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REGENERATIVE MEDICINE AS APPLIED TO GENERAL SURGERY

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Abstract

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 in order 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.

Keywords

regenerative medicine; tissue engineering; surgery; extracellular matrix; scaffold; stem cells

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

Hollander A, Macchiarini P, Gordijn B, Birchall M.
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Introduction

Regenerative medicine [RM] refers to a field in the health sciences that aims to replace or regenerate human cells, tissues, or organs in order to restore or establish normal function [1–3]. 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 the above. As such, RM brings together multidisciplinary teams including physicians, scientists, veterinarians, engineers, chemists, physicists, statisticians, mathematicians, and industry in new and productive ways [4, 5]. 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* [2].

Although the term RM was coined and appeared in the literature as recently as 1999 [2,3], *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 [2,6,7]. Recent reports on the fabrication and implantation in humans of bioengineered vessels [8–13], bladders [14], windpipe [15,16] and urethras [17], as well as the production of heart [18], liver [19–22] and lung [23,24] organoids, have provided glimpses of the immense intrinsic potential of RM as applied to surgical diseases.

Therefore, while we share the thought expressed by Hollander et al. that “never has there been a more exciting time to be involved in surgical science” [25], 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 non-healing 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 non-healing 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 10–15 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 (SC). It is important to note that while many potential skin therapies show promise in rodent models, some therapies may not be applicable to humans due to significant differences in wound healing mechanisms between thin-skinned 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", while 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 front-line 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 scarring [47] 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].

CEA 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 non-cultured 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]. Further, it is likely that non-scarring 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 grafting [80] or peripheral vascular bypass procedures [81], and the approximately 100,000 new individuals who will need hemodialysis access each year [82], over 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 non-thrombogenic, 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. While native arteries have rupture strengths >2000 mm Hg, Weinberg and Bell’s grafts failed below 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 greater than 2500 mm Hg. Neo-vessels were successfully implanted in a canine model initially [84].

The adaptation of biodegradable polymers, especially polyglycolic acid (PGA), has allowed researchers to begin to overcome the biophysical limitations of collagen based and *de novo* scaffolds. Niklason et al. 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 [85]. These constructs achieved burst pressures above 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. Decellurized 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], while 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, SMC 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 non-immunogenic, species matched constructs with burst pressures between 2600 and 3300 mm Hg that 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 (PCL) reinforced by woven PGA and seeded with bone marrow derived mononuclear cells prior to 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 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 [10]. 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, while updated results that are currently under review show a considerable decrease in the overall complication rate relative to preoperative care (McAllister T, L'Heureux N, unpublished data).

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 SC (iPS) hold the potential to generate large amounts of autologous building blocks for both in vitro and in vivo neovessel development.

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 treatment 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 following 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 one hour after intracoronary delivery of autologous bone marrow mononuclear cells by saline injection, <7% of the cells appeared in the myocardium whereas >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 in order to create a functional cardiac syncytium [91,100]. However, the integration of engrafted myocytes – as well as 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 addressed these problems by introducing better cell immobilization techniques that employed either a tissue engineering approach [101] or a simple biomaterial augmentation to direct cell injections [102]. Whereas a tissue engineering strategy requires the *ex vivo* formation of an 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 of a biomaterial scaffold and cultivated cardiomyocytes has been demonstrated recently [103]; 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 al*. 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 hydrogels [115] 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 – seem 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 tracheo-bronchial replacement [17, 18]. At present, the patient is well, active with normal lung function and, more importantly, does not require immunosuppressive drugs. The above seminal procedure 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 re seeded 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 in order to achieve an acceptable functional result from an airway and breathing perspective [121,122]. However, voice and swallowing functions remain sub-optimal due to 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]. Following 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 [Baiguera S, Macchiarini P, unpublished data].

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 disproportionate 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 based on the use of progenitor/SCs. It has been shown that bone marrow SC, such as MSC, 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, MSC have been shown to accelerate organ structural and functional recovery [128,129]. These studies demonstrated that systemically injected MSC 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 al. 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 [131]. More recently, Nakayama et al. produced an acellular kidney scaffold from adult rhesus monkey with preserved expression patterns of native ECM proteins [132]. 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 two 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 three-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, as well as preventing 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 two 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, as well as decellularized scaffolds such as small intestinal sub-mucosa (SIS) [136–139], and acellular bladder matrices [140–146]. While 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, as well as 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 seven 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 1/25/2011) revealed three studies sponsored by Tengion 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 submucosa [150] 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 (i.e., 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 five 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 non-seeded 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 above for 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 non-seeded 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 as well as 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 intra-portal 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 seven diabetic patients who received

allogeneic islet transplants and remained insulin-independent one year after transplantation [166]. Success of the Edmonton protocol was in part attributed to transplantation of islets isolated from 2–3 deceased donor pancreases. Unfortunately, intermediate outcomes in an international multi-center 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 al. has reported that adipose-derived SC 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 following 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 (Pdx1) 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]. iPS and amniotic fluid SC (AFSC) 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 in order 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, prior to 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 three-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 three-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 over 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 AFSC 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 immuno isolation. For example, efforts to breed pigs that lack expression of the galactose-alpha-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 immuno isolation 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, as well as 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 LDL receptor, the patient's hepatocytes were infused through the inferior mesenteric vein into the liver. LDL and HDL levels improved throughout the next 18 months and transgene expression was detected in a liver biopsy [191]. Following 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 due to poor quality, quantity and possibly toxicity of immunosuppression [203]. However, transplantation of a number of hepatocytes corresponding to 1–5% 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].

Due to the shortage of available human hepatocytes for transplantation, other cell sources have been used. Specifically, bone marrow derived MSC [204], hematopoietic SC [205,206] and fetal liver progenitor cells [207] have shown to improve, to a certain extent, the condition of cirrhotic patients. The latter cell type holds enormous potential for RM therapies due to 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, two 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 four 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].

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 eight 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, as well as for transplantation.

Gastrointestinal (GI) tract

The 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, while 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 panoply 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, PLA, PGA, composite PLA and PGA and PCL, 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 (b-FGF) to intestinal smooth muscle in a mouse model demonstrated maintenance of viability of implanted sphincteric smooth muscle as well as small intestinal smooth muscle [216,217]. A comparison of the delivery of various angiogenic growth factors demonstrated that the FDA-approved platelet derived growth factor 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 al. demonstrated that portions of the esophagus can be replaced with smooth muscles on PGA sheets in combination with fibroblasts keratinocytes [220]. Similarly, Hori et al. have repaired gastric wall defects in a canine model by using collagen sponge scaffolds [221]. Limitations of the above approaches include no demonstration of physiological functionality or gut motility.

Vacanti 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. Badylak et al. have implanted scaffolds made of SIS to replace segments of short bowel in canine models [223]. These approaches reported that the implantations met basic physiological demands, but display limited or no enteric neuronal repopulation.

Bitar et al. demonstrated neovascularization and successful implantation of bioengineered internal anal sphincter 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 [224]. This group has also recently co-cultured 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 as well as neuronal in-growth. Physiological function of the GI smooth muscle is derived from the restoration of smooth muscle as well as 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 VIP-ergic/nitrergic 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 oesophagus [226] 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 oesophagotracheal 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 orthopaedic 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 lab, 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 comprised of 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.

Due to 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 pre-clinical 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 SC [232] within muscle tissue, as well as the capacity of bone marrow- and adipose-derived SC to differentiate into muscle cells [233], has lead 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

Prior to recent advances in RM, the use of biomaterials was a cornerstone of surgical-based treatment of disease and injury in the cardiovascular and skeletal systems (e.g. heart valve replacements and joint or bone fixation). First generation materials such as bone cement, stainless steel and Dacron were used extensively due to 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 regeneration [243]; 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 factors [245]; suitable material properties including porosity, stiffness, and strength [246]; provision of physical/topographic cues to guide cell migration [247]; 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 three tissue systems – namely, nerve, blood vessels and bone – to show how the application of the biomaterial design criteria described above 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 acid [250] provide a favorable mechanical matrix to promote infiltration of Schwann cells and regeneration of axons. Materials containing inherent

binding sequences such as the aminoacid motif RGD [251], incorporation of covalently bound exogenous molecules [252], or achieving sustained release of soluble factors [253] 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 as well as 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 infiltration [254] 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 non-unions, 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 non-human primates [1, 259–263].

Literature shows that despite the involvement of both innate and acquired immunity, the innate compartment of the immune system seems 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 a foreign body reaction-like response [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 two 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, like – for example –, the one that envelopes medical devices like Port-a-cath months after implantation. On the other hand, 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 multi-protein 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*.

Abbreviations

RM	regenerative medicine
ECM	extracellular matrix
SC	stem cells
CEA	cultured epithelial autografts
ESC	embryonic stem cells
MSC	mesenchymal stem cells
SMC	smooth muscle cells
PGA	polyglycolic acid
PLLA	poly-L-lactic acid
PCL	poly-ε-caprolactone
TEVG	tissue engineered vascular graft
iPS	induced pluripotent
GI	gastrointestinal
SIS	small intestinal submucosa

IAS	internal anal sphincter
AFSC	amniotic fluid stem cells
MafA	musculoaponeurotic fibrosarcoma oncogene homolog A

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