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Engineering the matrix microenvironment for cell delivery and engraftment for tissue repair

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Abstract

Cell-based therapies represent promising strategies for tissue repair, particularly in cases in which host cells, due to disease, age, or excessive trauma, are unable to repair the defect or deficiency alone, even with additional delivered therapeutics. Current cell therapies fail to address long term engraftment or delivery timing and location and result in modest improvements with long term engraftment rates of less than 1%. In many cell therapy applications, an appropriate carrier must be used to deliver transplanted cells and promote cell engraftment and function for a successful outcome by providing the appropriate microenvironment for the interactions between transplanted and host cells. This review highlights important considerations for engineering the microenvironment for cell delivery and engraftment in tissue repair.

Cell-based therapies represent promising strategies for tissue repair, particularly in cases in which host cells, due to disease, age, or excessive trauma, are unable to repair the defect or deficiency alone, even with additional delivered therapeutics. This review highlights important considerations for engineering the microenvironment for cell delivery and engraftment in tissue repair. It is noted that different tissues have various requirements for cell delivery specific to the tissue type and application, for example directional cues in nerve axonal regrowth and shielding of transplanted pancreatic islets from the immune system while allowing for the transport of metabolites and biological factors.

Current cell therapies (selected cell-based clinical trials are highlighted in table 1) fail to address long term engraftment or delivery timing and location. For instance, cell therapies for myocardial infarction consist of a cell suspension delivered by injection or infusion and, although proved to have an excellent safety profile, efficacy has been inconsistent with modest improvements at best with long term engraftment rates of less than 1% as the majority of delivered cells die or are washed away within the first week [1]. These methods not only fail to retain cells at the delivery site [2], but they also fail to address the effects of specific delivery location and timing on efficacy, factors shown to influence stem cell survival and functional outcome in injuries such as traumatic brain injury [3]. Long term cell engraftment has been shown to correlate with enhanced therapeutic outcomes [4–6], and has also been shown to be greater in cases in which cells are delivered in an appropriate biomaterial carrier versus in media alone [7]. Thus, in many cell therapy applications, an appropriate carrier must be used to deliver transplanted cells and promote cell engraftment and function for a successful outcome. The ideal carrier would provide the appropriate

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microenvironment for the interactions between transplanted and host cells. For tissue repair, as shown in figure 1, the ideal carrier would result in 1. Transplanted cells integrated with host tissue and vascularization; 2. Replacement by healthy, normal tissue over the course of healing; and 3. Complete function restored.

Materials used as delivery vehicles include natural materials, synthetic polymers, and ceramics. Natural materials such as purified collagen, hyaluronic acid, alginate, and chitosan have been extensively used in regenerative medicine and tissue engineering. Materials derived from natural sources can be biologically active, promote cell adhesion and growth, and enzymatically or hydrolytically degradable. However, natural materials display lot-to-lot variability, risk of immunogenicity and pathogen transmission, and structural complexity that renders modifications difficult. Calcium phosphate ceramics, namely tricalcium phosphate, hydroxyapatite, and bioactive glasses, are of clinical interest in bone tissue engineering due to their similarities to the inorganic phase of bone. Although these ceramics have been shown to enhance osteoblast adhesion and differentiation, their use is limited due to their brittleness and difficulty in shaping for implantation, thus, most cell-delivery strategies that incorporate ceramics do so in combination with either natural or synthetic polymers as a composite material [8–10]. Synthetic polymers provide an alternative to natural materials as cell-delivery vehicles. Because of their defined chemical composition, synthetic materials are often reproducible and can be modified to control material properties such as degradation rate, mechanical properties, and shape. As most synthetic polymers lack cell adhesion sites, polymers usually need to be chemically modified to support cell adhesion and other bio-functionalities, and this yields the opportunity of engineering specificity into the material [7,11]. Tuning of the degradation rate is a strong advantage over ceramics and natural polymers, as synthetic polymers can be used to deliver therapeutic molecules at controlled and defined rates.

A particularly attractive class of materials for cell delivery is hydrogels. Hydrogels are water-swollen physically or chemically cross-linked polymer networks that can be engineered from natural materials such as alginate or synthetic polymers such as polyethylene glycol (PEG). Specific advantages of hydrogels are minimal adverse host reactions (e.g., biocompatibility), high water content, relatively mild reaction conditions, and opportunity for minimally invasive delivery as injectable carriers. Hydrogels also offer the advantage of allowing multiple factors to be incorporated in tunable ratios creating a biomimetic 3D niche in which mechanical properties, presentation of bioactive molecules, and degradation of the gel can be tuned independently as shown in figure 2. This review will highlight the main considerations for engineering synthetic hydrogel microenvironments for cell therapies, highlighting examples from bone regenerative medicine.

Matrix Stiffness/Polymer Mesh Structure

Bulk material stiffness has been widely accepted as a driving force behind stem cell fate in two-dimensional cultures [12,13], but recent studies have raised the question of whether bulk stiffness directly determines cell fate or if bulk stiffness affects ligand presentation which in turn alters cell behavior [14]. Several groups have shown that stem cell shape in 2D can regulate cell fate [12,15,16], however, Mooney has demonstrated that in 3D, cell morphology is not correlated with fate and that encapsulated cells interpret changes in matrix stiffness as changes in adhesive ligand presentation [17]. Although matrix stiffness and cell shape may play a role in determining cell fate *in vitro*, these findings are based on isolated, well-controlled cultures of, usually, one cell type. *In vivo*, the environment supplied by the carrier must not only provide directional cues to the transplanted cells, but allow for their engraftment as well as the infiltration of host cells. As bulk material stiffness is generally tuned by varying cross-linking density [18], polymer weight percent [18–20], or

cross-linking chemistry [20], it is difficult to decouple the effects of material stiffness on encapsulated cells from the effects of cross-linking density on proliferation, morphological changes, migration and matrix permeability, and the effects that those in turn have on cell fate. For example, increasing polymer weight percentage, which often increases cross-linking density, or holding polymer weight percent constant while increasing cross-linking density decreases diffusivity which limits nutrient transport and increases elastic modulus [18,19], which in turn decreases the proliferation rate of encapsulated fibroblasts [18] and the spreading of encapsulated C2C12 murine myoblasts [20]. For example, the inhibitory effect from increasing matrix density and stiffness on capillary formation in a fibrin clot results from reduced diffusivity rather than matrix elasticity. When pro-angiogenic factor-secreting fibroblasts were distributed throughout the 3D gel, inhibitory effects from increasing fibrin density were abrogated, and capillary formation was equivalent between different gel densities [21]. Undoubtedly, additional analyses are needed to clearly establish rational design rules for the effects of carrier mechanical properties and structure on cell delivery.

Matrix Degradation for Tissue Ingrowth and Therapeutic Release

Controlled degradation of the cell carrier is critical for tissue ingrowth and cell migration/outgrowth as well as delivery of biotherapeutics incorporated within the carrier. Engineering degradability into a cell carrier can be accomplished by incorporating cross-links that are enzymatically or hydrolytically degradable. Enzymes responsible for matrix degradation include matrix metalloproteinases (MMPs), which are the main class of enzymes used in remodeling the extracellular matrix (ECM), and hyaluronidase, the protease for hyaluronic acid which is found in the ECM of many connective tissues. Enzymatic degradation by cell-secreted hyaluronidase or MMPs can be achieved by incorporating cross-linking peptides that contain an MMP degradable site [11,22] or incorporating hyaluronic acid, mimicking the natural cell-controlled degradation of ECM. Degradable matrices can also be used for cell-mediated growth factor delivery with tunable degradation and release rates by capturing growth factors in a degradable cross-linked matrix [11,23,24] or by covalently or non-covalently tethering growth factors to the matrix using affinity-based and other motifs [25–27].

Vascularization

Many groups have reported a significant loss in transplanted cells within the first week after implantation [28,29]. By monitoring the kinetics of expression of three ischemic markers of transplanted hMSCs 30 days post-implantation, Becquart demonstrated that local tissue ischemia is a major cause for cell early cell loss [29]. This highlights the need for vascularization to promote transplanted cell survival and engraftment. A material must be degradable to allow for the infiltration of macrophages, fibroblasts and endothelial cells while promoting vascularization through either the release of growth factors or other soluble mediators. Many groups have demonstrated that both material degradability and angiogenic agents can work in concert to prolong the beneficial effects of angiogenic therapies. Cell-demanded release of encapsulated vascular endothelial growth factor (VEGF) by means of matrix-metalloproteinase mediated degradation of a synthetic matrix significantly increased the rate of reperfusion in hind-limb ischemia [27] and produced extensive vascularization with functional blood vessels in hydrogels implanted subcutaneously [27,30] and in the mouse cornea [31]. The effect of co-delivery of angiogenic factors and cells on the engraftment and survival of the transplanted cells is rarely addressed. Delivery of human bone marrow stromal cells on a VEGF-releasing biodegradable polymer scaffold into a femoral defect showed increased bone formation compared to the scaffold alone and hBMSC seeded scaffolds, but no comparison was provided on extent of engraftment or cell

retention between VEGF and no VEGF groups [32]. Many cell and growth factor co-delivery systems rely on genetically modified cells that produce a sustained supply of growth factors. There have been many successful *in vivo* studies in which cells provide a constant supply of growth factors rather than being the therapeutic agent themselves. For example, adipose-derived stem cells modified to express VEGF or bone morphogenetic proteins (BMPs) significantly enhance bone healing [33,34], but this approach risks deleterious effects from overexpression of target therapeutics [23,35] and still requires a proper carrier to retain cells at the defect site and promote cell survival. The host environment in a tissue injury is difficult to recapitulate *in vitro* due to multiple cell types, densely arranged cells, an inflammatory response, and vascular ingrowth needed to sustain transplanted cells. In order to recapitulate the cell density of tissues *in vitro*, cell density would need to be as high as 10^9 cells/cm³, and without a vascular system, this construct would fail due to inadequate nutrient supply. Multicellular 3D tissue constructs with perfused vascular networks will enable more accurate *in vitro* study of the interactions between tissues and cell delivery systems [36]. It is noteworthy to mention that although it has been shown that co-delivery of VEGF and cells can improve bone formation, few groups have directly addressed the effect of exogenous VEGF on cell engraftment and survival in bone regeneration, most likely due to focus being placed on the bone healing properties of VEGF rather than its pro-angiogenic effects on cell survival. Most *in vivo* cell-delivery studies in bone repair use new bone formation and/or mechanical testing as indicators of therapy efficacy and do not address cell engraftment or, at the bare minimum, provide evidence that some transplanted cells are present at the end of the study. Cell engraftment is rarely studied as a means to improve functional outcome which raises the question of whether cell engraftment is important if the overall outcome, i.e. functional restoration of the injured tissue, is improved.

Adhesive Interactions

An appropriate carrier must support the adhesion, migration, organization, and differentiation of the transplanted cells and host cells. These behaviors rely on an intricate interaction between various cues supplied by the extracellular environment which include insoluble molecules within the ECM such as proteins, soluble molecules such as growth factors, hormones, and cytokines, and cell-cell interactions. Integrins are a family of cell surface receptors that primarily mediate adhesion of cells to ECM proteins and also transduce signals across the cell membrane. Each heterodimeric combination exhibits unique binding characteristics and mediates cellular activities such as migration, proliferation, survival and differentiation [37]. Therefore, as a major mediator of important cellular responses, engineering integrin binding activity within a 3D environment may improve transplanted cell survival and function. As synthetic polymers such as polyethylene glycol have become a popular choice for biomaterials for their non-fouling properties, additional steps must be taken to functionalize these synthetic materials to promote cell adhesion. Encapsulating fibroblasts in a polyethylene glycol hydrogel with protease-degradable sites, RGD, neither or both demonstrated that both adhesive sites and degradability were required for cell spreading and migration within a 3D synthetic hydrogel carrier [22,27].

Cell Adhesion and Integrin Specificity

Materials may be functionalized to promote adhesion by 1. Incorporating small adhesive ligands, such as RGD oligopeptides [11,20,27,38]; 2. Components found in ECM such as collagen [39] or hyaluronic acid [40]; or 3. Functionalizing with small chemical groups, such as phosphates, to promote specific protein interactions [41]. There has been some degree of success using full length ECM proteins to functionalize biomaterials by tethering

or passive adsorption, but these proteins come with batch to batch variability, limited control over presentation, and difficulty in making modifications. For example, $\alpha_2\beta_1$ binds the sequence GFOGER, one of the major recognition sites on type I collagen [42], which makes up more than 90% of the organic phase of bone. When compared to full length type I collagen, the GFOGER collagen-mimetic peptide significantly improved *in vivo* peri-implant bone regeneration and osseointegration, as characterized by bone-implant contact area and mechanical fixation [43]. Use of a non-fouling material, such as PEG, allows for engineering integrin specificity in the cell carrier by incorporation of specific adhesive ligands. RGD, the shortest known adhesive sequence, is found in many proteins such as fibronectin, vitronectin, bone sialoprotein, and osteopontin [44], and promiscuously binds to a number of integrin receptors. Although RGD helps to mediate functional vascularization, the integrins specific for RGD may not be appropriate for every tissue engineering application. Emerging evidence supports a role for integrin binding specificity in directing cellular responses to biomaterials [45]. RGD-functionalized biomaterials used to deliver encapsulated cells to bone defects generally failed to promote bone healing [28,46]. When RGD is combined with synergy site PHSRN from fibronectin in the correct structural presentation, affinity for integrin $\alpha_5\beta_1$ increases 40-fold compared to RGD alone [47,48]. $\alpha_5\beta_1$ integrins have been shown to contribute to bone marrow cell adhesion, mineralization, and bone healing and $\alpha_5\beta_1$ is present on osteoblasts and osteoclasts during all stages of osteogenesis. Titanium implants functionalized with a fibronectin fragment specific for $\alpha_5\beta_1$ performed significantly better in an osseointegration study compared to RGD functionalization in terms of pull-out force and bone-implant contact area [48], consistent with other reports in the failure of RGD to promote functional osseointegration [49].

Ligand Structural Design and Valency

Many receptors require more than just ligand binding for efficient signal transduction. Current design of growth factor delivery generally fails to consider the interactions between growth factors and other components of the ECM. Integrin signals can overlap or even synergize with growth factor signaling, as some growth factor signaling cascades, such as platelet-derived growth factor-BB (PDGF-BB), fibroblast growth factor (FGF), and epidermal growth factor (EGF), require aggregation and ligation of integrins for activation [50]. Functionalizing a fibrin matrix with two fused fibronectin domains – the major integrin-binding domain, the 9th to 10th type III repeats, and a promiscuous growth factor binding domain, the 12th to 14th type III repeats – significantly enhanced the bone healing effects of PDGF-BB and BMP-2 in a calvarial defect compared to the growth factors or fused fibronectin domains delivered alone in fibrin [51]. Integrin recruitment and clustering plays an important role in the assembly of focal adhesions and maximizes signaling efficiency. García et al. investigated how nanoscale ligand clustering influenced osseointegration of titanium implants by engineering implant coatings that consisted of multimeric constructs of the $\alpha_5\beta_1$ -specific 7th to 10th type III repeats of fibronectin. They found that coatings of trimers and pentamers enhanced integrin-mediated adhesion and osteogenic signaling *in vitro* and, *in vivo*, required a 5-fold higher pull-out force compared to coatings presenting no ligand, monomers, or dimers [52]. In addition to cell-ECM interactions, intercellular communication is important for coordinating tissue functions and homeostasis, a feature that is generally lost in low-density 3D cell transplant constructs. Kohn et al. demonstrated that overexpression of connexin 43, the most prevalent subunit of gap junctions, in transplanted bone marrow stromal cells enhanced the magnitude and spatial uniformity of bone *in vivo* compared to nontransduced cells and enhanced the osteoinductive effects of BMP-7 [53]. Thus, an additional method to enhance cell delivery in tissue repair might be found in a ligand peptide that recapitulates signaling in gap junction intercellular communication.

Looking Forward

Recent studies with engineered synthetic hydrogels mimicking the extracellular matrix have provided significant insights into developing design rules for engineering the signaling environment for cell therapies for tissue repair. Although material stiffness may play a role in cell differentiation in well-controlled cultures *in vitro*, stiffness is generally coupled with degradability properties which, for *in vivo* cell delivery applications, modulate engraftment, proliferation, cell survival, and vascularization. The matrix environment should provide directional cues to the transplanted cells in terms of adhesive ligands, integrin specificity, and a carefully engineered growth factor microenvironment. Future cell-based strategies must consider the temporal and spatial complexities of the *in vivo* environment and the effect or role of cellular retention and engraftment for regenerative medicine. In addition to strategies previously mentioned, future considerations for engineering a cell delivery vehicle include dynamic or triggered changes in bioactivity [54,55], as well as multiple growth factor delivery for synergistic effects in directing cell fate [56]. Understanding, harnessing, and engineering these aspects into a cell-delivery vehicle may finally enable us to use cells to their full potential as a tissue repair therapy strategy.

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- Current cell-based strategies are significantly limited by biomaterial carriers.
- We highlight considerations for engineering the cell delivery microenvironment.
- We discuss strategies to improve cell delivery and engraftment in tissue repair.

Figure 1.

Schematic of cell delivery vehicle performance *in vivo* over time. A) Overview of integration of vehicle and transplanted cells into host tissue. B) Transplanted cells interact with host cells in many ways. Transplanted cells may release trophic or chemotactic factors that activate host cells and host cells in the injury site may release factors that drive differentiation or behavior of the transplanted cells as well as remodel the delivery vehicle and implant site. As cells degrade the matrix by releasing proteases, growth factors and other therapeutic molecules are released on demand. C) In response to specific adhesive ligands, growth factors, and/or soluble cues from the tissue environment, transplanted cells in the matrix undergo proliferation, cell-cell communication, migration and differentiation as the cells degrade the matrix. D) In response to pro-angiogenic growth factors (delivered or cell-secreted) and facilitated by adhesive ligands and matrix degradability, host vasculature infiltrates the matrix delivering nutrients to the transplanted cells and/or facilitates cell recruitment and trophic factor transport.

Figure 2.

Schematic of functionalized hydrogel preparation. A) Multi-arm polymer precursors are functionalized with adhesive ligands, growth factor tethering peptides (affinity-based or proteolytically labile-covalent cross-links), and growth factors. The addition of protease degradable cross-linkers creates a 3D hydrogel network. B) Alternatively, polymer chain precursors may also be functionalized with adhesive ligands and mixed with soluble growth factors or therapeutics to deliver untethered, but encapsulated molecules. Addition of cross-linkers creates a 3D hydrogel network. C) Cross-linking density or polymer weight percent may be tuned independently of functionalization. Increasing cross-link density or polymer weight percentage increases stiffness, but decreases diffusivity, pore size, swelling, and degradation rate.

Representative active clinical trials for cell-based therapies for tissue repair. Data were obtained from Stevens et al. [57], Langer et al. [58], and <http://www.clinicaltrials.gov>.

Table 1

Tissue	Application	Cell Source	Cell Type	Cell Carrier	Phase	Sponsor
Bone	Sinus Floor Bone Augmentation	Autologous	Adult multipotent cells	Beta-tricalcium phosphate	1/2	Univ. Michigan
	Nonunion fracture	Autologous	Osteoblastic cells	None	3	Bone Therapeutics
	Myocardial Infarction	Allogenic	MSC	None	2	Ostris
Heart	Myocardial Infarction	Autologous	BM-derived CD34+ SC	None	1	Amorcyte
	Myocardial Infarction	Autologous	Skeletal myoblast	None	2/3	Bioheart
	Myocardial Infarction	Allogenic	MSC	None	2	Stemmedica
	Myocardial Infarction	Autologous	BM-MNC	None	1/2	TotipotentRX Cell Therapy
	Ischemic Stroke	Allogenic	Multistem (proprietary adult SC)	None	2	Athersys
Cartilage	Osteoarthritis	Allogenic	MSC	None	2	Stempeutics
	Articular Cartilage Defect	Autologous	Chondrocytes	Collagen scaffold	3	Genzyme
	Articular Cartilage	Autologous	Chondrocytes	NOVOCART 3D (collagen matrix)	3	Tetec
	Meniscal Repair	Autologous	BM-SC	Cell bandage, proprietary	1/2	Azellon
	Cartilage Defect	Autologous	Chondrocytes	NeoCart, ex vivo TE construct	3	Histogenics
	Cartilage Defect	Autologous	Ad-MSC	None	1/2	RNL Bio
	Intervertebral Disc Degeneration	Autologous	Ad-MSC	None	1/2	RNL Bio
	Intervertebral Disc Degeneration	Autologous	Chondrocytes	NOVOCART disc, collagen matrix	1/2	Tetec
	Leg Ulcers	Allogenic	Keratinocytes, Fibroblasts	Collagen	4	Organogenesis
	Burns	Allogenic	UC-MSC, CB-MNC	None	1/2	Beike
Pancreas	Diabetes	Allogenic	cells	Alginate encapsulated	2	Universitair Ziekenhuis Brussel
	Diabetes	Xenogenic	Porcine islets	Alginate encapsulated	2	Living Cell Technologies
	Diabetes	Allogenic	MSC	None	2	Ostris
Other	Stroke	Allogenic	Neural stem cells	None	1	ReNeuron
	Stroke	Allogenic	MSC	None	1/2	Stemmedica
	Liver Cirrhosis	Allogenic	MSC	None	2	Stempeutics
	Spinal Cord Injury	Autologous	Ad-MSC	None	1/2	RNL Bio
	Spinal Cord Injury	Autologous	MSC	Bone marrow concentrate	1/2	TotipotentRX Cell Therapy

Tissue	Application	Cell Source	Cell Type	Cell Carrier	Phase	Sponsor
	Macular Dystrophy	Allogenic	ESC-derived retinal pigment epithelium cells	None	1/2	Advanced Cell Technology
	Critical Limb ischemia	Allogenic	MSC	None	2	Stempeutics
	Critical Limb ischemia	Autologous	HSC	Platelet rich plasma	1	Arteriocyte

Abbreviations: Mesenchymal stem cell (MSC), Bone marrow mononuclear cells (BM-MNC), Stem cell (SC), Adipose-derived mesenchymal stem cell (Ad-MSC), Umbilical cord-derived mesenchymal stem cell (UC-MSC), Cord blood-derived mononuclear cells (CB-MNC), Embryonic stem cell (ESC), Hematopoietic stem cell (HSC)