. Nakayama K, Takakuda K, Koyama Y, et al. Enhancement of peripheral nerve regeneration using bioabsorbable polymer tubes packed with fibrin gel. *Artif Organs*. 2007;31:500–508.
249. Sierpinski P, Garrett J, Ma J, et al. The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. *Biomaterials*. 2008;29:118–128.
250. Suri S, Schmidt CE. Cell-laden hydrogel constructs of hyaluronic acid, collagen, and laminin for neural tissue engineering. *Tissue Eng Part A*. 2010;16:1703–1716.
251. Yoshii S, Oka M. Collagen filaments as a scaffold for nerve regeneration. *J Biomed Mater Res*. 2001;56:400–405.
252. Dodla MC, Bellamkonda RV. Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. *Biomaterials*. 2008;29:33–46.
253. Taylor SJ, Sakiyama-Elbert SE. Effect of controlled delivery of neurotrophin-3 from fibrin on spinal cord injury in a long term model. *J Control Release*. 2006;116:204–210.
254. Ju YM, Choi JS, Atala A, et al. Bilayered scaffold for engineering cellularized blood vessels. *Biomaterials*. 2010;31:4313–4321.
255. Grasl C, Bergmeister H, Stoiber M, et al. Electrospun polyurethane vascular grafts: in vitro mechanical behavior and endothelial adhesion molecule expression. *J Biomed Mater Res A*. 2010;93:716–723.
256. Soletti L, Nieponice A, Hong Y, et al. In vivo performance of a phospholipid-coated bioerodible elastomeric graft for small-diameter vascular applications. *J Biomed Mater Res A*. 2011;96:436–448.
257. Hollister SJ, Lin CY, Saito E, et al. Engineering craniofacial scaffolds. *Orthod Craniofac Res*. 2005;8:162–173.
258. Lin CY, Schek RM, Mistry AS, et al. Functional bone engineering using ex vivo gene therapy and topology-optimized, biodegradable polymer composite scaffolds. *Tissue Eng*. 2005;11:1589–1598.
259. Badylak SF, Gilbert TW. Immune response to biologic scaffold materials. *Semin Immunol*. 2008;20:109–116.
260. Babensee JE, Anderson JM, McIntire LV, et al. Host response to tissue engineered devices. *Adv Drug Deliv Rev*. 1998;33:111–139.
261. Bayrak A, Tyralla M, Ladhoff J, et al. Human immune responses to porcine xenogeneic matrices and their extracellular matrix constituents in vitro. *Biomaterials*. 2010;31:3793–3803.
262. Daly KA, Stewart-Akers AM, Hara H, et al. Effect of the alpha-Gal epitope on the response to small intestinal submucosa extracellular matrix in a nonhuman primate model. *Tissue Eng Part A*. 2009;15:3877–3888.
263. Nilsson B, Ekdahl KN, Mollnes TE, Lambiris JD. The role of complement in biomaterial-induced inflammation. *Molecular Immunol*. 2007;44:82–94.
264. Anderson JM. Biological responses to biomaterials. *Annu Rev Mater Res*. 2001;31:81–110.
265. Rock KL, Latz E, Ontiveros F, et al. The sterile inflammatory response. *Annu Rev Immunol*. 2010;28:321–342.
266. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol*. 2010;10:826–837.