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REVIEW

# *In situ* tissue regeneration through host stem cell recruitment

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**The field of tissue engineering has made steady progress in translating various tissue applications. Although the classical tissue engineering strategy, which involves the use of culture-expanded cells and scaffolds to produce a tissue construct for implantation, has been validated, this approach involves extensive cell expansion steps, requiring a lot of time and laborious effort before implantation. To bypass this *ex vivo* process, a new approach has been introduced. *In situ* tissue regeneration utilizes the body's own regenerating capacity by mobilizing host endogenous stem cells or tissue-specific progenitor cells to the site of injury. This approach relies on development of a target-specific biomaterial scaffolding system that can effectively control the host microenvironment and mobilize host stem/progenitor cells to target tissues. An appropriate microenvironment provided by implanted scaffolds would facilitate recruitment of host cells that can be guided to regenerating structural and functional tissues.**
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## INTRODUCTION

Surgical reconstructive procedures often require the use of additional tissues, such as autograft, allograft or xenograft, in order to restore normal anatomical and functional tissue configurations. However, these materials are often associated with complications such as donor site morbidity, limited availability and host tissue reactivity.[1,2] Cell-based tissue engineering has emerged as a promising approach to overcome these limitations, as this technology enables the fabrication of functional tissues or organs that could be used for reparative procedures in patients.[3] The basic approach is to create bioengineered tissues or organs by combining patient's own cells with a natural and/or synthetic biomaterial scaffold under suitable culture conditions, resulting in tissue constructs that can be implanted *in vivo*. However, this approach requires a donor tissue biopsy and extensive cell expansion steps before implantation for therapy. Moreover, isolated tissue-derived primary cells are often heterogeneous and difficult to standardize. Thus, obtaining a reliable and reproducible cell source has been one of the challenging elements of cell-based approaches. This has motivated the development of a new strategy that eliminates the *ex vivo* cell manipulation before implantation, and this approach would decrease the time, effort and resources required to generate a tissue/organ substitute.

Recent progress in tissue engineering and regenerative medicine has adopted the concept of utilizing endogenous cells for *in situ* tissue regeneration. The principle of *in situ* tissue regeneration is to utilize the body's own biologic resources and its reparative capability by using a target-specific biomaterial system to recruit host stem or tissue-specific progenitor cells to the site of injury. This novel approach would allow for a damaged tissue to be regenerated without the need for cell transplantation (Figure 1). When scaffolds incorporated with bioactive molecules are implanted *in vivo*, sustained release of the bioactive cues unlocks the body's own regenerative capability. In turn, this induces the mobilization of tissue-specific host stem/progenitor cells, drives proliferation and differentiation of these recruited cells into the targeted cell types and regenerates functional tissues. This review discusses the recent development of approaches for *in situ* tissue regeneration, particularly focusing on the strategies that enhance host stem or progenitor cells into the target-specific scaffolds, and present some of the applications of *in situ* tissue regeneration.

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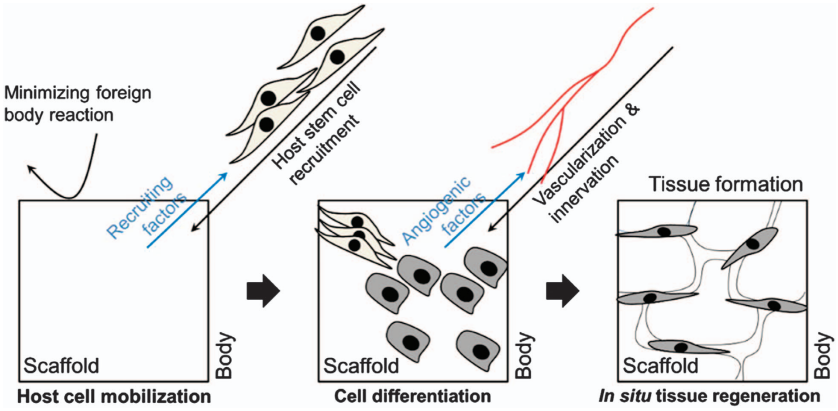


**Figure 1** A strategy for *in situ* tissue regeneration.

## BASIC CONSIDERATIONS FOR *IN SITU* TISSUE REGENERATION

The success of *in situ* tissue regeneration relies on effective recruitment of host stem or progenitor cells into the implanted biomaterial scaffolds and induction of the infiltrating cells into tissue-specific cell lineages for functional tissue regeneration. To achieve this, a target-specific scaffolding system, serving as a template, needs to be designed in order to enable ('instructs') the fate of the recruited host cells to proliferate and differentiate into a desired tissue type.[4] Sustained delivery of biological cues, such as bioactive molecules, from the implanted scaffold could play an important role in guiding host cells to form a well-integrated functional structure.[5] Moreover, a well-designed combination of biological cues with biomaterial scaffolds would provide appropriate microenvironments for efficient cellular specification within the implanted scaffold.

### Host cell sources for in situ tissue regeneration

It has been demonstrated that adult stem cells that contain self-renewal and differentiation capability can be isolated from various tissues and organs, including brain, liver, circulating blood, heart, skin, kidney, muscle and fat.[6–12] Most adult stem cells are quiescent and reside in a specialized microenvironment, which is called a 'stem cell niche'. In response to regulatory signals that originate from tissue injury, these stem cells become activated and begin repairing process. In addition to tissue-specific adult stem cells that are primarily responsible for tissue regeneration processes, bone marrow-derived stem cells have been identified as important cell sources that contribute their regenerating capacity to other tissues. The bone marrow harbors multiple distinct stem/progenitor cells that include hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). HSCs are responsible for the production of all circulating blood cells such as myeloid, erthyroid and lymphoid lineages. An important role of the HSC population for tissue regeneration is to provide paracrine bioactive factors to regenerative cells and occasionally transdifferentiate into desired tissue-specific lineages.[13] Another cell population contained in the bone marrow is stromal cells or MSCs that exhibit multipotent capabilities to differentiate into a variety of cell types *in vitro* and *in vivo*.

Interestingly, many reports have shown that MSC populations that express similar set of cell surface antigens can be isolated from bone, cartilage, muscle, bone marrow stroma, tendon, fat and other connective tissues,[14] and these MSC populations have been widely used in preclinical and clinical applications for tissue regeneration. These cells are known to modulate the immune system and/or provide trophic factors necessary for tissue-specific regeneration.[15] However, the specific identity of these MSC populations is still unclear and further studies are required for understanding the origin and contributions of these cells to tissue regeneration. EPCs[6] are another important cell source that are actively involved in promoting angiogenesis at the injury site. Facilitating neovascularization through EPCs is particularly beneficial in tissue regeneration and ischemic tissue injury.

Macrophages that play an important role in inflammatory response and foreign body reaction are found at the injury site during the tissue repair and remodeling process.[16] Recent reports have shown that macrophages are an important determinant during tissue remodeling in the context of regenerative medicine.[17] The proinflammatory macrophages, designated as an M1 phenotype, are involved in chronic inflammation and foreign body reactions,[18] whereas M2 macrophage phenotypes are associated with anti-inflammation, immunomodulation and tissue remodeling processes. A better understanding of the mechanisms underlying differential infiltration of the macrophage populations into scaffolds will aid in controlling the specific type of macrophage recruitment and may result in beneficial effects on the desired tissue regeneration.[17]

Although certain types of host cells have been identified in inflammatory responses and foreign body reactions, cell populations that infiltrate into biomaterial scaffolds are poorly understood. It is important to investigate the possibilities to use the body's biologic and environmental resources for tissue regeneration *in situ*. Recruitment of host cells into an implanted scaffold as part of tissue repairing process has been

examined. In our previous study,[19] poly(glycolic acid) nonwoven scaffold was used to address this dogma. This biomaterial implant was designed to enhance diffusion and accommodate host cell infiltrates into the highly porous structures. The results of this study showed that the number of host cells infiltrating into the implant increased for up to 3 weeks after implantation and began to decrease thereafter, as collagen accumulated to fill the pores of implanted scaffold. Interestingly, we observed that a small proportion of infiltrated host cells within the implants had multilineage potential (Figure 2). These results indicate that some of the host stem cells that mobilized into the biomaterial were multipotent, and given an appropriate microenvironment, they differentiated into tissue-specific cell lineages at the implant site.

**Biomaterial scaffolds for in situ tissue regeneration**

Creation of bioengineered tissue requires a scaffold, which provides structural support until the mobilized cells form functional tissue *in vivo*. Although the properties of scaffolds may vary depending on the targeting tissues, the general requirements of a scaffolding system are biological stability, biodegradability and temporal structural integrity. The scaffold's internal architecture should provide adequate permeability for establishing functional vascularization following implantation. The latter is critically important as this porous structure can not only facilitate space for the recruited cells to reside, but also permit incorporation of bioactive molecules and biophysical cues that enhance cell migration, proliferation and differentiation to produce a biofunctional host stem cell niche.[20] To design a tissue-specific scaffolding system for *in situ* tissue regeneration, the scaffolds should possess the ability to (1) regulate inflammation for minimized fibrotic formation, (2) utilize host microenvironment for recruiting host stem/progenitor cells and (3) control tissue-specific cell differentiation within the scaffold.

Biomaterials used for scaffolding can be naturally derived or synthetic polymers. Natural materials include polysaccharides and proteins. Polysaccharides that have been widely used for this purpose include cellulose, alginate, hyaluronic acid, starch, dextran, heparin, chitin and chitosan.[21] Proteins are the primary components of tissues or organs and have been used for various biomedical applications. Collagen, which is the most abundant protein in mammals, has been used as scaffold materials because of the ease of processing as well as its ability to induce minimal inflammatory and immune responses. Collagen has been approved by the US Food and Drug Administration (FDA) for many types of biomedical applications, including wound dressings and artificial skin.[22] Collagen can be configured into various structures such as films, fibers and sponges.[23–25] Decellularized collagen-rich tissue scaffolds have received much attention recently because of their ability to maintain microtissue architecture.[26,27] In addition, these acellular tissue matrices have been shown to support cell ingrowth and tissue regeneration.[28]

Synthetic polymeric biomaterials such as biodegradable polyesters, including poly(glycolic acid), poly(lactic acid) and poly(lactide-*co*-glycolide) (PLGA), are widely used in tissue engineering and regenerative medicine. The use of these polymers was approved by the FDA for human use in a variety of applications, including surgical sutures.[29] These biodegradable polymers are nontoxic during the degradation process *in vivo*, and are eventually removed from the body in the form of carbon dioxide and water.[29] These polymers possess thermoplastic properties and can be easily fabricated into various configurations with controlled microstructure and porosity using a number of processing techniques, including molding, extrusion,[30] solvent casting,[31] phase separation techniques and gas foaming techniques.[32,33] More recently, electrospinning techniques have been developed to quickly create highly porous scaffolds in various conformations, including nanostructures.[32,34–36] Other biodegradable synthetic

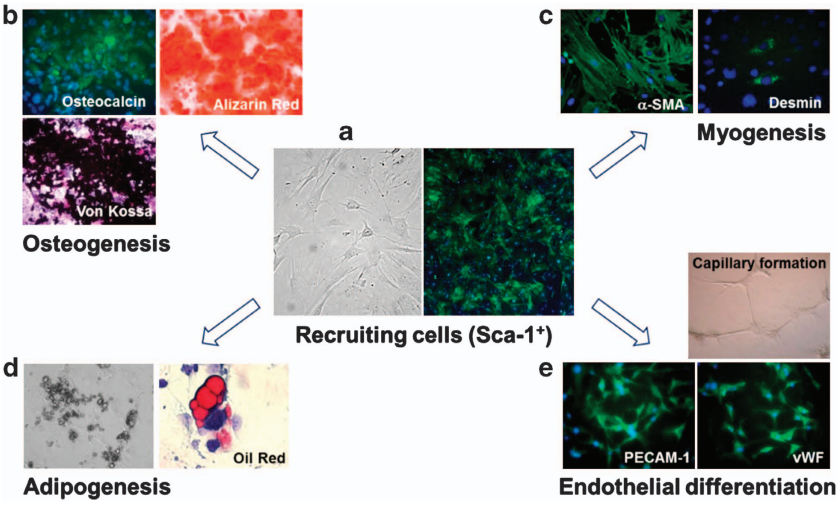


**Figure 2** Multidifferentiation capability *in vitro* of the infiltrated cells into the biomaterial scaffold: (**a**) Sca-1+ population of the cell infiltrate, (**b**) osteogenic, (**c**) myogenic, (**d**) adipogenic and (**e**) endothelial differentiation under appropriated culture conditions.[19] α-SMA, α-smooth muscle actin; PECAM-1, platelet/endothelial cell adhesion molecule; Sca-1, stem cell antigen-1; vWF, von Willebrand factor.

polymers used for tissue regeneration applications include poly(anhydrides) and poly(ortho-esters).[37]

**Biochemical signaling for in situ tissue regeneration**

A key to *in situ* tissue regeneration in the initial stage is proficient recruitment of host stem or progenitor cells into an implanted scaffold. However, adult stem cell populations in the body are generally too low in number to have a significant impact on acceleated tissue regeneration. In most cases of tissue regeneration, bone marrow-derived stem cells have contributed to regeneration, and therefore it is worthwhile to target these cells to be effectively mobilized into the peripheral blood system. During this mobilization, homing and engraftment process, other cytokines and chemoattractants are able to increase the efficacy of migration to the injury site. Table 1 shows several bioactive molecules used for *in situ* tissue regeneration.

Substance P (SP) is a neuropeptide that functions as a neurotransmitter and neuromodulator. A recent report showed strong evidence that released SP after corneal injury was able to mobilize a high number of stromal-like $CD29^+$ cells (MSC-like cells) from bone marrow into peripheral blood and drive migration and participation in the repair processes of the cornea injury.[38] This study shows that under the optimal culture conditions *in vitro*, the SP-induced $CD29^+$ MSC-like cells are able to demonstrate multidifferentiation capability by forming bone, cartilage and fat cells. These results indicate that SP is expected to play a positive role in tissue repair[39] based on the multipotency features. In terms of therapeutic aspects, the use of SP appears to be a cost-effective treatment because of high efficacy of host MSC-like cell mobilization with a single injection. A recent report also stressed positive roles of SP in reparative neovascularization.[40] In this report, patients with myocardial infarction showed high concentration of SP in the blood, which increased host progenitor cell mobilization, whereas suppression of SP levels resulted in a decreased number of host therapeutic progenitor cells. The study team concluded that SP-based nociceptive signaling may represent a possible target of regenerative medicine. Therefore, the use of stem cell-stimulating factors such as SP is a possible approach to accelerate the neovascularization process.

It is well known that retention of stem or progenitor cells in the bone marrow is through the interaction between the C-X-C chemokine receptor 4 (CXCR4) on the surface of stem/progenitor cells and stromal cell-derived factor-1α (SDF-1α) on the surface of bone marrow stromal cells.[41] When the retention axis is disrupted, the progenitor cells are released from the bone marrow stroma and mobilized into the peripheral blood. One of the mobilization-accelerating factors is granulocyte-colony-stimulating factor (G-CSF) and it has been widely used for clinical trials.[42] It was reported that $CD34^+$ HSCs can be effectively mobilized into the peripheral blood from the bone marrow through disruption of the SDF-1/CXCR4 axis.[41] In an approach for *in situ* tissue regeneration, several reports showed that G-CSF has been used as a single injection or directly incorporated into the implanted scaffold. A single injection of G-CSF for tissue regeneration has been conducted to accelerate EPC recruitment on implanted small-diameter vascular constructs.[43] It was shown that administration of G-CSF induced significant recruitment of $CD34^+$, $CD133^+$ EPCs into the vascular graft, generated endothelium and inhibited neointimal hyperplasia of a small-diameter heparinized decellularized vascular graft. A recent approach showed the efficiency of released G-CSF from a

**Table 1 Bioactive molecules used for *in situ* tissue regeneration**

| |
|---|
| *Stem cell-inducing/stimulating factor* |
| Substance P (SP) |
| Granulocyte-colony-stimulating factor (G-CSF) |
| CXCR4 antagonist (AMD3100) |
| Stem cell factor (SCF) |
| Parathyroid hormone |
| *Stem cell-homing/migration factor* |
| Stromal cell-derived factor-1 (SDF-1) |
| Protease-resistant SDF-1α |
| SDF-1α inhibitors |
| Diprotin A (inhibition of SDF-1α inhibitor (Dipeptidyl eptidase IV)) |
| Hepatocyte growth factor (HGF) |
| Monocyte chemotactic proteins (MCPs) |
| Matrix metalloproteinase-2 (MMP-2) |
| Galanin |
| *Collagen synthase inhibitors* |
| Matrix metalloproteinase inhibitors (MMPs) |
| Propyl hydroxylase |
| C-proteinase inhibitor |
| Halofuginone |
| *Tissue-enhancing factors* |
| Transforming growth factor-βs (TGF-βs) |
| Insulin-like growth factors (IGFs) |
| Fibroblast growth factor-1 (FGF-1) |
| Epidermal growth factor (EGF) |
| *Angiogenic factors* |
| Vascular endothelial growth factor (VEGF) |
| Fibroblast growth factor-2 (FGF-2) |
| Platelet-derived growth factor-BB (PDGF-BB) |
| TGF-βs |
| Angiogenin |
| Angiopoietin-1 (Ang-1) |
| Angiopoietin-2 (Ang-2) |
| Delta-like ligand 4 (Dll4) |
| *Innervation factors* |
| Brain-derived neurotrophic factor (BDNF) |
| Glial cell line-derived neurotrophic factor (GDNF) |
| Nerve growth factor (NGF) |
| Agrin |

hydrogel scaffold for enhancing EPC mobilization.[44] This group developed a hydrogel system incorporating G-CSF and showed that intramuscularly injected hydrogel significantly enhanced mobilization of $CD34^+CD31^+$ EPCs into the blood, as compared with a G-CSF bolus injection or hydrogel injection only. In addition, AMD3100, an antagonist of CXCR4, has been used singly or in combination with G-CSF to enhance mobilization of HSCs and progenitor cells.[45] Another report describes the use of AMD injection for the treatment of myocardial infarction. AMD treatment enhanced mobilization and recruitment of EPCs to the neovasculature.[46] In addition, a combined treatment of G-CSF and AMD has resulted in efficient mobilization of monocytes and stimulation of angiogenesis at ischemic sites.[47]

Stem cell factor is an endogenous ligand for the tyrosine kinase receptor c-*kit*, which is expressed on HSCs. Recombinant stem cell factor has been shown to act in synergy with G-CSF in mobilization of bone marrow-derived HSCs.[48] Regulation of selective mobilization of different populations of stem cells has been tested.[49] This study showed that treatment with CXCR4 antagonist (AMD3100) effectively mobilizes HSCs, but not EPCs or stromal cells. However, pretreatment with vascular endothelial growth factor (VEGF) resulted in EPC and stromal cell mobilization, whereas HSC mobilization was reduced. These results suggest that multiple intersecting signaling pathways regulate the proliferation and mobilization of bone marrow-derived stem cells for efficacious tissue regeneration.

Direct targeting of the stem cell niche is another approach to induce stem cell mobilization for promoting tissue regeneration. In bone marrow, one component of the HSC niche is osteoblasts. It was shown that stimulation of the parathyroid hormone receptor promotes osteoblast proliferation and secretion of paracrine factors that, in turn, resulted in an increase in the number of HSCs.[50] These studies indicate that direct targeting of osteoblasts can modify the activity of HSCs in the bone marrow.

In addition to stem cell-stimulating factors that mobilize host stem/progenitor cells in the body, bioactive molecules that induce engraftment of the mobilized host stem cells into desired tissues or organs for repair are considered as important cues for efficient *in situ* tissue regeneration.[51] One representative chemoattractant is SDF-1α that has been shown to attract MSCs[52] and HSCs to injured tissues through CXCR4 (SDF-1 receptor) expression.[53] In peripheral blood where the expression of CXCR4 is at a low level, a pool of both MSCs and HSCs can be maintained at a balanced level in distant parts of the body. However, tissue damage or other mobilization cues could mobilize these cells to peripheral blood and to the injury site. Thus, it is possible that sustained release of chemoattractants such as SDF-1α contained within an implanted scaffold could generate a high concentration gradient of these factors and drive efficient stem cell migration into the implant. For example, a previous report showed that local release of SDF-1α was observed from heparinized collagen sponge-enhanced recruitment of HSCs into subcutaneously implanted scaffolds. One recent study demonstrated an interesting approach to utilize the effects of SDF-1α on recruitment of host stem cell into implanted scaffolds.[54] This group incorporated SDF-1α into a biodegradable PLGA scaffold and implanted subcutaneously in mice. They showed that local release of SDF-1α induced efficient recruitment of host stem cells ($SSEA\text{-}4^+$ cells) in the implanted scaffold. In addition, the scaffolding system resulted in a reduced inflammatory response. This approach has also been applied to brain injury for the recruitment of neural progenitor cells.[55] For accelerated brain regeneration from cavitary brain lesions that fail to recruit endogenous neural progenitor cells, the authors developed an injectable scaffolding system that consists of gelatin-hydroxyphenylpropionic acid hydrogels and dextran sulfate/chitosan polyelectrolyte complex nanoparticles to deliver SDF-1α to the cavitary brain lesion region. They demonstrated the initial feasibility *in vitro* by showing that release of SDF-1α from the incorporated gel scaffold system significantly enhanced infiltration of neural progenitor cells, when compared with hydrogel only or vehicle controls, indicating that this scaffolding system is a promising approach for neural tissue repair.

SDF-1α is known to be easily damaged by matrix metalloproteinase-2. To address this issue, SDF-1α molecule was engineered to be resistant to protease activity and the engineered SDF-1α was tethered to self-assembling peptides to form nanofibers.[56] In this study the authors showed that the injected nanofibers containing protease-resistant SDF-1α molecules enhanced recruited EPCs, increased capillary density and functionally improved cardiac function. In an attempt to protect SDF-1α effects from endogenous inhibitors, an alternative approach showed that targeting of SDF-1α inhibitor improved SDF-1α effectiveness in stem cell engraftment into the damaged heart.[57] The dipeptidyl peptidase IV is known to cleave SDF-1α, therefore inhibition of dipeptidyl peptidase IV by the small-molecule diprotin A increases the concentration of SDF-1α in the heart following myocardial infarction. This resulted in increased progenitor cell recruitment to the ischemic myocardium and improved neovascularization and ventricular function.[57] Targeting of these inhibitors represents an effective strategy for regenerative medicine.

Monocyte chemotactic proteins (MCPs) are known to direct bone marrow-derived stem cells into the injury sites. One study shows that MCP-3 recruits MSCs into myocardial infarction site through activation of CC chemokine receptors.[58] In addition, MCP-1 and MCP-5 have been reported to be CCR2-activating chemokines that recruit bone marrow-derived macrophages into toxin-induced muscle injury site to restore angiogenesis and muscle regeneration.[59] A neuropeptide, galanin, has also been identified to play an important role in facilitating bone marrow-derived MSC migration through activation of galanin receptor.[60]

### Protein delivery system

When bioactive molecules are administered into the injury site as a bolus injection, most of the factors tend to lose their biological activities because of enzymatic digestion in the body. To overcome this limitation and maintain effective concentrations of molecules in the local microenvironment, sustained release of bioactive cues can be accomplished by encapsulation within a scaffolding system through physical or chemical binding. Release pattern of the incorporated bioactive factors can be controlled by scaffold modifications through changing physical properties, temperature, pH and material degradability. In particular, a scaffolding system for *in situ* tissue regeneration needs to possess an appropriate microenvironment that is able to recruit host stem and progenitor cells into the implant and support the expansion and differentiation into a desired tissue type. For this purpose, multiple factors need to be delivered to a target site because of the complexity of the microenvironment. In one study, a multiple protein delivery system was developed for accelerating vascularization and functional tissue formation, based on the fact that functional regeneration of tissues and organs is typically induced by the action of a number of growth factors.[61] The investigators reported that a new polymeric system facilitated the tissue-specific delivery of two or more growth factors, and enabled sustained release of bioactive molecules with different release kinetics for effective tissue regeneration. Similarly, a recent study demonstrated various delivery methods of bioactive molecules for controlled release over time.[62] Sustained release of multiple molecules mimics *in vivo* tissue regeneration and it contributes to effective and functional tissue regeneration. Another study used a gelatin-based scaffold to deliver four different bioactive molecules, namely VEGF, angiopoietin-1, keratinocyte growth factor and platelet-derived growth factor-BB.[63] The delivery of these molecules induced an increase in angiogenesis with a potential for promoting tissue regeneration.[63] However, the current delivery systems are limited to local delivery via release from the implanted scaffold[62] that results in accumulation of migrated cells mostly at the periphery of the scaffold, delaying cell infiltration into the interior of the scaffold. Consequently, the unbalanced cell localization prevents successful tissue regeneration. It is evident that a new delivery approach that facilitates efficient recruitment of host stem cells needs to be developed.

Toward this goal, we have developed a new delivery method, in which combined systemic and local delivery of multiple factors (SP and SDF-1α) were used to enhance recruitment of host stem cells such as MSC and HSC populations from two different compartments (circulating bloods and resident sources) into target scaffolds *in vivo*.[64] This strategy (Figure 3) consists of two steps: (1) to increase mobilization of bone marrow-derived MSCs and HSCs by systemic SP injection and (2) to enhance recruitment of two different populations of the SP-stimulated bone marrow-derived cells and resident stem cells into the implanted scaffolds via local release of SDF-1α from the scaffolds. Our results showed that this combination delivery system significantly enhanced host stem cells such as $CD29^+$ $CD45^-$ MSC-like cells, $CD146^+$a-$SMA^+$ pericytes and c-$kit^+$

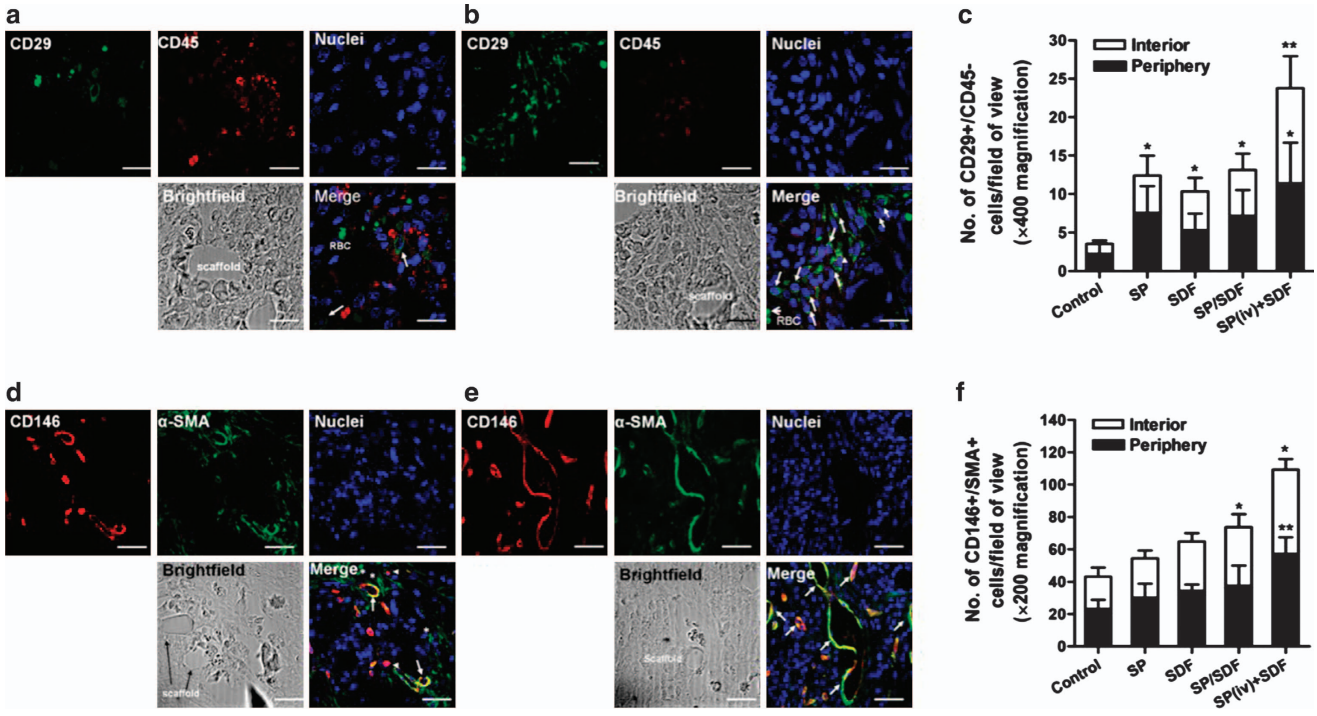


**Figure 3** Combination delivery system that uses systemic (a stem cell-stimulating factor, substance P (SP)) and local delivery (stem cell migrating factor, stromal cell-derived factor-1α (SDF-1α)); (**a**–**c**) $CD29^+CD45^-$ mesenchymal stem cell (MSC)-like cells and (**d**–**f**) $CD146^+$α-$SMA^+$ pericyte recruitment by the combination delivery system.[64] α-SMA, α-smooth muscle actin; RBC, red blood cells.

cell-included HSC population into the scaffolding system, indicating the effectiveness of the combination delivery system for *in situ* tissue regeneration.

## APPLICATIONS OF *IN SITU* TISSUE REGENERATION

The concept of *in situ* tissue regeneration has been translated into various therapeutic applications (Table 2). Various biomaterial scaffolds have been used for this purpose in the form of injection or implantation. Although many technologies are at the early stage in investigations, several technologies have been successfully performed in preclinical animal models and clinical applications with satisfactory outcomes.

### Bone

Osteogenic repair from bone loss has benefited from techniques of *in situ* tissue regeneration.[65] The required properties for a bone-specific scaffold are temporal and mechanical load bearing within the tissue defects. Moreover, it should minimize immune and/or inflammatory response. Biomaterials widely used for *in situ* bone regeneration include calcium phosphate, calcium sulfate and hydroxyapatite. As bone tissue is composed of these materials, it would be natural to consider using such materials for scaffolds for bone regeneration. This is because of their close chemical and crystal resemblance to the mineral phase of bone, demonstrating excellent biocompatibility and osteoconductivity.[66,67] The commonly used bioactive molecules for bone regeneration include bone morphogenetic protein-2, transforming growth factor-β, basic fibroblast growth factor and VEGF. In some cases, vital growth factors can be incorporated into the scaffolds to exert their osteoinductive and vascularization properties.[68–70] Biomaterials ranging from natural polymers, such as alginate, fibrin or gelatin, to synthetic polymers, such as poly(lactic acid) and PLGA, have been fabricated with either single or multiple bioactive molecules.[71] These scaffolds have demonstrated an ability to stimulate and induce neighboring bone marrow stromal cells and enhance bone tissue formation. Many clinical applications have been conducted for bone regeneration *in situ* using calcium phosphate cements, collagen gel or sponge combined with clinically approved bone morphogenetic protein-2.[72–74]

### Cartilage

Injured and damaged cartilage tissue can lead to severe arthritis because of low natural healing capability when compared with other types of tissues. In early studies, cartilage tissue constructs were easily produced by seeding chondrocytes onto scaffolds. However, when engineered cartilage constructs are implanted, a serious compatibility issue such as poor integration with the host tissue is often observed. As such, a recent study showed that successful cartilage regeneration can be achieved using a cell-free scaffolding system. In this study, scaffolds consisting of biodegradable PLGA polymer were incorporated with plasma and hyaluronic acid, and implanted into microfractured cartilage tissue.[75] Consequently, the implanted constructs facilitated the migration of bone marrow-derived stem cells that led to the formation of neo-cartilage tissue. More recently, Lee *et al.*[76] demonstrated that an entire articular surface of the synovial joint can be regenerated without cell transplantation using three-dimensional poly(ε-caprolactone) and hydroxyapatite composites fabricated by solid free-form technique. These scaffolds were incorporated with transforming growth factor-β3 and implanted into a rabbit model. Regeneration of new and avascular cartilage with vascularized subchondral bone tissue was evident. This result was shown to be effective in regenerating cartilage tissue by recruiting host stem cells to the site of the implants.[76]

### Skeletal muscle

Muscle tissue is the largest tissue mass in the body. Skeletal muscle accounts for ~45% of total body weight. Skeletal muscle tissue contains bundles of myofibers that function by contracting with motor nerve stimulation. Minor muscle injury because of exercise and weight lifting is easily restored by natural regenerative processes. However, if >20% of the muscle is lost, spontaneous recovery will not occur, leading to loss of muscle function. If the injury is not properly treated, skeletal muscle weakness and atrophy will occur.[77]

Cell-based approaches have offered new opportunities for restoring muscle function because of severe muscular injuries. Muscle satellite cells[78,79] have been identified as a cell source for muscle tissue regeneration owing to their self-renewal capabilities and muscle-specific differentiation following muscle injury. In addition to muscle satellite cells, several other stem cell populations, such as muscle-derived stem cells,[80] pericytes,[81] muscle-resident macrophages,[82,83] EPCs[53] and bone marrow-derived MSCs[84] have been used for muscle tissue engineering. The roles of these cell populations are critical for efficient muscle regeneration, by promoting angiogenesis and maturing neovasculatures, secreting myogenic trophic factors and modulating inflammation for reduced fibrosis.[82,85]

Several studies have been performed to regenerate muscle tissue *in situ*.[86] In one study, an alginate gel-based dual delivery system was used to deliver insulin-like growth factor-1 and VEGF for the enhancement of functional muscle regeneration. The roles of sustained release of insulin-like growth factor-1 and VEGF from the scaffold are mobilization and manipulation of satellite cells and inducing efficient angiogenesis for functional muscle regeneration, respectively. In another study, a collagen-based sponge scaffold was used to treat rabbit hind limb muscle injury. At 24 weeks after implantation, the control group (without scaffold) showed poor structural regeneration with severe scar tissue formation at the site of injury, whereas the scaffold-implanted group showed mild focal adhesions and new muscle tissue formation.[87] One important consideration for muscle regeneration *in situ* is how to create aligned and organized muscle fibers within the implanted scaffold. Alignment is critical for newly regenerated muscle fibers to exert normal physiological muscle function in response to

**Table 2 Recent therapeutic applications of *in situ* tissue regeneration**

| *Biomaterials* | *Bioactive factors* | *Experimental design* | *References* |
|---|---|---|---|
| *Bone* | | | |
| Alginate | BMP-2 | Rat muscle | 88 |
| Fibrin | Heparan sulfate | Rat cranial defect | 89 |
| Gelatin | FGF-2 | Mouse maxillae | 90 |
| Gelatin | BMP-2 | Rabbit ulnar bone | 91 |
| Fibrin/HAp | BMP-2 | Mouse calvarial bone defect | 92 |
| Alginate/chitosan | BMP-7/liposome | Rabbit libia defect | 93 |
| P(HEMA-VP) gel | FGF-2 | Rabbit femoral defect | 94 |
| PLGA microparticle | BMP-2 | Rat cranium defect | 71 |
| CPC | BMP-2 | Monkey spine | 72 |
| ACS | rhBMP-2 | Clinical anterior lumbar | 73 |
| Type I collagen | rhBMP-7/rh-OP1 | Clinical fibular defect (critical-sized defect) | 74 |
| *Brain* | | | |
| Injectable (Gtn-HPA) hydrogels and dextran sulfate/chitosan PCNs | SDF-1α | *In vitro* 3D culture of NPCs | 55 |
| *Cartilage* | | | |
| PGA | Autologous serum/HA/microfracture | Sheep full-thickness cartilage defect | 75,95 |
| Collagen | | Rabbit articular cartilage | 96 |
| PCL/HAp | TGF-β3 | Rabbit articular cartilage and bone defect | 76 |
| *Cardiovascular* | | | |
| PGA knitted fiber, PLA/PCL sponge and PCL filament | | Canine left pulmonary artery (long term, 12 months) | 97 |
| PEG gel | VEGF and HGF | Rat myocardial infarction | 98 |
| Electrospun PCL | RGD polypeptides | Rabbit carotid artery | 99 |
| Knitted polyester graft | Fibronectin/SDF-1α | Sheep carotid artery | 100 |
| Electrospun PCL | CAG peptides | Rat carotid arterial replacement | 101 |
| Decellularized heart valve | CD133 antibody immobilization | Sheep heart valve replacement | 102 |
| PGA/PLA/collagen | | Porcine descending aorta, porcine pulmonary arterial trunk, canine ventricular outflow tract | 103 |
| PGA/PLA/collagen | | Canine carotid arteries | 104 |
| Porcine SIS/collagen | | Rabbit arterial bypass model | 105 |
| PEUU | | Rat myocardial infarction model | 106,107 |
| Alginate | | Rat myocardial infarction model | 108 |
| *Esophagus* | | | |
| UBM | | Rat abdominal esophagus | 109 |
| Rat gastric acellular matrix | | Rat abdominal esophagus | 110 |
| *Skeletal muscle* | | | |
| Collagen | | Rabbit muscle (vastus lateralis) | 87 |
| Alginate gel | VEGF/IGF-1 | Mouse | 86 |
| *Skin* | | | |
| Chitosan | | Porcine burned skin | 111 |
| *Spine* | | | |
| PGA/HA | Blood serum | Rabbit disc defect | 112 |
| Stomach | | | |
| Collagen/PGA | | Canine stomach | 113 |
| *Tooth or periodontium* | | | |
| Collagen | FGF-2/gelatin microsphere | Canine periodontal | 114 |
| PLGA | GDF-5 | Canine periodontal | 115 |

Abbreviations: ACS, absorbable collagen sponge; BMP, bone morphogenic protein; CAG, cysteine–alanine–glycine; CPC, calcium phosphate cements; 3D, three-dimensional; FGF, fibroblast growth factor; GDF-5, growth differentiation factor-5; Gtn-HPA, gelatin-hydroxyphenylpropionic acid; HA, hyaluronic acid; HAp, hydroxyapatite; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; NPC, neural progenitor cell; OP, osteogenic protein; PCL/HAp, poly(ε-caprolatone)/hydroxyapatite; PCN, polyelectrolyte complex nanoparticle; PEG, poly(ethylene glycol); PEUU, polyester urethane urea; PGA, poly(glycolic acid); P(HEMA-VP), poly(hydroxylethylmethacrylate-4-vinyl pyridine); PLA, poly(lactic acid); PLGA, poly(lactide-*co*-glycolide); PP, polyprolene; RGD, arginine–glycine–aspartic acid; SDF-1α, stromal-derived factor-1α; SIS, small intestine submucosa; TGF-β3, transforming growth factor-β3; UBM, urinary bladder matrix; VEGF, vascular endothelial growth factor.

nerve stimulation. Recently, a scaffolding system consisting of unidirectionally aligned fibers was developed by electrospinning techniques using poly(ε-caprolactone) and collagen as base materials.[36] When skeletal muscle cells were seeded on the scaffolds, cellular alignment along the polymer fibers was evident with formation of organized muscle fibers upon differentiation. This result indicates that fabrication of muscle-specific scaffolds may be important for *in situ* muscle tissue regeneration.

## CONCLUSIONS

*In situ* tissue regeneration holds great potential to provide new therapeutic options for functional tissue regeneration. In order for this approach to be successful, stem cells need to be directed to the target sites, and appropriately guided to proliferate and differentiate into the cell type of interest within the microenvironment provided by biomaterial scaffolds. A variety of tissue-specific biomaterials and bioactive molecules have been identified and combined to promote stem and progenitor cell mobilization. As such, the concept of *in situ* tissue regeneration has been demonstrated in multiple tissue systems. However, continued development of effective tissue-specific scaffolding systems that provide powerful cues for stem cell activation and recruitment is needed in order to achieve functional tissue regeneration *in situ*. A better understanding of the complex interactions and pathways of the biomolecules that are involved in the targeted tissue regeneration is necessary in order to achieve effective therapeutic outcomes for translation into the clinic.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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