Biomaterials Approach to Expand and Direct Differentiation of Stem Cells

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Stem cells play increasingly prominent roles in tissue engineering and regenerative medicine. Pluripotent embryonic stem (ES) cells theoretically allow every cell type in the body to be regenerated. Adult stem cells have also been identified and isolated from every major tissue and organ, some possessing apparent pluripotency comparable to that of ES cells. However, a major limitation in the translation of stem cell technologies to clinical applications is the supply of cells. Advances in biomaterials engineering and scaffold fabrication enable the development of *ex vivo* cell expansion systems to address this limitation. Progress in biomaterial design has also allowed directed differentiation of stem cells into specific lineages. In addition to delivering biochemical cues, various technologies have been developed to introduce micro- and nano-scale features onto culture surfaces to enable the study of stem cell responses to topographical cues. Knowledge gained from these studies portends the alteration of stem cell fate in the absence of biological factors, which would be valuable in the engineering of complex organs comprising multiple cell types. Biomaterials may also play an immunoprotective role by minimizing host immunoreactivity toward transplanted cells or engineered grafts.

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INTRODUCTION

Stem cells, whether derived from embryos, fetuses, or adults, seem poised to dominate the next frontier of human regenerative medicine and cellular therapy. Over the last 15 years, major advances have been made in the isolation, culture, and the induction of differentiation of stem cells from various sources. Stem cells have now been identified in every major organ and tissue of the human body. Concomitant with these discoveries are intense efforts to understand the molecular mechanisms underlying the decision of stem cells to enter mitotic dormancy, undergo self-renewal, or differentiate terminally. An understanding of these molecular mechanisms would help realize the tremendous therapeutic potential of stem cells. To this end, state-of-the-art technologies have been developed to interrogate genome-wide gene expression in stem cells in an effort to establish the cause–effect relationship between the biologic states of stem cells and the molecular signatures that they manifest. Recent studies uncovered novel mechanisms by which stem cell fate is regulated, implicating the participation of stem cell-specific microRNAs[1] and fate reprogramming factors that can act cell autonomously.[2] In addition to the discovery of new genes, the functions of definitive stem cell markers such as Nanog, Oct4, and Sox2 are rapidly being elucidated. Continued discoveries in the cell and molecular biology of stem cells will facilitate their application, the most exciting of which would be in regenerative medicine and cell therapy.

The chronic shortage of donor organs and tissues for transplantation has provided the impetus for intense research in the field of tissue engineering (TE). Unlike pharmacology and physiotherapies that are mainly palliative, TE and cellular therapy seek to augment, replace, or reconstruct damaged or diseased tissues. The advent of various enabling technologies coupled with paradigm shifts in biomaterial designs, promises to change the fundamental landscape of TE. In recent years, biomaterials design has evolved from the classical, first-generation material-biased approach that favored mechanical strength, durability, bioinertness, or biocompatibility to third-generation, biofunctional materials that seek to incorporate instructive signals into scaffolds to modulate cellular functions such as proliferation, differentiation, and morphogenesis. To impart bioactivity to these biomaterials, their surfaces may be adorned with signaling molecules such as glycosaminoglycans, proteoglycans, and glycoproteins normally associated with the extracellular matrix (ECM) on cell surfaces, or they may be loaded with soluble bioactive molecules such as chemokines, cytokines, growth factors, or hormones that are released and act in a paracrine manner. Advances in conjugation chemistries have now widened the options for modifying natural biopolymers or synthetic biomaterials. The development of smart biomaterials that can respond to specific stimuli such as temperature,[3] pH,[4] electrical signals,[5] light,[6] and metabolites such as glucose[7] and adenosine triphosphate[8] can be employed to control properties

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such as drug release, cell adhesiveness, phase behavior, and mechanical parameters such as permeability, volume, and electrical conductivity.

THE ROLES OF BIOMATERIALS IN STEM CELL TE

With the possibility of therapeutic cloning becoming a reality,[9] there is an urgency to develop technologies that can precisely control the behavior of stem cells in culture. Central to these technologies would be the probable inclusion of biomaterials as an important component. For instance, the recent report of the successful transplantation of a urinary bladder engineered from autologous urothelial and muscle cells in human patients,[10] made possible by culturing these cells in a poly(D,L-lactide-co-glycolide) (PLGA) scaffold, heralds the arrival of the era of whole organ TE. Advances in biomaterial research will undoubtedly facilitate the transformation of this concept into reality. Biomaterial scaffolds can play a number of specific roles in TE applications using stem cells.

Biomaterials as defined systems for stem-cell derivation and expansion

A fundamental bottleneck that must be overcome to exploit stem cells for TE is the adequate supply of cells. This problem will become more critical when the engineering of bulk tissue or complex organs is contemplated, particularly when autologous tissue production is desired. Such goals would necessitate the maintenance of large quantities of undifferentiated cells to provide sufficient starting material. The long doubling time of most types of stem cell weighs directly on this problem. The doubling time of stem cells ranges from 36 h for human embryonic stem cells (ESCs) to an estimated 45 days for human hematopoietic stem cells (HSCs) (**Table 1**). Although it is generally believed that human ESCs can divide indefinitely, there is evidence to suggest that other stem cell types are subjugated to Hayflick's limit when cultured *in vitro*. Human mesenchymal stem cells (MSCs) appear to show signs of senescence after the ninth passage in culture with a decline in differentiation potential from passage 6 (ref. 18). The recent identification of a population of adult MSCs (multipotent adult progenitor cell, MAPC), with a self-renewal and multipotent differentiation potential very similar to that of ESCs, raises hope for a source of renewable autologous stem cells. These cells can be expanded *in vitro* up to 120 cell divisions without losing their stem cell potential.[19] However, as these cells occur at low frequency, extensive *in vitro* expansion would be required to obtain a sufficient number of cells for therapeutic purposes.

Table 1 Doubling time of human stem cells

	Average doubling time	Refs.
ESC	35 h	11, 12
HSC	45 weeks	13
MSC	1.3–16 days	14, 15
NSC	4 days	16
EGC	3.2 days	17

ESC, embryonic stem cell; EGC, embryonic germ cell; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; NSC, neural stem cell.

Although a number of commercially available cell culture matrices such as Matrigel and Cartrigel have produced encouraging results, the animal origin of these products renders them undefined and precludes their widespread use in human clinical applications. A recent trend favors the use of animal-free products, with recombinant human substitutes for such animal products emerging as an attractive alternative. Concerns about exposure of human tissues to xenogenic products have been substantiated experimentally. Besides the risk of contamination by adventitious infectious agents, there has been evidence to suggest that human cells could incorporate and express immunogenic molecules present in animal products. Human ESCs cultured with animal feeders or serum products could take up and express Neu5Gc, a non-human sialic acid, from the culture medium.[20] As most healthy human adults have circulating antibodies against Neu5Gc,[21] transplantation success would be compromised if ESCs previously exposed to Neu5Gc had been used to develop the donor tissues. Synthetic biomaterials could play a significant role in meeting the demands for well-defined systems for derivation and maintenance of ESCs.

Biomaterial substrates for clonal expansion of genetically engineered stem cells

An important potential clinical application of stem cells is their use in cell replacement therapy for inherited genetic disorders. Using viral vector transduction, stem cells can be manipulated *in vitro* to correct genetic aberrations or deficiencies. When transplanted into patients, such cells might restore normal tissue function. As the sites of viral vector insertion are largely random in distribution, there is a risk of neoplastic transformation of individual transduced clones.[22] This risk may, however, be managed by the safe design of viral vectors. Alternatively, a preselection step for clones that do not harbor deleterious insertions, followed by a thorough preclinical evaluation of these clones in animals, may minimize the risk. *Ex vivo* expansion of preselected clones can be achieved in a bioreactor fabricated from a suitable biomaterial to produce sufficient cells to engraft a patient.

Biomaterials for differentiation of stem cells

The plasticity of ESCs represents a proverbial double-edged sword for its use in clinical application. Although clearly a desirable property owing to the tremendous differentiation repertoire that it accords, it also poses a risk of tumorigenicity. Undifferentiated cells that retain pluripotency give rise to tumors known as teratomas. Hence, it is critical for any therapeutic strategy employing a stem cell-based approach to ensure complete and irreversible differentiation of stem cells into the desired progenitors or terminal target cell type. This may be accomplished by supplementing the appropriate trophic factors in the culture medium, or delivering them from a scaffold in a controlled manner. Different technologies have been developed to incorporate drug delivery function into a scaffold. Proteins, peptides, or plasmid DNA can be loaded into microspheres and uniformly dispersed in a macroporous polymeric scaffold, or they can be encapsulated in a fiber before forming a fibrous

scaffold.[23,24] This biomaterials-based approach to provide a local and sustained delivery of growth factors would be particularly valuable for the tissue development of ES-seeded scaffolds *in vivo*.

The mechanical properties of a scaffold or culture surface can also exert significant influence on the differentiation of the seeded stem cell. By exerting traction forces on a substrate, many mature cell types such as epithelial cells, fibroblasts, muscle cells, and neurons sense the stiffness of the substrate and show dissimilar morphology and adhesive characteristics.[25] This mechanosensitivity has recently been extended to the differentiation of MSCs.[26] When cultured on agarose gels with increasing crosslinking densities, human MSCs differentiated into neuronal, muscle, or bone lineages according to the stiffness of the matrix which approximated that of brain, muscle, and bone tissue, respectively. Highlighting the importance of matrix elasticity in dictating stem cell fate, this study also suggests an interesting biomaterial approach to influence the differentiation of stem cells.

Biomaterials as cell carriers for *in vivo* stem cell delivery

The loss of implanted cells can arise due to cytotoxicity or failure of the cells to integrate into host tissue, which presents a significant challenge to current approaches to tissue regeneration. Sites of injury or diseased organs often present hostile environments for healthy cells to establish and repopulate owing to the heightened immunological surveillance and the high concentration of inflammatory cytokines at these sites. Therefore, an additional role for TE scaffolds is to insulate their cellular cargos from the host immune system, obviating the need for a harsh immunosuppressive regime to promote the survival of grafts. Alginate-based biomaterials have been found to immunoprotect encapsulated cells and preliminary studies have demonstrated their feasible use as a vehicle for stem cell delivery.[27] The incorporation of immuno-modulatory molecules into biomaterial designs may represent another strategy to tackle the issue of immunorejection.

STRATEGIES FOR STEM CELL-BASED TE

Stem cell-based TE offers clear merits over conventional TE strategies using mature cells. Conventional replacement therapies using autografts, allografts, or xenografts suffer from a host of drawbacks such as scarcity of donor source, donor site morbidity, risk of lateral transmission of pathogens, and graft-versus-host rejection. In contrast, stem cell-based approaches circumvent these drawbacks, yet introduce the advantages of scalability. A major unmet challenge in TE has been the synthesis of complex grafts that are comprised of multiple cell types. Stem cell-based TE provides one approach to this challenge. This concept was demonstrated by the engineering of an articular condyle with both cartilaginous and osseous components by differentiation of a single population of MSCs in a polyethylene glycol-based hydrogel scaffold.[28]

From an engineering standpoint, current approaches for the derivation of stem cell-based implantable grafts can be summarized into four possible strategies (**Figure 1**). In the most common strategy, stem cells are amplified by *ex vivo* expansion and differentiated into the target cell type before being seeded into scaffolds to constitute the grafts. In cases where instructive signals are incorporated into the scaffolds, differentiation can take place *in situ* in the scaffolds. In the second strategy, stem cells are amplified and differentiated directly in the scaffold before implantation. This strategy is likely more suited to adult stem cells. In the third strategy, stem cells are partially differentiated into progenitor cells either before or after seeding into scaffolds to give rise to proto-tissues. When implanted, these constructs transiently release progenitors that migrate into surrounding regions, where they undergo terminal differentiation, integrate, and contribute to regeneration of the lesioned areas. Prolonged release of stem/progenitor cells may be achieved when a suitable scaffold is used to maintain them in a partially differentiated state. Injectable grafts, composed of pristine or stimulated stem cells encapsulated in biodegradable hydrogels, constitute the fourth strategy. This strategy is attractive for soft tissue repair or treatment of solid tissues with critical size defects that are too fragile for surgical intervention.

EMERGING TRENDS IN STEM CELL TE

Micro/nanopatterned biomaterials to direct stem cell differentiation

The influence of surface features or topography on cellular growth, movement, and orientation has long been recognized.[29–33] Basement membranes, which serve as the basic substrata for cellular structures throughout the vertebrate body, are not smooth structures but, rather, are covered with grooves, ridges, pits, pores, and the fibrillar meshwork of the ECM, composed predominantly of intertwined collagen and elastin fibers with diameters ranging from 10–300 nm. Besides providing tensile strength and mechanical rigidity to the basement membrane, the fibrillar meshwork of protein fibers along with glycosaminoglycans also furnish binding sites for the less abundant cell-adhesion molecules. Natural stem cell niches, such as the bone marrow compartment, are replete with instructive ECM molecules secreted by stromal cells. The ECM is, however, not a completely amorphous entity but one that possesses a certain degree of quaternary organization. ECM fibers are arranged in semi-aligned arrays with which cells interact. At the tissue level, ordered topographical organization is more evident. For example, parallel-aligned fibrils are found in tendon, ligaments, and muscles. Concentric whorls are observed in bone, and mesh-like and orthogonal lattices are present in the skin and cornea, respectively. Therefore, it is not unexpected that cells respond to topographical cues. Studies revealed that not only are the dimensions of the topographical features important, but also their conformation—whether they are ridges, grooves, whorls, pits, pores, or steps[34–37]—and, more intriguingly, even their symmetry.[38]

The advent of micro- and nanofabrication technologies has made it possible to take apart and study independently the topographical and biochemical contribution to the cellular microenvironmental niche. Using technologies borrowed directly from the semiconductor and microelectronics industries, a plethora of techniques has been developed for creating patterned surfaces to investigate cellular behavior as diverse as cell–matrix and cell–cell interactions, polarized cell adhesion, cell differentiation in response to surface texture, cell migration,

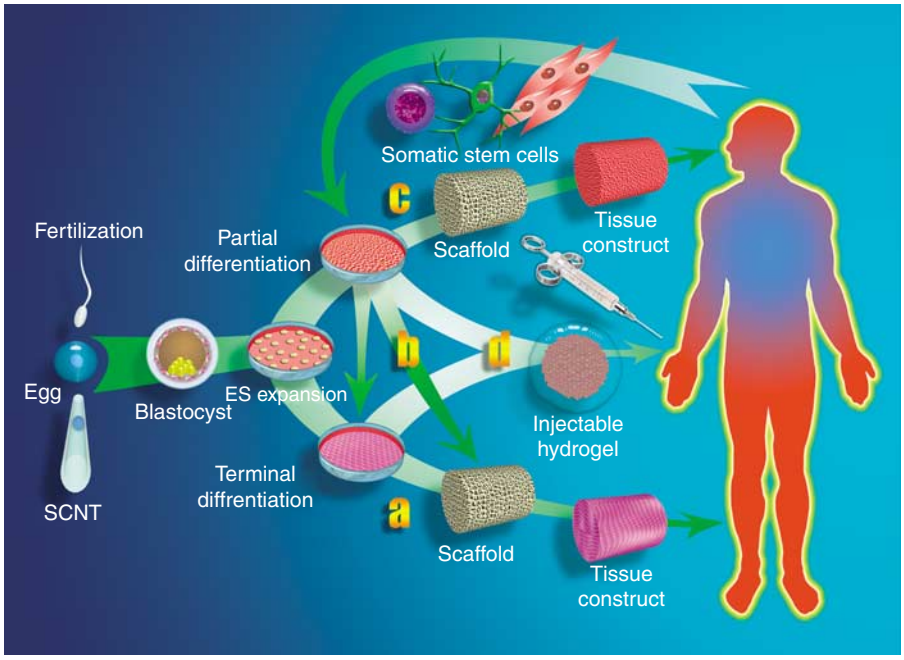

Figure 1 Multiple roles for biomaterials in stem cell TE. Biomaterials play different roles at various stages in the application of stem cells to TE. ESCs may be derived from blastocysts obtained by either fertilization or somatic cell nuclear transfer under xeno-free conditions on biomaterial substrates. Derived stem cells can be expanded in culture on biomaterial-based bioreactors. Tissue scaffolds can be tailored according to the specific goals of the intended therapy. (**a**) Expanded ESCs can be differentiated terminally into mature cell types before seeding into scaffolds to construct tissues or whole organs. Alternatively, expanded stem cells may be partially differentiated into committed tissue progenitors (proto-tissues) that undergo terminal differentiation in seeded scaffolds (**b**) before or (**c**) after implantation into the body. In the latter case, the progenitor cells may continue to proliferate and migrate outward from the implanted graft to repair lesioned areas. (**d**) Injectable grafts for both soft and hard tissue regeneration may be produced by encapsulating progenitor or fully differentiated cells in biodegradable hydrogels. Somatic stem cells isolated from pediatric or adult patients can similarly be expanded in a biomaterials-based culture system before being applied as described for ES-derived cells.

mechanotransduction, and cell response to gradient effects of surface-bound ligands. Patterning techniques, such as chemical vapor deposition, physical vapor deposition, electrochemical deposition, soft lithography, photolithography, electron-beam lithography, electrospinning, layer-by-layer microfluidic patterning, three-dimensional (3D) printing, ion milling, and reactive ion etching, have been reviewed in detail by several authors.[39–45] These techniques, coupled with computer aided design tools and rapid prototyping technologies,[46] have opened up the possibility to tailor TE scaffolds with precisely controlled geometry, texture, porosity, and rigidity.

Micro- and nanoscale patterning techniques are particularly suitable for probing stem cell interaction with their microenvironment because they allow for levels of precision compatible with the delicate regulatory control of stem cell fates. Osteoblasts have proved to be a convenient model for studying cell–topography interaction as they are overtly responsive to gross topography of biomaterials.[47] Osteoblasts displayed anisotropic behavior when cultured on nano-patterned grooves fabricated on a polystyrene surface, using a combination of Langmuir—Blodgett lithography and nano-imprinting,[48] or on micropatterned grooves using hot embossing imprint lithography.[49] Cells were observed to align, elongate, and migrate parallel to the grooves. The depth of the grooves was found to influence the alignment of the cells, with 150-nm grooves inducing a statistically higher degree of alignment compared to 50-nm grooves.[48] Expression of an osteoblastic phenotype was most prominent on patterned surfaces deposited with calcium phosphate, highlighting the synergy between topography and surface chemistry. Fibrinogen coating on microgrooved surfaces fabricated from a biodegradable blend of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(L/D,L-lactic acid)-enhanced osteoblast alignment along the grooves.[50] Micropatterning of the ubiquitous RGDS adhesive peptide, as well as the osteoblast-specific KRSR peptide, produced ordered arrays of adhered osteoblasts.[51] Given the responsiveness of osteoblasts to topography, it is not surprising that the success of integration of endosseous implants is dependent on their surface topography.[52]

Substrate patterning holds particular utility in neural TE because repair of neurological injuries often requires directional guidance in terms of neuronal growth, migration, neurite projection, or synapse formation. Adult hippocampal progenitor cells (HPCs), cocultured with postnatal rat type-1 astrocytes, extended axially along the grooves of micropatterned polystyrene substrates chemically modified with laminin.[53] Directionally aligned poly(L-lactide) (PLLA) nanofibrous scaffolds fabricated by electrospinning induced neural stem cells (NSCs) to align themselves parallel to the fibers.[54] Microcontact printing of neuron-adhesive peptides using poly(dimethylsiloxane) soft-lithography provides a valuable tool for studying axonal guidance and neurite formation in developmental neurobiology.[55]

TE of skeletal muscle could also potentially benefit from micro- and nanopatterning technologies. Skeletal muscle is a highly organized structure consisting of long parallel bundles of

multinucleated myotubes that are formed by differentiation and fusion of myoblast satellite cells. Under normal culture conditions, on conventional tissue culture polystyrene, myoblasts grow in monolayers with fibroblastic morphology. However, in the presence of organized topographical cues, such as aligned nanofibers or micropatterned substrates, myoblasts fuse and assemble into elongated myotubes.[56]

Scaffold-based nanoparticle delivery system

Nanotechnology has provided new ways for functionalizing TE scaffolds with bioactive factors (drugs, proteins, or nucleic acids). Rather than doping the factors directly into the bulk material during scaffold fabrication, these factors can first be encapsulated in nanoparticles that are then dispersed into the bulk material. The factors are delivered to cells when the nanoparticles are released during scaffold degradation. Such a delivery system offers several advantages: (1) by prudent selection of nanoparticle shell material, the rate of factor release can be more tightly regulated because encapsulation in nanoparticles can limit diffusion. The rate of factor release would depend on the degradation rate of the scaffold, the size and density of the nanoparticles, as well as the nature of the nanoparticles; (2) the factors can be protected from external degradation before delivery to cells, which is important for labile agents such as growth factors, plasmid DNA, and siRNA; (3) encapsulation in nanoparticles can resolve solvent incompatibility issues between the cargo and the scaffold bulk material.

Harnessing developmentally important molecules for TE

As the demands of TE enter higher levels of sophistication, new biomolecules are recruited into the repertoire of factors used to alter stem cell fates. Increasingly, factors that play regulatory roles during early embryogenesis and morphogenesis are being studied for stem cell culture and differentiation. Notable examples are factors involved in the Notch, Wnt/β-catenin, bone morphogenetic protein, fibroblast growth factor, and activin/nodal signaling pathways. Many of these pathways are intrinsically active in cell signaling between stem cells and also between stem cells and their natural cellular niches. Members of the Wnt protein family promote self-renewal of HSCs[57] and MSCs[58] and induce neural differentiation of human ESCs.[59] Activin A alone is sufficient to maintain long-term self-renewal and pluripotency of human ESCs in feeder- and serum-free cultures.[60] As the roles of these molecules in stem cell biology become better understood, they can be incorporated into TE scaffolding design so as to harness their effects upon stem cell differentiation and tissue development.

THE DEVELOPMENT OF BIOMATERIALS FOR STEM CELL EXPANSION AND DIFFERENTIATION

ESCs

Expansion of ESCs. Until recently, the expansion of human ESCs was performed exclusively on feeder cell layers. However, recent reports of defined, feeder-free formulations for the derivation and maintenance[12,61–63] of human ESCs promise to change this scenario. Biomaterials-based expansion of human ESCs has now become a distinct possibility, as has large-scale culture of human ESCs in bioreactors. This will hopefully lead to the alleviation, if not elimination, of the two major obstacles to the widespread implementation of ES technologies in the clinic, which are concerns about exposure to animal components as well as consistency in both the quality and quantity of cell supply.

Biomaterials-based expansion has been achieved with murine ESCs. A number of studies described the use of hydrogel polymers as a support substrate for the maintenance of murine ESCs and embryoid body (EB) formation. Harrison *et al.*[64] evaluated the effects of modified aliphatic poly(α-hydroxy esters) such as poly(D,L-lactide), PLLA, poly(glycolide), and PLGA on murine ESC propagation in leukemia-inhibitory factor-conditioned media. Alkali treatment of the substrate surface, which cleaves the polyester backbone to present carboxyl and hydroxyl groups, increases hydrophilicity and significantly increases the proliferation of mature ESCs. Murine ESCs cultured on electrospun nanofibrillar polyamide matrix (Ultra-Web) showed greatly enhanced proliferation and self-renewal compared to culture on two-dimensional tissue culture surfaces, highlighting the effects of 3D topography.[65] Molecular analysis of the cultured cells revealed the activation of the small GTPase Rac, and the phosphoinositide 3-kinase pathway, which are both associated with stem cell self-renewal and upregulation of Nanog, a homeoprotein required for maintenance of pluripotency. It was postulated that the 3D microarchitecture of Ultraweb mimicked the ECM/basement membrane so as to activate stem cell proliferation and self-renewal.

Human ESCs have been expanded *in vitro* as cell aggregates known as EBs. Culture of human ESCs in a slow-turning lateral vessel bioreactor yielded up to a threefold increase in EB formation compared to static dish cultures.[66] Subsequently, the formation of human EBs within a 3D porous alginate scaffolds was reported.[67] There is, however, a tendency for cultured human EBs to undergo spontaneous differentiation, particularly vasculogenesis.[67,68] A good understanding of the factors affecting ESC self-renewal and maintenance and the underlying gene regulatory and signal transduction mechanisms will be instrumental in directing future designs of biomaterials for ES expansion.

Differentiation of ESCs. Achieving production of specific tissues from ESCs will require precise control of their differentiation. This would involve both physical and biochemical cues acting in concert. The versatility of such a concept was demonstrated by the induction of human with ESC differentiation into distinct embryonic tissue types within a biodegradable 3D polymer scaffold made from a 50:50 blend of PLGA and PLLA.[69] The type of tissue produced depended on the differentiation growth factor that was supplemented. Retinoic acid and transforming growth factor β induced ESC differentiation into 3D structures with characteristics of developing neural tissues and cartilage, respectively, whereas activin-A or insulin-like growth factor induced liver-like tissues. Although cell

seeding was carried out in the presence of Matrigel or onto scaffolds precoated with fibronectin, it was shown that neither Matrigel nor fibronectin alone could potentiate the effects observed with the PLGA/PLLA scaffolds. It was therefore hypothesized that the mechanical stiffness conferred by the scaffold acted synergistically with the Matrigel or fibronectin to enhance human ESC differentiation and 3D organization. Furthermore, it was shown that tissue constructs made with the scaffolds integrated well into host tissues when transplanted into severe combined immunodeficiency (SCID) mice. Supplementation of retinoic acid, nerve growth factor, or neurotropin 3 induced neural rosette-like structures throughout the scaffolds.[70] Nerve growth factor and neurotropin 3 induced the expression of nestin, a marker of neural precursor cells, as well as the formation of vascular structures. Pure PLLA scaffold was a suitable carrier for *in vivo* mineralization of human ESCs in SCID mice.[71]

HSCs

Despite almost three decades of extensive research into HSC expansion and self-renewal, a stable and reliable expansion system for human HSCs has yet to be achieved. This is probably due to the extreme sensitivity of true HSCs to their immediate micromilieu. Minute fluctuations in cytokine concentrations, oxygen tension, temperature, and cell–ECM interactions are sufficient to set in motion irreversible differentiation cascades that lead to depletion of HSCs in culture.

Stroma- and cytokine-free expansion of HSCs/hematopoietic progenitor cells (HPCs) using a porous biocompatible 3D scaffold was first described by Bagley *et al.*[72] Scaffolds fabricated from tantalum-coated porous biomaterials (TCPB matrix or Cellfoam) presented a microarchitecture reminiscent of bone marrow *trabeculae*. Culture of bone marrow HPC on TCPB in the absence of cytokine augmentation maintained progenitor phenotype and multipotency up to 6 weeks, a considerably longer period compared then with cultures grown on fibronectin-coated plastic dishes, bone marrow stroma cocultures, and other 3D devices. In particular, culture on TCPB matrix led to a 1.5-fold expansion of HPC numbers following 1 week in culture and a 6.7-fold increase in colony-forming ability following 6 weeks in culture. Supplementation with low concentration (ng/ml) of stem cell factor and Flt3-ligand, but not interleukin 3, markedly enhanced the effects of TCPB matrix in maintaining the multipotency of HPCs.[73] The use of low concentrations of cytokines in *ex vivo* expansion of HSCs/HPCs has clinical relevance as it has been shown that exposure of these cells to high, non-physiological levels of cytokines before transplantation diminishes their ability to engraft into bone marrow.[74] Improved expansion outcome was also observed for cord blood-derived $CD34^+$ cells cultured on TCPB scaffolds.[75] Culture on TCPB scaffold for 2 weeks yielded a threefold increase in the number of nucleated cells and a 2.6-fold increase in colony-forming units. Both $CD45^+$ and $CD34^+$ cells increased threefold in number. Additionally, expanded cells were capable of engrafting sublethally irradiated, non-obese diabetic/SCID mice.

More recently, the effects of surface-immobilized cell adhesive peptides and polypeptides on the proliferation and differentiation of purified cord blood $CD34^+$ cells were investigated.[76,77] Fibronectin covalently grafted onto 3D poly(ethyleneterephthalate) (PET) non-woven scaffolds markedly improved the maintenance of the $CD34^+$ phenotype, multipotency, and non-obese diabetic/SCID engraftment efficiency of cultured cord blood $CD34^+$ progenitor cells compared to fibronectin-grafted two-dimensional scaffolds or tissue culture plastic controls. It was hypothesized that immobilized fibronectin synergized with the 3D topography of the modified scaffolds to create a biomimetic microenvironment for $CD34^+$ proliferation and maintenance.

Purified cord blood $CD34^+$ HSCs cultured in reconstituted collagen I fibrils in the presence of Flt3-ligand, stem cell factor, and interleukin 3 for 7 days of culture showed increased number of colony-forming units, although the total expansion factor of $CD34^+$ cells was slightly lower compared to control suspension cultures, suggesting that collagen I scaffold performed better at preserving the multipotency of the $CD34^+$ cells.[78] Gene-expression profiling of the cultured cells revealed the upregulation of more than 50 genes in the presence of collagen I. Among these, genes for several growth factors, cytokines, and chemokines (*e.g.*, interleukin 8 and macrophage inhibitory protein 1α) were confirmed using quantitative polymerase chain reaction. In addition, higher expression of the negative cell-cycle regulator BTG2/TIS21 and an inhibitor of the mitogen-activated protein kinase pathway, DUSP2, underline the regulatory role of the ECM. Together, these data show that the expansion of $CD34^+$ cord blood cells in a culture system containing a 3D collagen I matrix induces a qualitative change in the gene-expression profile of cultivated HSCs.

MSCs

MSC expansion. MSCs have been extensively studied for TE owing to their potential to differentiate into osteogenic, chondrogenic, and adipogenic tissues, which are major targets for reparative medicine. In addition, recent evidence demonstrated their potential for neural trans-differentiation both *in vitro*[79–81] and *in vivo*,[82,83] and for differentiation into smooth muscle cells.[19,15] Adherence to tissue culture plastic has been used as a criterion for selection of MSCs from other cell types during their purification from bone marrow and umbilical cord blood. Although tissue culture plastic could support extensive proliferation of MSCs, continuing efforts are being made to develop an optimal substrate for MSC expansion. Clinical-scale expansion of MSCs is achievable using bioreactor culture.[84]

MSC differentiation. Although much has been learned about the roles of biological factors in inducing MSCs differentiation, the roles played by the physical environment in this process are only emerging. Surface chemistries of substrates alone appear sufficient to alter the differentiation of MSCs. Although unmodified and $-CH_3^-$- modified silane surfaces supported MSC maintenance, $-NH_2$- and $-$SH-modified surfaces promoted osteogenic differentiation, and $-$COOH- and $-$OH-modified surfaces promoted chondrogenic differentiation.[85] Mechanical signals such as local stresses (tensile, compressive, shear), geometry, topography, and cell–cell contact have a direct influence on the differentiation of MSCs.[86] McBeath *et al.*[87] demonstrated that the fate of MSCs differentiation can be altered

by manipulating cell shape using a micropatterned adhesive substrate. Enforced spherical cell morphology led to preferential adipogenic commitment, whereas a flattened morphology induced osteoblastic commitment. Cell shape was further shown to influence the differentiation fate via cytoskeletal mechanics, most probably transduced by RhoA signaling.

Biomaterials for osteogenic differentiation of MSCs. A wide range of biomaterials has been tested to harness the osteogenic potential of MSCs for bone TE. Constituents mimicking natural bone have often been incorporated into biomaterial design to stimulate ossification. Calcium and phosphate ions are important components during the mineralization phase of the ossification process. Materials composed of calcium phosphate such as hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP; $Ca_3(PO_4)_2$) are attractive candidates for bone substitutes. HA is a natural component of bone and has been clinically tested for orthopedic and periodontal applications.[88,89] HA coating has been shown to improve the outcome of prosthetic implants.[90] Porous HA ceramics supported bone formation by marrow MSCs *in vitro*[91] and *in vivo*.[92] A number of unique characteristics of HA contributes to its osteoconductive property. HA is known to strongly adsorb fibronectin and vitronectin, ligands for the integrin family of cell adhesion receptors that play key roles in mediating adhesion of MSCs and osteoblast precursors.[93] In addition, when used in blends with other polymers, HA particles exposed on the surface of scaffolds favor focal contact formation of osteoblasts.[94] A bone-like mineral film consisting mainly of calcium apatite, when introduced onto the surface of poly(lactide-co-glycolide) substrate, could achieve the same effect as when HA was incorporated into the bulk material.[95] It is also believed that HA degradation products create an alkaline microenvironment and provide electrolytes necessary for mineralization of ECM by osteoblasts during bone formation. This microenvironment then recruits surrounding cells to acquire an osteoblastic phenotype and to participate in the ossification process.[96]

Composites of HA with other polymers have been evaluated as osteoconductive substrates. Scaffolds fabricated from a composite consisting of HA/chitosan-gelatin promoted initial cell adhesion, supported 3.3-fold higher cellularity and could maintain higher progenicity of MSCs compared with chitosan-gelatin alone.[97] Biphasic calcium phosphate ceramics, composed of a mixture of HA and β-tricalcium phosphate, are considered to be more bioactive[98] and more efficient than HA alone for the repair of periodontal defects[99] and certain orthopedic applications.[100] A macroporous form of biphasic calcium phosphate can promote bone formation and has a degradation rate compatible with bone ingrowth kinetics.[99,101] Mineralized collagen sponges constructed of cross-linked collagen-1 fibers coated with non-crystal HA improved cell seeding and induced osteogenic differentiation of human MSCs.[102] When seeded with fibrinogen hydrogel into a polycaprolactone-HA composite scaffold, human MSCs differentiated efficiently into osteoblasts under osteogenic medium conditions.[103]

Other forms of calcium phosphate-containing material that have been assessed for osteoconductivity are octacalcium phosphate and α-tricalcium phosphate. Tissue constructs of various conformations including two-dimensional cell sheets and 3D blocks were achieved with rat MSCs seeded on octacalcium phosphate crystal microscaffolds.[104] Macroporous α-TCP was demonstrated to support osteogenesis from human MSCs.[102]

Bioactive glass fibers possess several characteristics attractive for bone TE. Firstly, they spontaneously initiate precipitation of HA on their surfaces, which renders them osteoconductive. Secondly, their fibrillar nature mimics the porosity of bone material and also the fibrillar organization of collagen fibrils that are orthogonally distributed within natural bone. Bioactive glass integrated well with surrounding bone tissue when used as defect fillers. Composites of bioactive glass with other biodegradable polymers, such as phospholipase, facilitated the formation of crystalline HA on the surface, which was conducive for MSC proliferation and differentiation into osteoblasts.[105]

Bone ECM components profoundly influence the activity of MSCs. Bone matrix consists primarily of fibronectin, collagen types I and IV, laminin, and the glycosaminoglycans heparan sulfate, chondroitin sulfate, and hyaluronan.[106] Recent evidence suggests that the different response of MSCs to different 3D polymeric scaffolds may be determined by the adsorptivity of the polymer for various ECM components present in the culture medium.[107] For example, polycaprolactone mediates MSC attachment primarily via adsorbed vitronectin, whereas PLGA does so via adsorbed type-I collagen. Incorporation of these components into bone TE scaffolds provides a way to control the behavior of MSCs more precisely. Scaffolds composed of hyaluronan, a major glycosaminoglycan found in bone ECM, have been demonstrated to modulate the expression of molecules associated with the inflammatory response as well as that of bone remodeling metalloproteinases and their inhibitors by human MSCs.[108] This finding has a significant impact on the construction of bone grafts for clinical use. Human MSCs cultured on a poly(3-hydroxybutyrate) fabric scaffold, immobilized with chondroitin sulfate, displayed phenotype and gene expression consistent with extensive osteogenesis.[109] Honeycomb collagen scaffolds fabricated from bovine dermal atelocollagen provided a superior surface for MSC proliferation and osteoblastic differentiation compared to a tissue culture plastic control.[110]

Biomaterials for chondrogenic differentiation of MSCs. Conventional TE of cartilage suffers from an inadequate supply of autologous chondrocytes.[111] Deriving chondrocytes from MSCs has become an attractive alternative. A wide spectrum of natural and synthetic biomaterials has been investigated for chondrogenic differentiation of MSCs. Several studies have described the use of natural polymers such as silk,[112,113] cellulose,[114] hyaluronan,[115] hyaluronic acid,[116] agarose,[117] and marine sponge fiber skeleton.[118] In addition, hybrid polymers, composed of synthetic and natural polymer blends, or of different natural polymers and their derivatives, have been tested. For example, (PLGA)-gelatin/chondroitin/hyaluronate scaffolds proved to be superior as a carrier of autologous MSCs in repairing full-thickness cartilage defects in rabbits compared with PLGA scaffolds.[119] Cho *et al.*[120] developed an injectable

thermosensitive hydrogel from a copolymer of water-soluble chitosan and Poly (*N*-isopropylacrylamide) (WSC-*g*-PNIPAAm) for chondrogenic differentiation of human MSCs. When injected into the submucosal layer of the bladder of rabbits, cells entrapped in the copolymer underwent further chondrogenesis and formed tissue resembling articular cartilage composed of a mixture of hyaline and fibrous cartilage and other tissue components.

Electrospun polycaprolactone nanofibrous scaffold has proven to be an interesting substrate for chondrogenic differentiation of MSCs.[121] Richardson *et al.*[122] demonstrated the potential of a biodegradable PLLA scaffold as a chondroactive substrate for MSCs-based TE of intervertebral discs. They had shown earlier that contact coculture of chondrocyte-like cells from the nucleus pulposus of the human intervertebral disc with MSCs could recruit MSCs to differentiate into nucleus pulposus cells.[123] Guo *et al.*[124] reported repair of large articular cartilage defects with implants of autologous MSCs seeded onto β-TCP scaffolds in an ovine model.

NSCs

In mammals, adult neurons lose their proliferative potential. The central nervous system, therefore, has limited regenerative capacity when inflicted with lesions resulting from trauma, stroke, or neuropathological conditions. Clinical trials using transplantation of fetal brain cells to treat neurodegenerative diseases such as Parkinson's disease has raised questions regarding the effectiveness of this strategy.[125] Repair of neurological injuries in the central nervous system is complicated by the presence of natural inhibitors of nerve regeneration, notably neurite outgrowth inhibitor and myelin-associated glycoprotein. Thus, a subset of therapeutic strategies for spinal cord injury is focused primarily on creating a permissive environment for regeneration by targeting these inhibitory proteins. The peripheral nervous system retains limited capacity for self-repair if the injuries are small. Larger injuries, however, require nerve grafts usually harvested from other parts of the body. TE using NSCs provides a viable and practical alternative for cell therapy of the central nervous system and peripheral nervous system.[126] However, there is a critical need for technologies to expand NSCs on a large scale before their use in the clinic can become commonplace. In the mammalian brain, NSCs originate from two specific regions, the subventricular zone and the dentate gyrus area of the hippocampus.[127] Evidence suggests that NSCs are widely distributed in the adult brain.[128] In addition, reprogramming of oligodendrocyte precursors[129] and astrocytes[130] could also give rise to multipotent NSCs. Recently, directed differentiation of human ESCs[131,132] and MSCs[133] into neuronal lineages has emerged as an alternative source of cells for neural TE and neuroscience research.

Pioneering work on large-scale culture of human NSCs was performed in suspension bioreactors.[134] However, nutrient and oxygen transfer constraints limit the size of NSC aggregates, known as neurospheres, which form in suspension cultures.[135] Propagation of NSCs in static cultures was achieved in the presence of basic fibroblast growth factor and/or epidermal growth factor, but passaging of the cells necessitated continuous mechanical dissociation of neurospheres.[136]

Many surgical procedures for treating brain lesions such as tumor and blood clot removal result in volume loss, creating cavities that should ideally be filled if recovery of neuronal integrity is desired. In addition, neurodegenerative diseases and hypoxic–ischemic injuries lead to necrotic and/or scar tissue formation that occludes normal cognitive and motor functions. Restoration of these functions would necessitate replacing the necrotic or scar tissue with healthy cells, a futuristic concept known as reconstructive brain surgery. Successful delivery and incorporation of NSCs for cell replacement therapy of the brain hinges upon the use of a suitable carrier material. Similarly, the repair of transected spinal cord or peripheral nerve injuries with engineered grafts would depend upon proper selection of an ideal nerve conduit to bridge the injury site. Of the different types of biomaterials, resorbable polymers appear to be the most suitable candidates to fulfill these roles.

Encouraging results from several studies raised optimism about the potential of neural TE in clinical applications. Using a biodegradable blend of 50:50 PLGA and a block copolymer of PLGA-polylysine, Teng *et al.*[137] fabricated a bilayered scaffold with outer and inner microarchitectures to mimic the white and gray matter of the spinal cord, respectively. The inner layer was seeded with NSCs and the construct was inserted into a laterally hemisected lesion of the rat spinal cord. Animals implanted with the scaffold-NSC constructs displayed improved recovery of hindlimb locomotor functions compared with empty scaffold and cells-only controls. The recovery was attributed to a reduction in tissue loss from secondary injury processes, diminished glial scarring and, to a certain extent, reestablishment of axonal connectivity across the lesion supported by the scaffold-NSC construct. An interesting finding was that an implanted poly(glycolide)-based scaffold-NSC construct could establish bidirectional feedback interactions with the brain in a reciprocal manner to mediate repair of an ischemia-induced lesion.[138] It is worth mentioning that a novel self-assembling peptide nanofiber scaffold implanted alone without cell cargo could support axonal regeneration through the site of an acute brain injury and could restore functional neuronal connectivity in the severed optic tract in animal models.[139]

A self-assembling peptide nanofibrous scaffold, functionalized with a high density of the neurite-promoting laminin epitope, IKVAV, could rapidly induce differentiation of seeded neural progenitor cells into neurons, but at the same time suppressed the development of astrocytes.[140] In another study, rat neural progenitor cells entrapped in a 3D collagen matrix rapidly expanded and spontaneously differentiated into excitable neurons and formed synapses.[141] Porous foam matrices prepared from poly(styrene/divinylbenzene), using a high internal phase emulsion templating and coated with poly(D-lysine) or laminin, promoted neurite outgrowth from human embryonal carcinoma stem cell-derived neurons.[142]

Endothelial progenitor cells

Neovasculogenesis, or the formation of blood vessels postnatally, is now thought to be attributed mainly to the activity of endothelial progenitor cells (EPCs). Ever since their isolation from peripheral blood mononuclear cells was first reported,[143]

EPCs have been identified from various sources including bone marrow,[144] umbilical cord blood,[145] vessel walls,[146] and fetal liver.[147,148] Resident EPC populations in bone marrow constitute a natural reservoir of cells that can be rapidly mobilized upon acute demand following major vascular insult.[149]

The potential application of EPCs for therapeutic vasculogenesis is widely recognized.[145,147,150] Direct infusion of endothelial stem/progenitor cells from various sources for neovascularization has been evaluated extensively in preclinical and clinical studies (reviewed in ref. 151). Early strategies for developing vascular prostheses focused on the delivery of angiogenic growth factors such as vascular endothelial growth factor, fibroblast growth factor-2, and DNA encoding these factors to induce ingrowth of microvessels from the host vasculature *in situ*. *In vitro* preendothelialization was hypothesized to create an antithrombogenic barrier for the devices, thereby preventing thrombus occlusion. Artificial grafts were seeded with differentiated endothelial cells (ECs)[152] or ECs in combination with other cell types such as smooth muscle cells.[153]

Owing to their undifferentiated state, EPCs retain the potential to remodel and integrate into the site at which they are transplanted. Kaushal *et al.*[154] implanted grafts constructed from decellularized iliac vessels preseeded with EPCs in a sheep model. EPC-seeded grafts remained patent for 130 days, whereas non-seeded grafts occluded within 15 days. Furthermore, explanted EPC grafts exhibited contractile activity and nitric-oxide-mediated vascular relaxation that were similar to native arteries. EPCs have also been employed in intraluminal endothelialization of small-diameter metallic stents.[155] In variations of the experiment, EPCs were used for surface endothelialization of whole metallic stents coated with a photoreactive gelatin layer[156] or endothelialization of a small-diameter compliant graft made of microporous segmented polyurethane and coated with photoreactive gelatin.[157] The EPC layer displayed antithrombogenic properties similar to that of mature ECs. EPC-endothelialized small-diameter compliant grafts, molded from type-I collagen and strengthened with segmented polyurethane film, remained patent for up to 3 months in a canine implantation model.[158] Living tissue patches comprising umbilical cord myofibroblasts and EPCs seeded on poly(glycolide)/P4HB mesh scaffolds have been fabricated for potential application in pediatric cardiovascular repair.[159] Fibrin coating of polymer scaffolds has been shown to promote the attachment of EPCs.[160] Mature ECs derived from cord blood EPCs have also been explored for endothelialization of vascular grafts.[161] Recent scaffold fabrication techniques, in particular aligned, coaxial electrospinning holds particular promise for the engineering of vascular grafts. In addition to providing a surface texture ideal for cell attachment and alignment, combinations of polymers can be selected to recapitulate the viscoelastic properties of natural vessels as well as to selectively promote the growth of EPCs and smooth muscles cells to generate a more biomimetic graft.

Embryonic germ cell-derived primordial germ cells

Human embryonic germ (EG) cells are a potential alternative to ESCs as a source of pluripotent stem cells for cell therapy and regenerative medicine. EG cells are derived by the adaptation of primordial germ cells to survive and self-renew in culture.[17,162] Despite the lower ethical acceptance of EGs owing to their controversial origin and the difficulty of maintaining well-defined EG lines *in vitro*, there is evidence to suggest that they follow a different epigenetic program than ESCs, and this may accentuate their importance as an alternative stem cell source in the future.

Thus far, only a limited number of studies have investigated the potential use of EGs for TE. Yim and Leong[163] reported evidence of neuronal differentiation of EG-derived EBs cultured on a cellulose acetate nanofibrous scaffold surface-decorated with nerve growth factor. Culture on a biodegradable scaffold, composed of poly(epsilon-caprolactone-co-ethyl ethylene phosphate) and unmodified cellulose acetate, led to enhanced proliferation of EBs.[164] Extended culture (10 months) on the two scaffolds produced cellular outcomes, with EBs cultured on poly(epsilon-caprolactone-co-ethyl ethylene phosphate) scaffold secreting copious amounts of ECM while showing down-regulation of the expression of neural markers. This study highlighted the fact that the architecture and biodegradability of the scaffolds play an important role in determining the fate of EG cells in cell culture.

Adipose-tissue-derived stem cells

Adipose tissue–derived stem cells (ADSCs) display much the same surface markers as bone marrow–derived MSCs with the exception of the presence of VLA-4 expression and the absence of the expression of its receptor, CD106. Consistent with this phenotypic similarity, the two cell types exhibit an almost indistinguishable differentiation repertoire. Under suitable culture conditions, ADSCs differentiate along classical mesenchymal lineages, namely adipogenesis, chondrogenesis, osteogenesis, and myogenesis.[165,166] Interest in ADSCs lies primarily in their potential as an alternative to bone marrow MSCs. Although they occur at frequencies comparable to those of their bone marrow counterparts, the extraction protocol for ADSCs is deemed less invasive than that for bone marrow harvests. Additionally, these cells may prove valuable in treating conditions associated with bone marrow failure.

The capacity of ADSC to differentiate along various lineages, when seeded into polymeric scaffolds, has been evaluated both *in vitro* and *in vivo*. In an attempt to find the minimal sequence of laminin sufficient to promote ADSC attachment on TE scaffolds, Santiago *et al.*[167] covalently immobilized RGD, YIGSR, and IKVAV peptide sequences on a polycaprolactone surface. ADSCs were found to adhere most avidly to a IKVAV-modified surface. ADSCs cultured on scaffolds formed by agglomeration of chitosan particles, showed evidence of osteogenic and chondrogenic differentiation.[168] Encapsulation in agarose hydrogels and gelatin scaffolds was permissive for chondrogenic differentiation of ADSCs.[169] ADSCs seeded in HA/TCP scaffolds or in collagen/HA–TCP composite matrix showed definitive osteogenesis when implanted into SCID mice.[170] In side-by-side comparison to bone marrow MSCs, ADSCs in atelocollagen honeycomb-shaped[171] or β-TCP scaffolds[172] showed no distinguishable differences in osteogenic differentiation either *in vitro* or when implanted into nude mice.

Adipose TE using ADSCs is currently being contemplated as a viable alternative strategy in plastic, corrective, and reconstructive surgery. Trials using mature autologous adipose tissue have only met with limited success because of tissue resorption[173] and ensuing calcification.[174] A confounding factor is that mature adipocytes are terminally differentiated and postmitotic.[175] ADSCs are speculated to circumvent some of these drawbacks. Animal studies have provided proof-of-concept for this approach. *In vivo* adipogenesis has been demonstrated with implanted ADSCs seeded in collagen,[176] hyaluronic acid,[177] phospholipase,[178] PLGA,[179] and phospholipase/poly(glycolide) composite[180] scaffolds. A consensus from these studies is that a polymeric scaffold is beneficial for adipose tissue formation from implanted ADSCs.

In addition to classical mesenchymal lineages, ADSCs have been shown to be capable of crossing developmental boundaries and to trans-differentiate into skeletal muscle,[165] cardiomyocytes,[181] neurons,[182] and ECs.[183] Although some of these cells have been tested in scaffold-free cell therapies, their use in biomaterials-based TE offers areas for exploration.

Other stem/progenitor cells with potential for TE applications

A number of more recently identified stem/progenitor cells provide interesting subjects for research and are probable candidates for organ-specific TE. The recent report of the isolation of human renal progenitor cells from adult kidney[184] is set to launch a new branch of TE. End-stage renal failure is a catastrophic disease usually leading to death. Conventional treatments such as kidney transplantation and renal dialysis have severe limitations and are often associated with considerable morbidity. Although the idea of a tissue-engineered kidney is not novel,[185] the use of renal stem cells could allow for the construction of a new organ *de novo* as well as for prospects for creating an autologous organ. Microporous scaffolds and the implementation of microfluidic technologies could be envisaged to take the lead in this arena.

TE of a functional pancreas has been an area of intense research for several decades. Multipotent adult pancreatic progenitor cells identified recently[186] will provide momentum to make this goal achievable in the near future. Other newly discovered stem/progenitor cells that have broadened the cellular arsenal for regenerative medicine include liver,[187] retinal,[188] skeletal muscle,[189] hair follicle,[190] and dentine pulp[191] stem cells.

CHALLENGES TO STEM CELL TE

In spite of justified optimism, several major challenges remain to be met. Foremost is the problem of mass transport during scale-up of engineered tissue constructs. Any TE modality that aspires toward clinical translation must consider vascularization. This hurdle is currently viewed as the limiting factor to the size of tissue constructs that can realistically be achieved. Supply of nutrients and oxygen to cells located deep in bulk tissue or complex organs must be resolved in order for them to be maintained in the body for any meaningful duration. Thrombogenic occlusion of microconduits or micropores introduced into biomaterial constructs is a common problem faced in tackling this limitation. The incorporation of antithrombogenic molecules into biomaterials is one of the strategies employed to overcome the problem. Alternatively, angiogenic factors can be incorporated into biomaterials to induce *de novo* vasculogenesis and/or angiogenesis from tissues surrounding the implants. Spontaneous vasculogenesis observed under certain conditions, such as in human ESC EBs growing in suspension cultures,[66,68] lends hope to surmounting this challenge.

Another challenge is the requirement for innervation. In fact, this requirement has been the major obstacle in the development of an implantable hybrid liver assist device. The liver is richly innervated via both the sympathetic and parasympathetic pathways from the hypothalamus and adrenal glands, which regulate functions such as blood flow through the hepatic sinusoids, solute exchange, and parenchymal function. Innervation is also required by other organs such as muscles, the pulmonary system, the kidney, and endocrine glands. Therefore, selection of biomaterials and the design of a tissue construct for repairing these organ systems would have to take into account the provision for innervation.

Organ systems are not composed of a homogenous cell type, but rather an assembly of different cell types either intermingled together or partitioned into discrete sublocations. Each of these cell types may have unique substratum requirements. Engineering of complex organs would, therefore, need to cater to each component cell type. A challenge remains to find the correct balance between the biological and physical properties of the scaffold material to suit each cell type. In this respect, TE using stem cells has clear advantages, because the plasticity of the cells can allow for *de novo* formation of tissues depending on scaffold composition. *In situ* remodeling at the interface between different cell types, akin to events that occur between germ layers during embryogenesis, can give rise to new tissues. This may theoretically relax the stringency for precise substratum requirements.

The creation of relevant disease models to evaluate the efficacy of the engineered tissue constructs is as important as overcoming the engineering hurdles. Often, small rodent models with mechanically or pharmacologically induced lesions do not accurately recapitulate human disease conditions, causing disparate outcomes between preclinical and clinical trials. Non-human primate models may in theory, provide the most relevant animal models, but these are not readily available for practical and ethical reasons. The creation of non-human primate models for various human diseases by gene targeting and nuclear transfer has been proposed.[192,193] However, cloning of monkeys remains unsuccessful to date. Success in this arena may positively impact stem cell TE.

SUMMARY AND FUTURE PERSPECTIVES

The field of TE has entered an exciting new chapter, where experimental technologies are being aggressively explored for clinical translation, signifying a veritable "coming of age" of the field. The convergence of two important disciplines, that of biomaterials engineering and stem cell research, promises to revolutionize regenerative medicine. With this merger, several concepts that would have been deemed far-fetched a few years ago are now being actively pursued. Among these concepts are

brain reconstructive surgery, tailor-made autologous body replacement parts, and cybernetic prosthesis. The future of stem cell TE is undoubtedly technology driven. New applications and improvement upon current designs will depend heavily on innovations in biomaterials engineering. Concomitant with this, progress in stem cell biology will be imperative in dictating advances in stem cell TE. A better understanding of the molecular mechanisms by which substrate interactions impact stem cell self-renewal and differentiation is of paramount importance for targeted design of biomaterials. Discoveries in the fields of developmental biology and functional genomics should also be parlayed for broadening the repertoire of biological molecules that can be incorporated into biomaterials for fine-tuning stem cell activities. With the merger between the two powerful disciplines—biomaterials engineering and stem cell biology—a new drawing board now lies before us to develop therapies that could hopefully help the world population age more gracefully.

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