Osteogenic differentiation of adipose-derived stromal cells: Advancements and future directions for bone tissue engineering

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Received: October 26, 2015
Published online: January 04, 2016

Adipose-derived stromal cells (ASCs) present a promising cell source for tissue engineering applications. ASCs are attractive due to their abundance and ease of procurement through subcutaneous liposuction and subsequent isolation from lipoaspirates. ASCs are also preferable to more controversial stem cell sources due to ethical considerations and potential for teratoma formation. Recent approaches to promote osteogenesis have incorporated potent agents such as exogenous osteogenic growth factors, substrate-induced differentiation using mechanical properties of scaffolds, and genetic modification using viral vectors, RNAi, and nanotechnology. In this manuscript we review current literature on methods for the differentiation of ASCs for the purpose of improving bone tissue engineering.


Introduction

Bone fractures account for approximately 10 million of all clinical and emergency visits annually in the United States alone [1]. Although many of these injuries heal without the need for major intervention [2], an estimated 2.2 million cases worldwide require reconstruction using tissue transplants [3]. Autologous bone grafts from the patient’s own iliac crest remain the gold standard for treating skeletal defects [4], as they enhance osteogenesis, in which transplanted cells produce new one; facilitate osteoconduction, where the graft provides a trellis for migration of new tissue into the defect; and promote osteoinduction, in which proteins and cytokines found in the graft attract host mesenchymal stem cells and promote osteoblastic differentiation [5]. Although autologous grafts have had relatively successful clinical outcomes, nearly 10% of bone harvests are associated with major complications [6]. Limited supply and donor-site morbidity have also hindered the efficacy of autologous bone grafting [7]. Allogeneic bone grafts taken from other individuals provide an abundant source of donor tissue, but issues of immunorejection, graft-versus-host disease, and disease
transmission have also rendered this treatment less than ideal [1]. Artificial replacements such as metals have also shown inadequate host tissue integration, wear, and risk of infection [8]. Ceramics are often too brittle as well for replacements in regions of high stress or torsion [9]. Thus, an alternative means of treatment is necessary for effective skeletal defect reconstruction.

Tissue engineering, as defined by Langer and Vacanti over two decades ago, “is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function” [10]. To successfully develop tissue substitutes, studies have identified three crucial components that constitute the “triad” of tissue engineering: (1) cells to produce or replace lost tissue; (2) a biocompatible scaffold; and (3) tissue-inducing substances that will induce specific cell phenotypes [11]. Tissue-inducing substances are often cytokines, growth factors, or other bioactive materials that guide either transplanted or endogenous cells down a particular lineage for specific tissue differentiation [12-14], and scaffolds like hydrogels, meshes, and other polymeric matrices which provide structural support for tissue growth and a network for cell proliferation [15, 16]. Cell sources can range from fully differentiated populations to pluripotent stem cells depending on the desired application [17, 18]. Mesenchymal stromal cells (MSCs) have recently gained popularity in tissue engineering due to their multipotency and ease of harvest [19-21]. In this review, we highlight the use of adipose-derived stromal cells (ASCs) for bone tissue engineering and discuss various strategies to induce osteogenic differentiation.

**Adipose-derived stromal cells**

ASCs present a promising cell source for tissue engineering applications. Although there has been much ambiguity in the terminology used to describe these plastic-adherent, multipotent cells, the International Fat Applied Technology Society (IFATS) has accepted “adipose-derived stromal/stem cells” to define this population [22]. ASCs have become attractive due to their abundance and ease of procurement through subcutaneous liposuction and subsequent isolation from liposapirates [23, 24]. ASCs are also preferable to more controversial stem cell sources, such as embryonic or induced-pluripotent stem cells, as they do not carry ethical implications or risk of teratoma formation [25]. ASCs also have the capacity to differentiate along several lineages, including bone, fat, cartilage, tendon, and muscle [26-28]. Recent studies, however, have shown that ASCs consist of a variable, heterogeneous population, and cell sorting for specific surface markers may help for particular avenues of tissue differentiation and therapeutic applications [29].

Differentiation of these cells is often induced using osteogenic differentiation media (ODM), which consists of a basal media such as DMEM supplemented with various factors like fetal bovine serum (FBS), ascorbic acid, B-glycerophosphate, and dexamethasone [23, 29, 30]. Although ODM can be easily made from its constitutive ingredients or purchased from various companies (Life Technologies A10072-01, USA; Lonza PT-3002, USA), recent approaches to promote osteogenesis have incorporated more potent agents, such as exogenous treatment with osteogenic growth factors, substrate-induced differentiation using mechanical properties of scaffolds, and genetic modification using viral vectors, RNAi, and nanotechnology [11, 13, 31]. We herein review recent applications of these methods involving ASCs for the purpose of improving osteogenesis for tissue engineering.

**Growth factors**

Growth factors, traditionally a major workhorse for regenerative medicine, are proteins or steroidal hormones found in the body that can stimulate cell growth and guide differentiation. In the context of bone, Bone Morphogenetic Protein 2 (BMP-2), a member of the Transforming Growth Factor beta (TGF-ß) superfamily, has long been known to promote new bone formation [32]. Recent studies have applied this factor to ASCs both in vitro and in vivo to amplify osteogenesis using multi-modal approaches that fully incorporate the triad of cells, scaffolds, and tissue-inducing substances. Fan et al. reported the successful use of BMP-2 loaded onto an apatite-coated chitosan/chondroitin sulfate (CS) scaffold to promote healing of rat mandibular defects using noggin-suppressed mouse ASCs (mASCs) [33].

A multi-modal approach to bone tissue engineering confers synergistic effects. Lu et al. demonstrated that preconditioning with BMP-2 for three days enhanced osteogenesis in human ASCs (hASCs) cultured on a novel glass nanoparticle scaffold, which further promoted gene expression of osteogenic markers like RUNX2, COL1A, Osteopontin, and ALP [34, 35]. It was proposed that the short pretreatment period of hASCs with BMP-2 mimicked the transient physiological increase in BMP-2 levels following bone fracture and holds clinical value by minimizing risks associated with introducing exogenous growth factors into the body [36, 37].

Although BMP-2 has often been cited as a potent growth factor in bone regeneration, other members of the BMP family have recently received attention for their osteogenic effects. One group has demonstrated a synergistic
“cross-talk” between BMP-6 and vascular endothelial growth factor (VEGF). hASCs treated with BMP-6 and VEGF upregulated Osterix and COL1A expression in vitro [38]. Given that Osterix and COL1A have proven critical for osteoblast differentiation and proper bone formation, these findings highlight the efficacy of novel growth factor and cytokine combinations for optimal osteogenesis [39, 40].

By taking advantage of this protein “cocktail” approach, Tajima et al. showed that platelet-rich plasma (PRP) taken from peripheral blood dramatically improves healing in a rat calvarial defect model when used in combination with rat ASCs (rASCs) compared to either cell or PRP treatment alone [41]. PRP contains numerous factors necessary for cell proliferation, differentiation and wound healing, such as VEGF, TGF-β1, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) [42]. The accessibility of peripheral blood eliminates the need for recombinant protein synthesis and allows for autologous PRP applications in the clinical setting [43].

Substrates

While the use of growth factors on ASCs to induce osteogenesis has proven both potent and effective, there is a growing body of research devoted to harnessing the mechanical and topographical properties of cell substrates to direct lineage differentiation. A landmark study by Engler et al. discovered that stem cells are highly sensitive to the microenvironment surrounding them, and softer gels with lower elastic moduli (~0.1-1 kPa) induced neuronal commitment, whereas stiffer gels (~40 kPa) promoted osteogenic differentiation [44, 45]. Banks et al. recently illustrated the powerful influence of cell-substrate interactions using porcine ASCs (pASCs) on collagen-GAG scaffolds of varying stiffness loaded with or without BMP-2. The study found that after a certain threshold BMP-2’s effects became insignificant compared to those of scaffold stiffness [46].

This complex interaction between mechanical and chemical cues in scaffolds has sparked studies seeking a synergistic “sweet spot” for directed differentiation [47]. Nii et al. cultured hASCs on three-dimensional hydrogel scaffolds of varying stiffness loaded with different concentrations of fibronectin. The study found a non-linear relationship between the two factors with an ideal intermediate of both stiffness and fibronectin levels for enhanced osteogenesis [48]. This may have enormous implications for tissue engineering as future strategies begin mixing multiple decoupled factors.

Substrate stiffness has been of significant interest to tissue engineers for scaffold design and application, though studies have also demonstrated the potential for surface topography manipulation to further influence cell differentiation [49]. Xia et al. recently synthesized a ceramic scaffold with nanorod and microrod topographical properties to better mimic the native bone milieu surface, and rASCs seeded onto the scaffold demonstrated improved osteogenesis as well as angiogenesis both in vitro and in vivo in a rat calvarial defect [50].

Rather than synthesizing novel scaffolds to recapitulate the natural environment of tissue, an alternative approach utilizes cell-derived extracellular matrices (ECM) for tissue culture which contain all of the proteins and GAGs necessary for cell support [51]. In one study, researchers cultured bone marrow-derived MSCs and harvested their ECM (BM-ECM). They found that hASCs cultured in BM-ECM demonstrated upregulation of osteogenic markers and increased deposition of bone-specific ECM proteins/GAGs when implanted subcutaneously in immunodeficient mice in comparison to hASCs cultured on traditional culture plastic [52]. This study further supports the body of literature citing the origin-specific effects of ECM-derived scaffolds for differentiation, such as cartilage-derived ECM promoting chondrogenesis [53, 54].

Genetic modification

A more direct method of inducing osteogenic differentiation involves the modification of relevant genetic material within a cell, such as genomic alteration or translation inhibition, and has long been under scrutiny for stem cell and tissue engineering applications [55]. Advances in manipulation of gene expression ex vivo has allowed researchers to induce endogenous upregulation of specific growth factors and transcription factors, whereas high doses of exogenous recombinant factors were previously necessary for similar results [56].

Atluri et al. demonstrated the use of polyethylenimine (PEI), a cationic polymer-based transfection reagent, to create nanoplex-mediated delivery of fibroblast growth factor 2 (FGF-2) and BMP-2 plasmids into hASCs in vitro. They found that co-delivery of these plasmids improved osteogenic differentiation by measure of osteocalcin and Runx-2 gene expression than either plasmid alone [57]. The nanoplex approach holds clinical value as well due to reduced immunogenicity. Additionally, transfection ex vivo mitigates the toxic and systemic risks inherent to delivery of supraphysiologic levels of cytokines [58].

Non-viral transfection suffers from reduced efficiency of gene delivery. Liao et al. have reported the construction of
baculoviral vectors for BMP-2 and microRNA miR-148b that have both high transduction efficiencies and osteogenic differentiation capabilities in hASCs [59]. The baculovirus is particularly attractive as a transduction agent as it is nonpathogenic in mammalian cells and is therefore neither toxic nor able to replicate in humans [60]. hASCs treated with both BMP-2 and miR-148b demonstrated increased expression of osteogenic markers in vitro and nearly complete healing of calvarial defects in nude mice [59].

Rather than upregulating particular genes using recombinant DNA, alternative strategies have focused on gene inhibition through RNA interference (RNAi). TWIST1 is a transcription factor critical in regulating proper bone formation, in which mutations often lead to craniofacial deformities, as seen in Saethre-Chatzen syndrome [61]. Quarto et al. demonstrated that silencing of this gene using small hairpin RNA (shRNA) delivered via lentiviral particles in hASCs led to increased osteogenic differentiation by enhancing downstream osteoinductive pathways such as BMP [62].

Small interfering RNA (siRNA) is another RNAi approach to inhibit specific genes through non-viral transfection [63]. Schneider et al. delivered siRNA using Lipofectamine™ to silence chordin and noggin, antagonists of BMP-2, in hASCs to promote osteogenesis. A single treatment with siRNA was sufficient for enhanced osteogenic differentiation as evidenced by increased ALP activity and mineralization in vitro. This characteristic makes siRNA particularly effective as a transient treatment to allow for normal temporal bone growth after an initial phase of accelerated fracture repair [64].

Conclusions

As the elderly population increases and the number of annual bone fractures - already in the tens of millions - continues to rise, new methods for treatment and reconstruction will become imperative [65]. While autologous bone grafting, the current gold standard, has had clinical success, issues of donor site morbidity and limited autologous supply have left much to be desired for patients and physicians alike. Inherent risks associated with allogeneic transplants, such as disease transmission or immunogenic response, also limit the efficacy of this form of treatment. Inadequate osseointegration and inferior mechanical robustness have likewise hindered artificial bone replacements as an ideal remedy. Tissue engineering holds promise for bone reconstruction through skeletal regeneration and tissue replacement. ASCs in particular have enormous clinical potential due to their abundance, ease of autologous procurement, and ability to differentiate into a variety of adult tissues. Numerous studies have shown the efficacy of osteogenic differentiation of ASCs through genetic modification, substrate stiffness induction, and growth factor treatment for enhanced bone formation, but many additional steps are needed to bring this promising technology from the bench to the bedside. The potential negative effects of supraphysiologic cytokine levels and viral vectors in human subjects are still unknown and unpredictable. Further research and rigorous clinical trials, however, may redefine our “gold standard” for the treatment of skeletal defects through regenerative tissue engineering.

Conflicting Interests

The authors have declared that no conflict of interests exist.

References


