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Cell Delivery Mechanisms for Tissue Repair

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Many cell populations, derived from both adult tissues and embryonic stem cells, show promise for the treatment of a variety of diseases. Although the major effort in stem cell therapies in the past has been identifying potentially therapeutic cells, it is now clear that developing systems to deliver these cells and promote their efficient engraftment will provide an equally challenging task. More sophisticated pretransplantation manipulations and material carriers may dramatically improve the survival, engraftment, and fate control of transplanted stem cells and their ultimate clinical utility.

Introduction

Cell therapies promise to provide cures to a multitude of diseases and disorders, frequently via tissue repair, which is the focus of this review. Stem cell populations, in particular, are appealing therapeutic agents due to their typically rapid and extensive proliferation and their potential to customize the cells to the patient. A stem cell has two characteristics in its basic form: the ability to self-renew and the ability to maintain a differentiated and functional cell type for the life of an organism. The ability of a stem cell to form multiple functional cell types is an additional, but not necessary, characteristic of many of the currently studied embryonic and adult stem cells (Wagers and Weissman, 2004). There has been tremendous progress over the past decade in the identification of a variety of cell populations isolated from adult tissues that are capable of contributing to the rebuilding of multiple tissues and organs (e.g., cardiac and neural) (Filipczyk et al., 2007; Lee et al., 2007), the development of techniques to direct the differentiation of embryonic stem cells to desirable cell populations (e.g., pancreatic beta cells; Porat and Dor, 2007), and even recently the ability to reprogram human adult cells into ES cell-like pluripotent cells (Takahashi et al., 2007; Yu et al., 2007). The major challenge facing this field is to transition rapidly from the identification of candidate cell populations to the development of effective delivery approaches.

Potential cell therapies are most typically introduced to the body via an injection of cells, suspended in an appropriate medium, either into the systemic circulation or directly into the tissue of interest (Figure 1). A wide variety of cell populations has been transplanted in this manner, including various myoblast populations, neural stem cells, hepatocytes, various bone marrow-derived cells, and ES cells differentiated down various pathways (Hofmann et al., 2005; Skuk et al., 2007; Evans et al., 2007; Thuret et al., 2006; Porat and Dor, 2007). These studies have confirmed the ability of transplanted cells to participate in the repair of damaged or diseased tissue and demonstrated proof of principle for this new therapeutic approach. The results of many studies, however, are also making clear that at least some of the transplanted cell populations regulate regeneration via secretion of trophic factors, instead or in addition to directly participating in building of the tissue (Mangi et al., 2003; Capone et al., 2007). This latter mechanism suggests that appropriately distributing transplanted cells in the host tissue may be critical to their utility. In either case, however, studies have uniformly demonstrated large-scale death of the transplanted cells, extremely poor engraftment (typically <3% cells engraft), and loss of control over the fate of the transplanted cells after their introduction into the body. The spread of the cells from the injection site is also typically quite restricted, seriously limiting the scale of tissue repair in many situations. Together, these issues are likely responsible for the limited clinical success of this approach to date and the repeated finding that success is greatest in small tissue volumes (e.g., rodent models).

It may be possible to dramatically improve the impact of transplanted stem cell populations by borrowing concepts from the tissue engineering field originally developed for the transplantation of differentiated cells (Langer and Vacanti, 1993; Griffith and Naughton, 2002). In particular, the tissue engineering field routinely makes use of material carriers, functioning as synthetic analogs of the extracellular matrix, to provide a substrate for transplanted cell adhesion, to control the localization of the cells in vivo, and to serve as a template for the formation of new tissue masses from the combination of transplanted cells and interfacing host cells (Figure 1) (Lutolf and Hubbell, 2005; Sands and Mooney, 2007). These functions may prevent anoikis in the transplanted cells and also regulate their gene expression. Further, the material's ability to orchestrate the host response to the transplanted cells (e.g., vascularization) may dramatically improve their survival and function in the host environment. Clinical success to date in tissue engineering approaches to cell transplantation is mainly in the skin arena (Auger et al., 2004), but proof of principle for the engineering of many tissue/organ types has been demonstrated in both small and large animal models (Nerem, 2007).

This review will provide an overview of two complementary approaches, both the foci of active investigation, to enhance the success of tissue repair strategies with cell transplantation: manipulation of the biology of cells in advance of their transplantation and the development of material carriers to manipulate transplanted cells in vivo and orchestrate the host response. Select examples of these two approaches will be described to

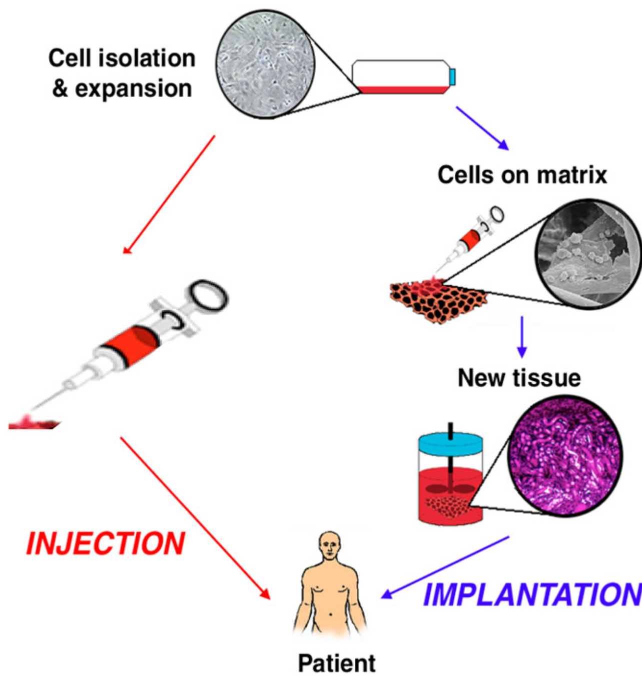

Figure 1. Current Strategies for Cell Transplantation
Cells are multiplied in culture prior to transplantation or used with minimal manipulation after harvesting and then delivered by one of two strategies. Most commonly (left branch), cells suspended in medium are directly injected into the defective tissue or into the systemic circulation. The cells are expected to home to the site of interest or stay at the site of injection, depending on the particular application, but in either case, little guidance is provided to the cells by the transplant system. Alternatively (right branch), cells may be allowed to adhere to a material carrier, typically a biodegradable polymer, in vitro, allowed to proliferate and differentiate within a bioreactor, and subsequently be implanted or injected on the material to localize the cells to a specific anatomic site and signal to the adherent cells. The material serves as a template to guide tissue formation and typically is designed to degrade in concert with deposition of new extracellular matrix and cell proliferation (Taken from Lee and Mooney, 2001; used with permission of the American Chemical Society [ACS]).

illustrate the various directions taken to date. Future directions in this field, and current challenges to progress, will also be examined. This review will not specifically examine cell transplantation approaches to treat blood disorders or cancer, as although these share certain objectives and challenges with tissue repair, there also exist considerable divergences between these areas of application.

Manipulating Cells prior to Transplantation

Two general approaches have been taken for cell therapies. The first has been rapid cell isolation and reapplication of the cells with minimal manipulations, preferably without either the patient or cells leaving the operating room (e.g., Moseley et al., 2006). The second, more common approach has been cell isolation, transport to the lab, and extensive in vitro manipulation before reimplantation. Advantages of the former approach include minimal cost and minimal oversight by regulatory agencies such as the FDA, whereas the latter approach is costly and requires manipulation of the cells under current Good Manufacturing Protocols (cGMP) in clean rooms with extensive documentation and close regulatory supervision (e.g., De Bie, 2007). The significant advantage of the latter is the ability to manipulate the potentially therapeutic cells in vitro to "improve" their ability to treat the targeted disease once reimplanted. The following is a brief summary of issues related to both the source of the therapeutic cells and their in vitro manipulation prior to transplantation, using examples primarily from the area of skeletal and cardiac muscle repair.

Influence of Cell Source

Skeletal muscle has a well-defined muscle-specific progenitor called the satellite cell, which is activated after muscle fiber damage and which can rapidly repair the damage in situ in normal healthy adults. These cells can be readily isolated from humans by simple muscle needle biopsy protocols, can be expanded in vitro to billions of cells, and can be reinjected in an attempt to treat diseases, such as Duchennes muscular dystrophy, in which the native cell population is depleted (Kapsa et al., 2003) or heart ischemia in which endogenous progenitor cells are missing (Menasche, 2007). In general, the results from these clinical trials to date have been disappointing, primarily due to rapid cell death after reimplantation. Recent in vitro studies have identified several other skeletal muscle stem/progenitor cells that may have a greater potential for skeletal or cardiac muscle repair than the satellite cell (reviewed in Peault et al., 2007). Studies are underway for their isolation, in vitro expansion, and reimplantation to determine their repair potential (Sampaolesi et al., 2006; Zheng et al., 2007). The in vivo repair capacity of these cells can be enhanced by in vitro chemical manipulation, such as exposure to nitric oxide before reimplantation (Sciorati et al., 2006). Incubation of human skeletal myoblasts in tissue culture with several growth factors, including IGF1, also improved their survival when implanted in vivo (Brimah et al., 2004). Sex-related differences in the stem cells used for skeletal muscle repair have been observed (Deasy et al., 2007) and may be an important consideration for other tissue types. Further, the methods used to isolate the stem cells can have a profound effect on their subsequent ability to regenerate into differentiated cells when transplanted in vivo. For example, adult mouse skeletal muscle satellite cells isolated without the use of proteolytic enzymes were several orders of magnitude more efficient in generating new muscle tissue in vivo than satellite cells isolated by typical enzymatic digestion techniques (Collins et al., 2005). In some cases, as few as seven satellite cells in their native extracellular matrix and implanted in a muscle bed can form hundreds of new postmitotic muscle fibers in vivo. Enzymatic damage to cell surface ligands or receptors may be the cause of this reduced repair capacity. Hematopoietic stem cells (HSCs) are also routinely isolated by nonenzymatic techniques.

Methods to differentiate human stem cells into cardiomyocytes have been widely studied to date because damaged adult heart tissue has an extremely poor regenerative capacity due to few local cardiomyocyte precursor cells (Beltrami et al., 2003). Functional cardiomyocytes arising from differentiating human embryonic stem (hES) cells were initially isolated and expanded in vitro, but at relatively low efficiency (Kehat et al., 2001). These studies required the use of an irradiated mouse cell feeder layer, which could prove problematic for clinical use because cultured cells can incorporate foreign xenogeneic proteins and generate a host immune response when implanted. Two other potential drawbacks of early methods to yield human cardiomyocytes

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are the relatively long time in vitro required to give rise to beating cells (up to 3 weeks) and the low percentage of embryoid bodies containing contractile cells (~10%). Recently, feeder layer-free tissue culture conditions to generate contractile cardiomyocytes have been described and used in subsequent in vivo transplant studies (Laflamme et al., 2005). These studies used Matrigel as a cell attachment substrate as well as tissue culture medium conditioned by mouse embryonic fibroblasts, both of which might also be problematic for clinical applications due to their potential to induce an immune response. Attempts to use FDA-approved matrix molecules, such as collagen (e.g., Zyderm), as a cell carrier can also be problematic because they can be highly antiangiogenic in vivo (Thorrez et al., 2006).

Chemical and Physical Manipulation

Many in vitro manipulations have been examined in an effort to improve stem cell differentiation and engraftment. DMSO was tested as a differentiation stimulant to enhance cardiomyocyte yield but was found to be ineffective, whereas the addition of retinoic acid to ES cell medium depleted undifferentiated cells and improved cardiomyocyte yield (Zandstra et al., 2003). Serial application in vitro of a mesendodermal inducing factor (activin A) followed by a cardiac tissue differentiation factor (BMP4) to hES cells led to a 50-fold increase in cardiomyocyte differentiation, compared to serum, and enhanced engraftment in vivo. Injection of these differentiated stem cells with Matrigel also had beneficial effects on cell survival in vivo (Laflamme et al., 2007), – possibly by preventing anoikis (Frisch and Screaton, 2001) (see next section). In vitro heat shock of the hES cardiomyocytes (30 min at 43°C 24 hr before implantation) was also found to have a profound effect on survival and grafting of the cells in vivo into undamaged hearts, with a 3-fold increase in the graft size due to a continued high rate of proliferation of the cells in vivo (Laflamme et al., 2005). Although the mechanism(s) by which this simple protocol enhances transplant efficiency is not fully understood, it is thought that these activated molecular chaperones (Hsp60, Hsp70, and Hsp90) limited cell death. Of course, continued proliferation of the transplanted cells in vivo must be somehow controlled to prevent the formation of teratomas.

Stem cells may require different manipulations ex vivo depending on the site into which they are to be subsequently transplanted. For example, cardiomyocytes derived from hES cells responded differently when implanted into an undamaged heart compared to an area of infarction (Laflamme et al., 2007). Epithelium-lined cysts occurred in the latter site, but not in the former. The cause of this difference is unknown but may reflect level of ischemia at the implant site, the state of the host repair process, and/or the amount of scar tissue present. Mechanical strain on the transplanted cells would also be different in the two sites and could play a role in determining the fate of implanted stem cells (Estes et al., 2004).

Stage of Cell Differentiation

The in vitro differentiation state of stem cells that leads to the best survival in vivo is unknown in most instances. This issue is also complicated by the possibility that differentiation of stem cells in vitro may help reduce the formation of teratomas in vivo (Laflamme et al., 2007) but limit cell survival. Transplantation of undifferentiated proliferating primary myoblasts led to rapid cell death within several days (Skuk et al., 2003), whereas implantation of differentiated postmitotic myofibers results in a slower process of cell death over weeks rather than days (Thorrez et al., 2008). Cardiac "tissue-restricted" progenitor cells (isl1+) have been isolated from the postnatal heart of rats, mice, and humans, and the differentiation of these adult-derived stem cells into the cardiomyocyte phenotype requires both membrane-bound factors (coculturing with fixed rodent myocytes) and soluble factors (conditioned medium from viable rodent myocytes [Laugwitz et al., 2005]). These early-stage progenitor cells may be more efficient than differentiated cardiomyocytes for the growth of new heart tissue after transplantation because they can give rise not only to cardiac cells but also to other cell types (endothelial and smooth muscle cells) required for cardiogenesis.

In Vitro Cell Growth in Three Dimensions

Bioreactors for stem cell growth in vitro may better reflect the in vivo environment and be useful for the growth, differentiation, and "conditioning" of stem cells for subsequent in vivo transplantation. Nutrient and oxygen availability can be better regulated in these systems than in monolayer cultures. Bioreactor spinner flask perfusion conditions have been established to increase the yield of cardiomyocytes per input ES cells (19.8-fold over static conditions) by encapsulating the differentiating ES cells in low melting temperature agarose to maintain an efficient cell density for optimal cell growth; it was also shown that transient exposure of the ES cells to hypoxic conditions improved cardiomyocyte yield 1.47-fold (Bauwens et al., 2005). Hypoxia can also augment the expansion of bone morrow-derived stem cells by regulating telomerase activity in the cells (D'Ippolito et al., 2004). Bioreactors have also been used to tissue engineer cardiomyocytes into contractile "tissues" (Radisic et al., 2004), and engineered cardiac tissue from neonatal heart cells can be implanted successfully as a tissue patch to improve cardiac function in rodent hearts (Zimmermann et al., 2006). Recently, hES cells were also engineered into contractile cardiac tissues (Rogge et al., 2007) and thus might represent a vast, reliable cell source for cardiac patch repair. Scaling up the engineered tissue approach for clinical applications will require significant improvements in the capacity to generate large three-dimensional tissue in vitro. Alternatively, it may be possible to promote the growth of small engineered tissues subsequent to their introduction in vivo. Bioreactor studies may prove vital for this approach.

Genetic Manipulation

Genetic manipulation in vitro to improve/enhance differentiation function and transplantation efficiency of stem cells is another active area of research. Transfection of ES cells with a neomycin-resistant transgene linked to a myosin heavy chain promoter was able to effectively enrich the ES cell population into cardiomyocytes at greater than 70% efficiency (Zandstra et al., 2003). Skeletal myoblasts, which have been transduced to express the gap junction protein connexin 43, formed an enhanced network of interconnected contractile cells in the heart (Roell et al., 2007), potentially providing a good source of autologous adult stem cells for heart repair. Genetically modifying stem cells to secrete soluble factors may also enhance implantation efficiency. For example, tissue engineering of human skeletal muscle with myoblasts genetically engineered to secrete vascular endothelial factor stimulated vascularization of the implant site (Thorrez et al., 2006).

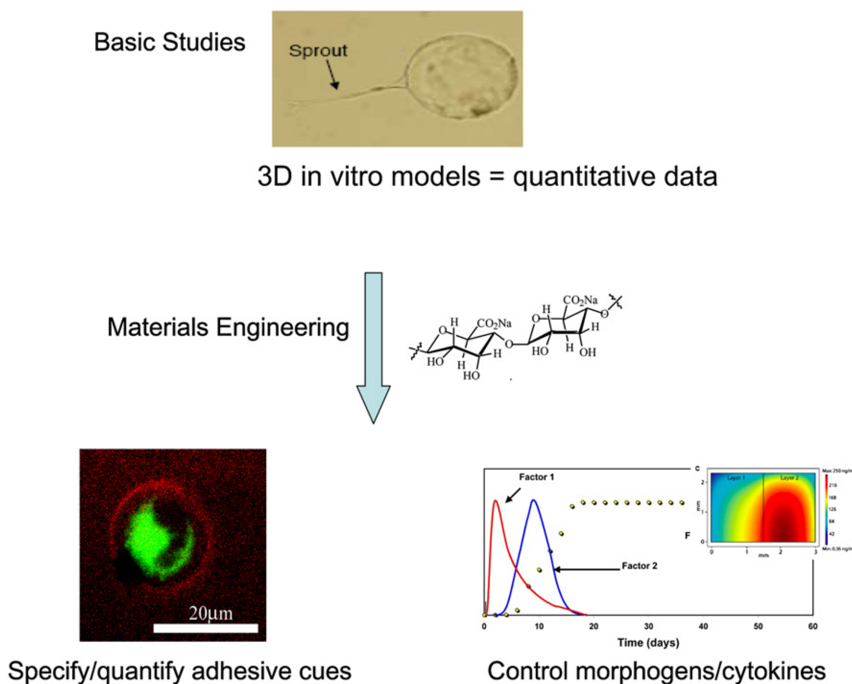

Figure 2. Design Strategy for Materials Utilized as Cell Delivery Vehicles
Design criteria are often derived by combining the known biology of the cell of interest (e.g., relevant adhesion molecules and morphogens) with in vitro studies using appropriate 3D models of tissue formation to develop quantitative relations between the cues and cell response. In this example, the sprouting of endothelial cells into a surrounding extracellular matrix-mimic (fibrin gel) from microcarrier beads (top image) may be used as a model of sprouting angiogenesis, and the relation between the level and direction of sprouting and morphogen concentration and spatial gradients may be quantified. These designs are then translated to materials systems, using the tools of chemistry and materials science and engineering. These materials provide the desired cues with appropriate spatiotemporal resolution to cells transplanted on the materials. Polymers, such as the polysaccharide structure shown, are most often used to create vehicles for cell transplantation. The two general types of cues designed into the material carriers include adhesive cues (e.g., specific adhesion ligands and number of bonds cells form with ligands) and control over time and space of the availability of single or multiple morphogens and cytokines to transplanted and host cells. The image of the cell (green structure on bottom, left) demonstrates FRET (red fluorescence) resulting from a cell specifically adhering to adhesion peptides coupled to the gel-forming polymer. The graphs (bottom, right) demonstrate waves of morphogen release over time from the polymers and resultant spatial gradients (inset) in surrounding tissue (images taken from Huebsch and Mooney, 2007 and Chen et al., 2007b).

Material Systems to Manipulate Transplanted Cells and Host Response

Materials for cell transplantation have been traditionally designed from a classic materials science perspective, in terms of providing appropriate physical (e.g., strength) and chemical (e.g., degradation rate) properties (Langer and Vacanti, 1993). More recently, however, the design space for these materials has expanded dramatically to include biological criteria that include a consideration of signals cells receive via adhesion to the carrier and of soluble signals (e.g., morphogens) available in the cellular microenvironment (Figure 2) (Lutolf and Hubbell, 2005; Kong and Mooney, 2007). These various cues are intended to orchestrate the host cell response, in addition to regulating the transplanted cell population(s).

Quantitative biological design criteria will be critical for materials used for stem cell transplantation, as both the types and quantities (e.g., concentrations, gradients in time and space) of the cues provided by the material will likely play a crucial role in signaling cell fate. An attractive approach to generating quantitative design criteria is to first develop a quantitative understanding of the relation between the cue and the target cell population response. This information can often be obtained by using three-dimensional (3D) cell culture models (Griffith and Swartz, 2006) such as those described in the last section, as they enable rapid screening of a typically large variable space. The tools of materials science and engineering are then utilized to design a material, most often a polymer, that provides this cue to transplanted and host cells in the desired quantity over time and space. For example, a 3D sprouting assay has been used to delineate the role of concentrations and gradients of various morphogens on angiogenesis in vitro and design material systems to promote angiogenesis in vivo (Chen et al., 2007a).

Materials to Regulate Adhesion

Adhesion to a substrate is required to prevent anoikis and allow transplanted cell survival over even short time frames, and manipulating the presentation of adhesive cues further allows one to regulate major cellular processes (e.g., migration, proliferation, and differentiation) over longer time scales. A variety of naturally derived ECM molecules (e.g., type I collagen and fibrin) are currently being used as cell vehicles due to their intrinsic cell binding capabilities, as are synthetic polymers to which adhesion is regulated by adsorbed proteins (Silva and Mooney, 2004). Synthetic peptides mediating adhesion can also be presented to cells as self-assembling hydrogels, coupled as side chains to polymer backbones, or as components of synthetic proteins that provide desirable combinations of cell interactive domains and overall physical/chemical properties (Gelain et al., 2006; Lutolf and Hubbell, 2005; Silva et al., 2004). The utility of approaches presenting specific adhesion peptides depends on the absence of nonspecific adhesion to enable a high ‘‘signal’’ to ‘‘noise’’ ratio in the system. Strikingly, even when an identical adhesive peptide is utilized at a constant overall density, its clustering and spacing on a nanometer scale can dramatically alter the cell response and enhance or diminish processes such as proliferation or differentiation (Maheshwari et al., 2000; Comisar et al., 2007). In addition to the chemistry of the cell-material interface, the mechanics of this interface are being recognized to play a key role in the cell response (Discher et al., 2005). In particular, the differentiation of adult tissue-derived progenitor populations in vitro is controlled in part by the stiffness of the adhesion substrate (Chen, 2004; Engler et al., 2006; Gwak et al., 2007). However, it is not clear if these findings result from an inductive or permissive effect (e.g., selection of a subset of the population); in addition, distinct roles may be played by adhesive cues in

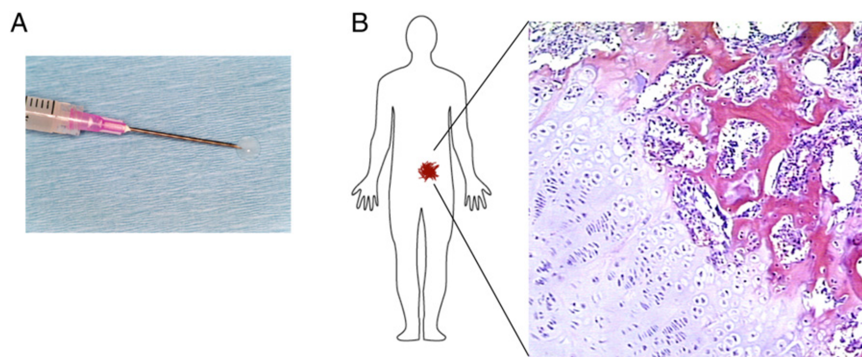

Figure 3. Cell Transplantation Vehicles
A variety of materials for cell delivery are under development, and these materials must typically function to deliver the cells in a minimally invasive manner, allow for delivery of single or mixed cell populations, and cue transplanted cells to organize into a new tissue capable of responding to host signals.
(A) Injectable hydrogels are often used to transplant cells with minimally invasive techniques and can localize the cells to a desired anatomic site and provide instruction to the cells.
(B) The challenges of engineering growth plate-like structures are representative of the complex function required of these materials. In this example, injectable gels presenting appropriate cell adhesion peptides were used to cotransplant mixed cell populations and cue the cells to reorganize into structures that both histologically resembled growth plates and were also functionally similar to growth plates in their ability to increase in size over time (Alsberg et al., 2002). Cartilage, mineralized tissue, and bone marrow were formed 26 weeks after transplantation into rodents in this study, and the organization and morphology of the cells were virtually identical to those found in normal growth plates (image taken from Alsberg et al., 2002).

more primitive versus more committed cell populations (Hsiong et al., 2007). The development of more sophisticated tools to probe and quantify molecular level events at the cell-material interface (Kong et al., 2006; Sniadecki et al., 2007) will significantly improve our ability to understand and ultimately regulate these events via materials designed to trigger predefined signal transduction pathways.

Controlling Regeneration with Adhesive Cues

Controlling the adhesive cues presented to transplanted cells has dramatically impacted their survival and their ability to both form new tissue structures and participate in regeneration of damaged tissues (Alsberg et al., 2002; Davis et al., 2005; Hill et al., 2006). The density of Notch ligand binding sites, for example, can impact the engraftment of human cord blood progenitor cells (Delaney et al., 2005). Hydrogels can be introduced into the body by minimally invasive techniques (Figure 3A) and have been used to present specific adhesive peptides in desirable patterns to transplanted cells, promoting the formation of complex, tissue-specific structures (Figure 3B) (Alsberg et al., 2002). However, the ability of progenitor populations, as contrasted to committed cell populations, to participate in regeneration has been found to be dependent in some studies on the provision of a more complex microenvironment (e.g., adhesive ligands in concert with appropriate growth factors; see next section) (Simmons et al., 2004; Huang et al., 2005a). There has not yet been a definitive demonstration that the mechanical properties of the adhesion substrate regulate tissue formation by transplanted cells in vivo. This likely relates to the difficulty in separating mechanical and degradative properties of materials, and the material degradation rate clearly plays an important role in the ability of transplanted cells to deposit new matrix and remodel the tissue (Alsberg et al., 2003; Benoit et al., 2006). The development and application of new materials in which the degradative and mechanical properties can be decoupled will undoubtedly aid these studies and potentially provide another control point for cell fate following transplantation.

Regulating Presentation of Soluble Cues

The local presentation of soluble cues (e.g., morphogens) from material carriers may be utilized to regulate the fate of transplanted and host cell populations as they together repair tissues. Extensive research has been performed over the last 30 years to develop suitable microenvironments for HSC expansion and directed differentiation (reviewed in Panoskaltsis et al., 2005). Attempts to mimic the HSC niche in vitro have utilized exogenous growth factors in combination with 2D and 3D scaffolds and bioreactor perfusion systems. Other stem cell repair systems may also need to simulate growth-differentiating factors in a pharmokinetic manner that mimics the natural stem cell niche by providing exogenous growth factors to the cells on 2D and 3D scaffolds. In the simplest approach, cytokines, morphogens, and growth factors that regulate survival, proliferation, and/or differentiation may be physically encapsulated, covalently coupled, or associated via secondary bonding with the cell transplantation vehicle. The factors may be subsequently presented in vivo with high local concentrations, defined gradients, and low systemic exposure, via release controlled by either material degradation or factor diffusion from the material (Tessmar and Göpferich, 2007). Specific examples of this approach include improvements in the efficacy of transplanted cardiomyoctes in mediating myocardial infarction via presentation of IGF from the carrier (Davis et al., 2005), presenting TGF-β and BMP-4 to enhance the ability of transplanted mesenchymal stem cells to form bone (Simmons et al., 2004; Park et al., 2007), and EGF delivery to increase keratinocyte-driven epidermis regeneration (Gwak et al., 2007). Release of antiapoptotic cytokines could potentially also be used to enhance the short-term survival of transplanted cells, as demonstrated for resident host cells (Hérodin et al., 2003). Appropriate compartmentalization of multiple agents can provide their simultaneous or sequential delivery (Chen et al., 2007b), which may allow multiple distinct stages of cell differentiation and tissue formation to be orchestrated by the material.

Local Gene Therapy for Regeneration

In place of direct protein delivery, the cell delivery vehicle may be designed to provide oligonucleotides (e.g., plasmid DNA [pDNA] and siRNA) to mediate local morphogen production. Sustained release of pDNA from material carriers and surface presentation of pDNA can dramatically increase transfection levels in vivo (Bonadio et al., 1999; Shea et al., 1999; Houchin-Ray et al., 2007). More recently, the condensation of pDNA into small particles, using polycations, prior to encapsulation into polymers

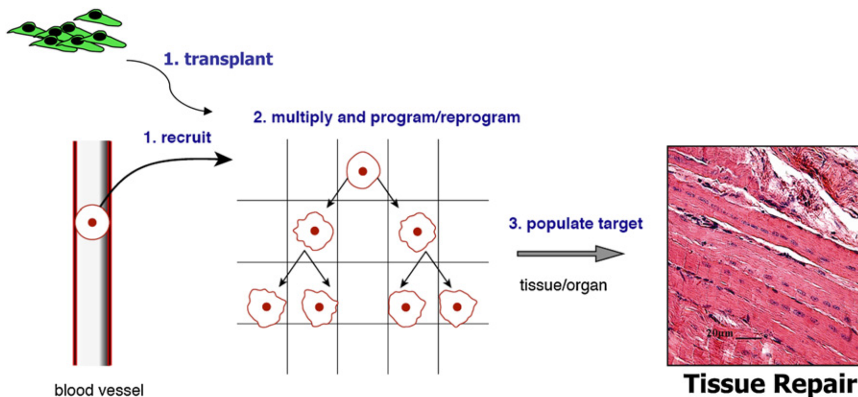

Figure 4. Programming Transplanted or Resident Cells
Appropriately designed material systems may be used to either transplant cells or recruit resident cell populations, using appropriate signaling (1). In either case, once cells are in contact with the material system, it may be designed to program the cells in situ by the presentation of appropriate adhesive cues, morphogens, and/or genetically modifying the cells (2). Programming would often include activating the cells to a highly proliferative state in order to generate large numbers of daughter cells, controlling the state of differentiation, and ultimately promoting a continuous dispersion of appropriately differentiated daughter cells to the tissue immediately adjacent or at a distance to the material. This concept was recently demonstrated with muscle-derived satellite cell populations, as a material carrier providing appropriate adhesion ligands and growth factors was demonstrated to activate the cells into a proliferative state, prevent their terminal differentiation while within the material, and provide an efficient repopulation of damaged muscle tissue by the transplanted cells, with a significant increase in muscle regeneration (3) (image taken from Hill et al., 2006).

has been demonstrated to significantly increase tranfection levels, via increased uptake efficiency and sustained presentation to cells (Huang et al., 2005b). Importantly, microenvironmental cues, including the density and organization of adhesion ligands and matrix mechanical properties, strongly regulate the ability of cells to uptake and express pDNA (Kong et al., 2005, 2007), again emphasizing the importance of designing the overall microenvironment of transplanted cells, and not simply one element in isolation of the other variables. Although not addressed to date, the controlled delivery of siRNA to transplanted cells holds significant promise to regulate tissue repair.

Host Integration

A significant challenge to the successful implementation of cell therapies is the integration of the new cells with the vascular, lymphatic, and nervous systems of the host. Toward this end, factors released from delivery vehicles may diffuse into the surrounding tissue to create gradients (Silva and Mooney, 2007) in order to activate host cells and guide their ingrowth into the cell transplantation site. The need for vascularization is motivated by the observation that transplanted cells will rapidly die if they are placed a significant distance from the host vasculature (Skuk et al., 2007), and one approach to address this issue is to drive angiogenesis at the site of cell transplantation. Material systems that provide local and sustained presentation of angiogenic molecules have been demonstrated to enhance vascularization and perfusion of the implant site (Sun et al., 2005; Trentin et al., 2006; Rajangam et al., 2006) and increase the survival of transplanted cells and tissue formation (Huang et al., 2005a). This process may be further manipulated by sequential delivery of factors involved in the initiation (e.g., VEGF) versus maturation (e.g., PDGF) of vessels, allowing temporal and spatial control over vascular bed formation (Richardson et al., 2001). Alternatively, transplantation of endothelial cells or progenitors in concert with the cell type of interest may also contribute to the formation of a new vascular network that enhances cell survival and tissue formation (Nor et al., 1999; Levenberg et al., 2005). Homing of circulating endothelial progenitor cells may also be promoted by providing appropriate cues (Hristov et al., 2007) from the cell delivery vehicle. Although there has been little effort to actively drive formation of a new lymphatic system (Niklason et al., 2002), the systems and approaches developed to drive angiogenesis may prove useful for this application. In contrast to the intensive efforts to drive vascularization at the sites of cell transplantation, there has been relatively little progress toward understanding and regulating innervation in engineered tissues. A number of approaches to nerve regeneration, often involving electrical, chemical, or topographic stimulation (Gomez and Schmidt, 2007; Kapur and Shoichet, 2004), are being actively pursued, but only a few studies have noted structures suggestive of nerves in tissues forming from transplanted cells (Oberpenning et al., 1999; Grikscheit et al., 2004). Recently, reinnervation of engineered muscle tissue was noted when cells were implanted adjacent to transected nerve (Dwahan et al., 2007). A lack of innervation will likely prevent full function and lead to atrophy in many situations, even if phenotypically normal tissue structures are formed initially by transplanted cells. The recent recognition of significant molecular crosstalk between the nervous and vascular systems (Zacchigna et al., 2008) during development and regenerative processes may provide multiple targets to simultaneously enhance innervation and vascularization.

Summary and Future Directions

Historically, most cell transplantation studies have utilized simple cell injections or infusions, but there has been considerable progress recently in the development of more sophisticated strategies that utilize pretransplant cell manipulations and material carriers to alter cell fate in vivo. Identification of the appropriate cell type(s), soluble and insoluble cues needed to direct the fate of embryonic and adult stem cells, as well as genetic and tissue engineering of the cells will likely be important steps in the future manufacturing of successful clinical products. The fundamental questions of which approach or combination of approaches will lead to the most successful cell engraftment, survival, and physiological functioning will ultimately depend on the disease to be treated, the method/site of implantation, as well as the health status of the individual patient to be treated and will require extensive additional basic research. Material carriers may be particularly useful to quantitatively control and manipulate not only the fate of the transplanted cells but also host cell populations (e.g., vascular progenitors) that may contribute to tissue repair. Although current systems demonstrate incremental

advances in transplanted cell utility, strategies that integrate multiple cues into one system may provide dramatic improvements in the future.

A number of significant challenges remain for material systems used as carriers for transplanted cells. There exists a substantial variable space inherent to these systems as they combine various cues (e.g., types, densities, organization of adhesion ligands, presentation of various cytokines, and growth factors), and the development of large-scale screening approaches (Anderson et al., 2004; Fok and Zandstra, 2005) will be required to thoroughly and systematically examine this space in order to design optimal material systems. This will likely require better 3D in vitro model systems, and a combined experimental and in silico approach (Vaziri and Gopinath, 2008). The successful vascularization of tissue repair sites using delivery of angiogenic molecules or cells is rapidly increasing the size scale of tissue repair (e.g,. from hundreds of microns to millimeter scale in thickness). However, it will likely be necessary to engineer complete vascular networks, containing both conducting and distributing vessels, if one is to repair centimeter-thick tissues, and microfabrication techniques that predefine vascular trees over this size scale may provide a path toward this goal (Borenstein et al., 2007). The identification of specific pathways that actively resolve inflammation (Serhan et al., 2007) may also provide new targets for material-regulated vascularization and, more broadly, microenvironmental control over transplanted cell fate. Clearly, the use of allogeneic cells may dramatically increase the utility of stem cell therapies, and it may be possible to design the material delivery systems to also manipulate the host immune system to induce tolerance (Reddy et al., 2006).

Cell delivery systems in the future will likely continue to take inspiration from the specialized microenvironments or niches, which contain and controllably activate stem cell populations within tissues (Mitsiadis et al., 2007). It may even be possible to design cell transplantation vehicles that serve to localize and maintain the viability of the transplanted cells, activate the cells to a proliferative state, and induce daughter cells to migrate outward over time to provide a continuous flow of cells at the optimal differentiation state for their participation in regeneration (Figure 4). These types of systems may also be designed in the future to enhance the homing of exogenous (Min et al., 2006) or endogenous progenitor cells (Hristov et al., 2007; Goldman, 2007) prior to local, material-controlled programming and redispersion to sites of tissue repair. These two concepts may be useful for a wide array of stem cell populations.

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