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REVIEW

Review of biophysical factors affecting osteogenic differentiation of human adult adipose-derived stem cells

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Abstract Developing bone is subject to the control of a broad variety of influences in vivo. For bone repair applications, in vitro osteogenic assays are routinely used to test the responses of bone-forming cells to drugs, hormones, and biomaterials. Results of these assays are used to predict the behavior of bone-forming cells in vivo. Stem cell research has shown promise for enhancing bone repair. In vitro osteogenic assays to test the bone-forming response of stem cells typically use chemical solutions. Stem cell in vitro osteogenic assays often neglect important biophysical cues, such as the forces associated with regular weight-bearing exercise, which promote bone formation. Incorporating more biophysical cues that promote bone formation would improve in vitro osteogenic assays for stem cells. Improved in vitro osteogenic stimulation opens opportunities for "preconditioning" cells to differentiate towards the desired lineage. In this review, we explore the role of select biophysical factors—growth surfaces, tensile strain, fluid flow and electromagnetic stimulation—in promoting osteogenic differentiation of stem cells from human adipose. Emphasis is placed on the potential for physical microenvironment manipulation to translate tissue engineering and stem cell research into widespread clinical usage.

Keywords Human stem cells · Adipose-derived mesenchymal stem cells (ASCs) · Differentiation · Osteogenesis · Bone tissue engineering · Biophysical signals

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Introduction

Stem cells derived from adult human tissue attract considerable attention due to the potential for stem cell therapies. Bone tissue engineering stem cell therapies harness the regenerative power of stem cells to replace or assist healing of damaged bone. Adipose tissue-derived mesenchymal stem cells (ASCs) are under increasing clinical investigation as they are easier to isolate and culture than bone-marrow derived mesenchymal stem cells (BMSCs) (Mesimäki et al. 2009; Lendeckel et al. 2004). To confirm stem cell functional ability, in vitro osteogenic assays stimulate and evaluate bone cell-like responses. These include intracellular calcium increase, osteogenic gene upregulation, and calcium deposition. The ability of cells to promote therapeutic bone formation is different from, but linked to, the ability to produce these bone cell-like responses. In general, cells will be transplanted in stem cell form on some scaffold. The in vivo environment surrounding the implanted cell–scaffold construct will affect the subsequent development of the stem cells. The ideal scheme for bone tissue engineering with stem cells involves an in vitro environment that enables prediction and control of stem cell behavior in vivo after transplantation. The aim of this review is to analyze recent publications on the role of biophysical factors that drive osteogenic differentiation of human ASCs in vitro. Earlier reviews are summarized in Table 1. The diversity of these reviews highlights the need for a meta-review to integrate information from a broad range of sources and build a coherent description of the biological basis for osteogenic responses. Practical limitations may lead stem cell researchers to supplement standard osteogenic induction with only one form of biophysical stimulus. So, it is important to understand the effects of each biophysical stimulus separately. We describe findings for each type of osteogenic stimulus in two categories: (1) substrate stimulus applied through manipulation of substrate on which

Table 1 Topics covered by previous reviews

Authors	Title	Aim
Bodle et al. (2011)	Adipose-derived stem cells in functional bone tissue engineering: lessons from bone mechanobiology	To summarize the current knowledge of mechanotransduction in ASC lineage specification and how this information has been used in bone tissue engineering with ASC
Levi and Longaker (2011)	Concise review: adipose-derived stromal cells for skeletal regenerative medicine	To define ASCs, to describe the isolation procedure of ASCs, to review the basic biology of their osteogenic differentiation, discuss cell types and scaffolds available for bone tissue engineering, and to explore imaging of ASCs and their potential future in human skeletal tissue engineering efforts
Liu ety al. (2010)	Mechanisms for osteogenic differentiation of human mesenchymal stem cells induced by fluid shear stress	To briefly review how hMSCs respond to fluid flow stimuli and focus on the signal molecules involved in this mechanotransduction
McCullen et al. (2010a)	Musculoskeletal mechanobiology: interpretation by external force and engineered substratum	To outline many of the main mechanotransduction mechanisms known to date, and describe recent literature examining effects of both external forces and cell-substrate interactions on musculoskeletal cells
Scherberich et al. (2010)	Adipose tissue-derived progenitors for engineering osteogenic and vasculogenic grafts	To summarize studies supporting development of vasculogenic/ osteogenic grafts generated from adipose- derived cells
Teo et al. (2010)	Nanotopography/mechanical induction of stem cell differentiation	To discuss the effect of physical nanotopography on stem cell differentiation and the current theories on the topography induction of stem cell differentiation and the nuclear integration of biophysical signals

ASCs adipose-derived stem or stromal cells, *hMSCs* human mesenchymal stem cells

cells are seeded; and (2) active stimulus involving continuous application of strain, fluid shear, or electromagnetic fields. Factors that influence differentiation are summarized in Fig. 1. For each type of stimulus, we sketch a model that can describe processes underlying osteogenic induction (illustrated in Figs. 2, 3, 4 and 5, below). Next, we consider differences in strategies for both promoting and evaluating osteogenic differentiation so as to resolve apparent conflicts in the different studies. Finally, we explore hypotheses on the molecular mechanism, the cellular processes that produce a response,

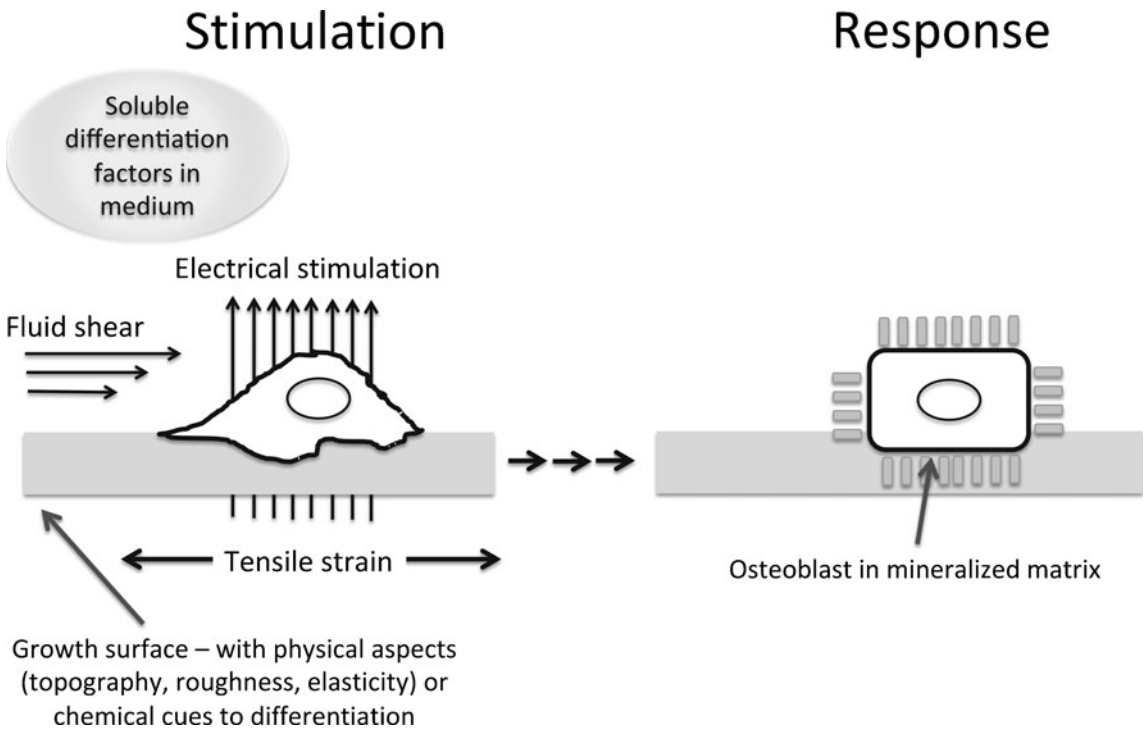

Fig. 1 A schematic illustrating the factors influencing differentiation of human stem cells reviewed in this article

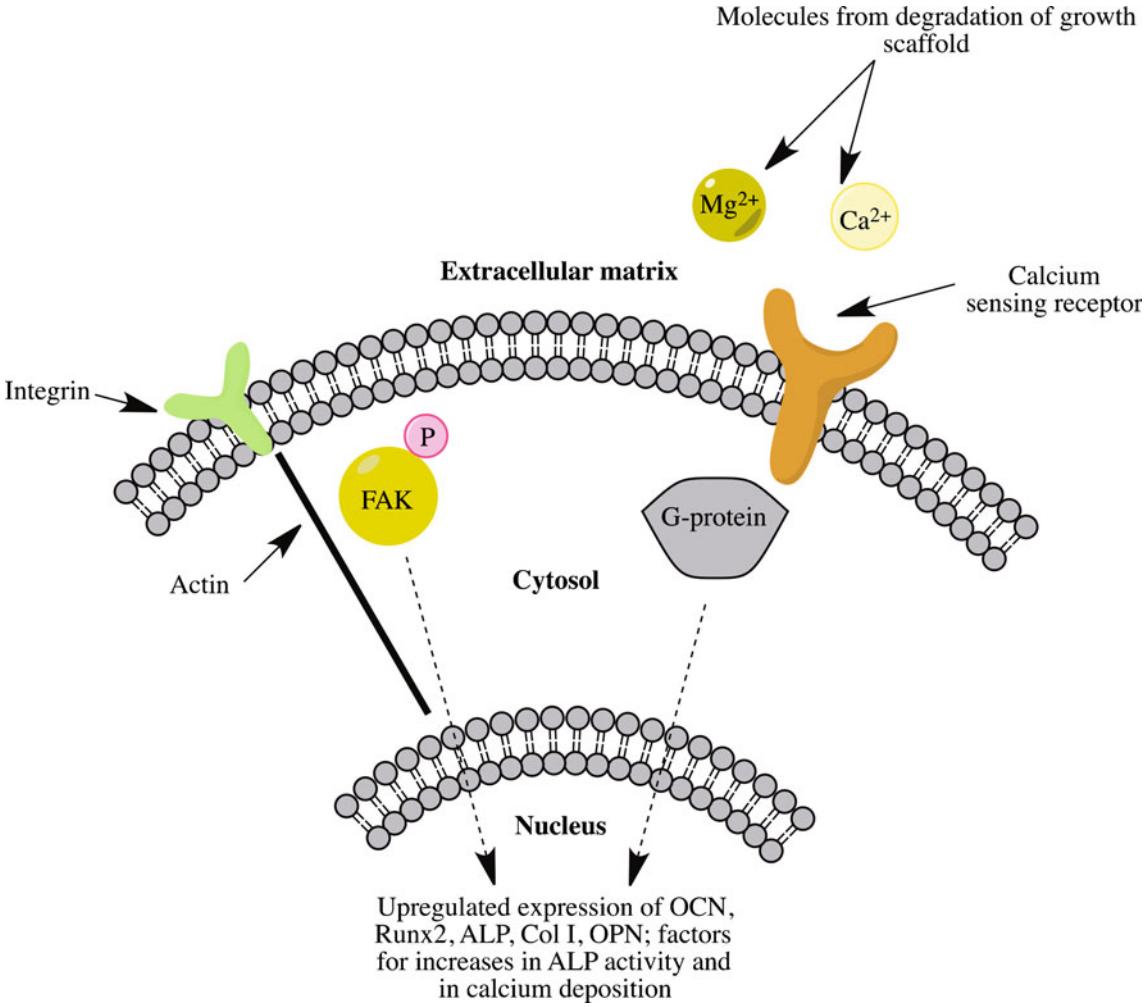

Fig. 2 A schematic summarizing hypotheses of substrate as a regulator of osteogenesis in ASCs. Two proposed mechanisms for scaffold-induced osteogenesis: (1) altered behavior of focal adhesions, a mechanism relying on direct connections between ECM and nucleus through FAK activation; and (2) an indirect mechanism, stimulation by scaffold ions entering through, e.g., a calcium receptor

and how knowledge of this mechanism can be applied to "programming" ASCs in bone tissue engineering.

Substrate stimulus

Most cells are anchorage-dependent, so the nature of the growth surface, substrate or scaffold, always plays a key role in influencing cell behavior. The ideal scaffold for bone engineering must first have good osteoconductivity; i.e., it must support the attachment, survival and appropriate distribution of the osteogenic cells. An ideal scaffold should also have good osteogenic ability, i.e., the scaffold should be able to promote the formation of new bone by cells that attach to it. The chemical composition and material properties of scaffolds have an important influence on their osteoconductivity and osteogenic ability. Substrate stimulus is manipulation of growth surface properties to produce a specific reaction. Table 2 highlights studies that have used substrate stimulus for driving ASCs osteogenic differentiation. These studies describe scaffold composition, pore size and porosity as key variables in scaffold-stimulated bone generation using ASCs.

The chemical composition of a scaffold influences its degradability and may regulate bone remodeling. Bone remodeling is the natural process of reshaping bone in which new bone is formed and mature bone is absorbed (Hao et al. 2010). The degradation rate of scaffolds used in bone healing should be high enough to yield the formation of new bone, but slow enough to provide support until new bone formation is complete. "Bioceramics" such as tricalcium phosphate (TCP) and hydroxyapatite (HA) have a mineral structure that mimics bone and have been applied to clinical use since the 1980s (Calori et al. 2011; Nandi et al. 2010). Bioceramics have mechanical properties similar to bone, but their brittleness can be problematic (Kim et al. 2011). Polymeric materials commonly used for bone tissue engineering, including poly (ε-caprolactone) (PCL) and

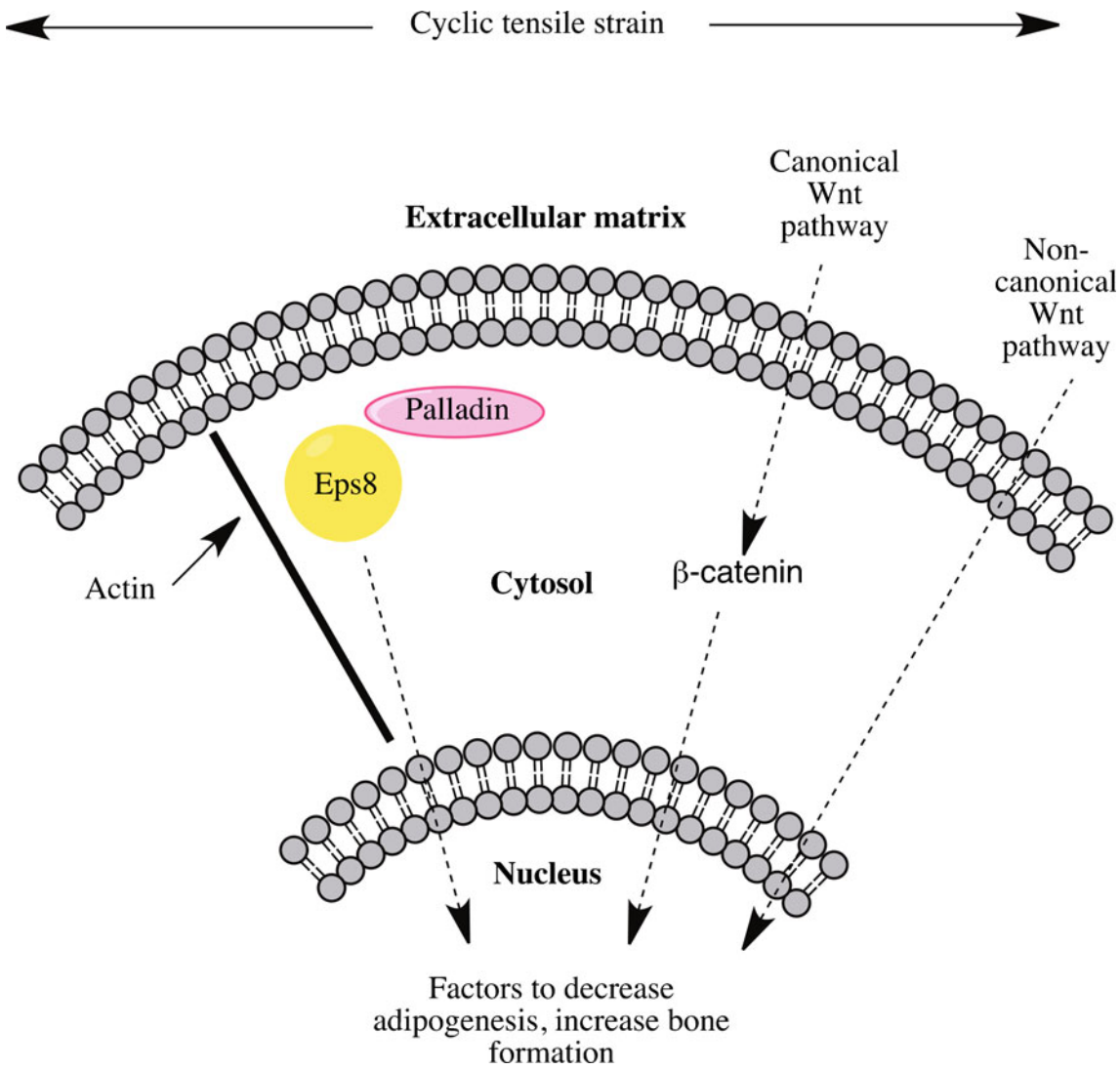

Fig. 3 A schematic summarizing hypotheses of the regulation of mechanical stretching-mediated induction of bone formation using ASCs. Three proposed mechanisms for osteogenesis in strain experiments: (1) altered actin interaction based on elasticity, (2) altered actin interaction based on strain-upregulation of the protein palladin, and (3) stretching inhibition of pparγ signaling through Wnt pathways. Notably, disrupting actin does not disrupt strain induction, supporting theories of osteogenic mechanisms independent of cytoskeleton

poly(glycolic acid) (PGA), have predictable and reproducible properties and can be less brittle than ceramics (Kim et al. 2011; Burg and Kellam 2000). Another advantage is that polymers may be delicately shaped to present cues for osteogenesis on the nanometer scale (Reed et al. 2009; Sefcik et al. 2008; Teo et al. 2010). But, polymeric materials may present biocompatibility problems (Hao et al. 2010). Naturally derived polymers such as collagen have the advantage of biocompatibility, but may be too weak mechanically for general bone tissue engineering (Kim et al. 2011). Composite materials attempt to take advantage of the strengths of multiple materials. For instance, a gel can be used as a "cell carrier", filling large holes in a rigid scaffold, and providing a soft support for cells in vulnerable first stages, but maintaining a large space for bone formation in later stages (Hao et al. 2010; Liu et al. 2008). The growth surfaces of neighboring cells may influence ASC osteogenesis indirectly through paracrine signaling (Lu et al. 2011). A wide variety of scaffolds have been investigated for in vitro bone tissue engineering from human ASCs, including: the bioceramic, bioactive glass (Haimi et al. 2009); the polymeric material, poly lactide-co-glycolide (PLG) (He et al. 2010); a composite of poly(ι-lactic acid); and TCP (McCullen et al. 2010b). Other scaffold materials have included a form of TCP called β-TCP, a new bioceramic called akermanite and collagen I, shaped into a honeycomb structure.

Studies including the investigation of atelocollagen honeycomb scaffolds for ASC osteogenesis report enhancement of osteogenesis with osteogenic medium, but less osteogenesis

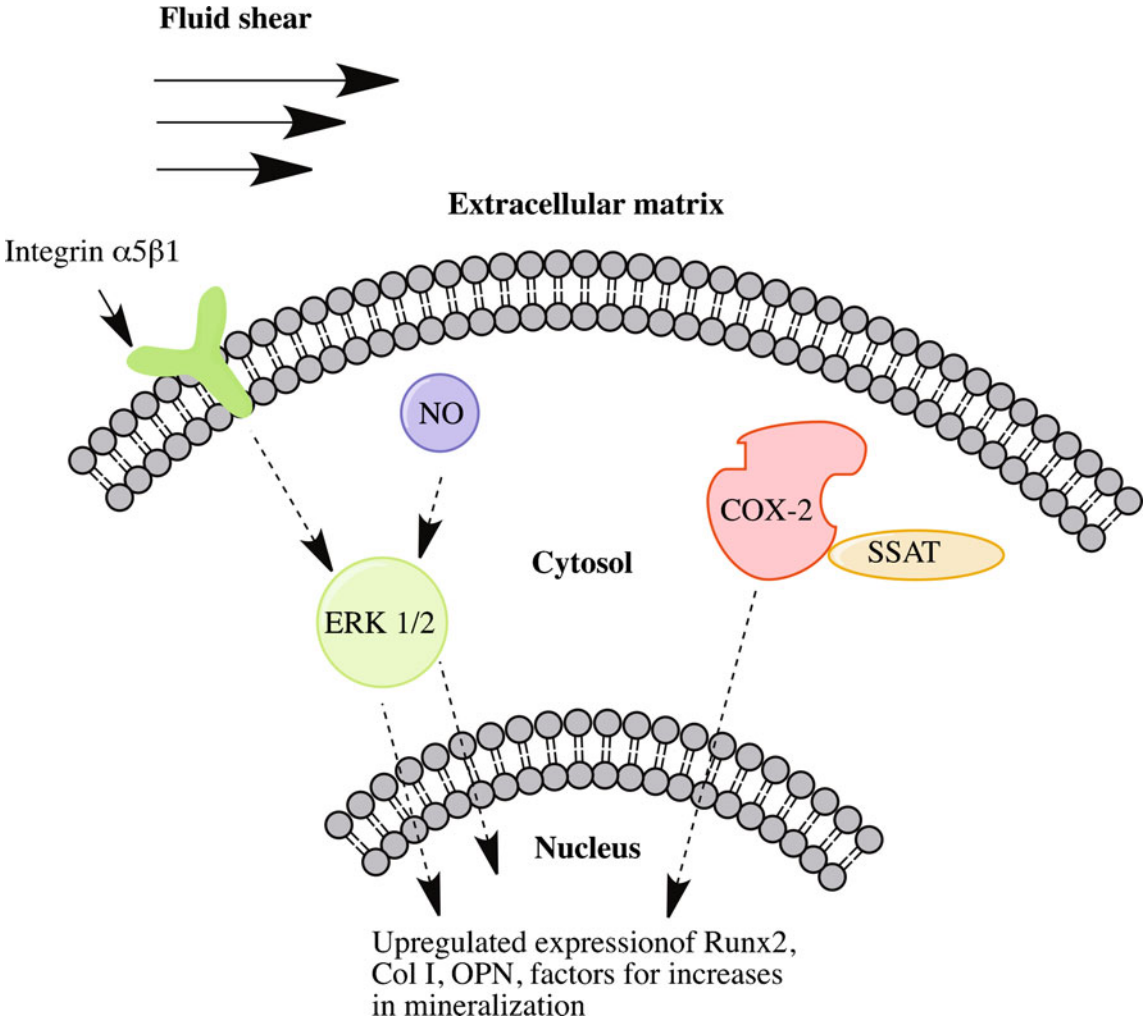

Fig. 4 A model of fluid shear stress-induced osteogenesis of adipose-derived stem cells. Mechanical loading increases ERK1/2 activation, NO production and expression of COX-2, Runx2, integrin α5β1, and the polyamine-regulating SSAT. NO and Cox-2 are reported to be involved in bone mechanical adaptation. NO and integrin α5β1 participate in ERK 1/2 activation

enhancement as compared with β-TCP scaffold. Two groups used in vitro and in vivo assays for studies using atelocollagen scaffolds for bone engineering with ASCs. Hattori and colleagues characterized osteogenic capacity in vitro by measurement of secreted osteocalcin and by SEM evaluation of cells filling scaffold holes after 4 and 8 weeks (Hattori et al. 2006). For evaluation of in vivo osteogenesis, an immunohistochemistry assay was performed to identify osteocalcin in histological sections after 2 weeks culture in osteogenic medium followed by 8 weeks subcutaneous implantation in mice. To gauge osteogenic capacity in vitro, Kakudo and colleagues assessed mineralization and alkaline phosphatase activity at day 14 after induction, also measuring calcification with scanning electron microscopy (Kakudo et al. 2008). This group used qRT-PCR to verify increase in expression of osteogenic marker transcription factor Runx2 with respect to control at days 7 and 14 after induction. For evaluation of in vivo osteogenesis, they implanted cell-scaffold constructs into mice subcutaneously after 14 days culture in osteogenic medium or control growth medium. After 8 weeks, cell–scaffold constructs were excised and tested with Von Kossa and immunostaining for osteocalcin. Hattori and colleagues describe better osteoconductivity on a β-TCP scaffold for ASCs, as compared with scaffolds of hydroxyapatite (HA) and atelocollagen honeycomb scaffolds (Hattori et al. 2006). Kakudo and colleagues showed better osteogenesis in cells cultured with osteogenic chemical supplements as compared to control growth medium (Kakudo et al. 2008). Taken together, the results of these studies suggest that β-TCP is a better osteogenic scaffold material for ASCs than HA and that atelocollagen scaffolds hold promise for ASC bone engineering applications where their low mechanical strength is acceptable.

Results of a study by Liu and colleagues showed better osteogenic differentiation with a scaffold of the newly designed bioceramic akermanite as compared with β-TCP

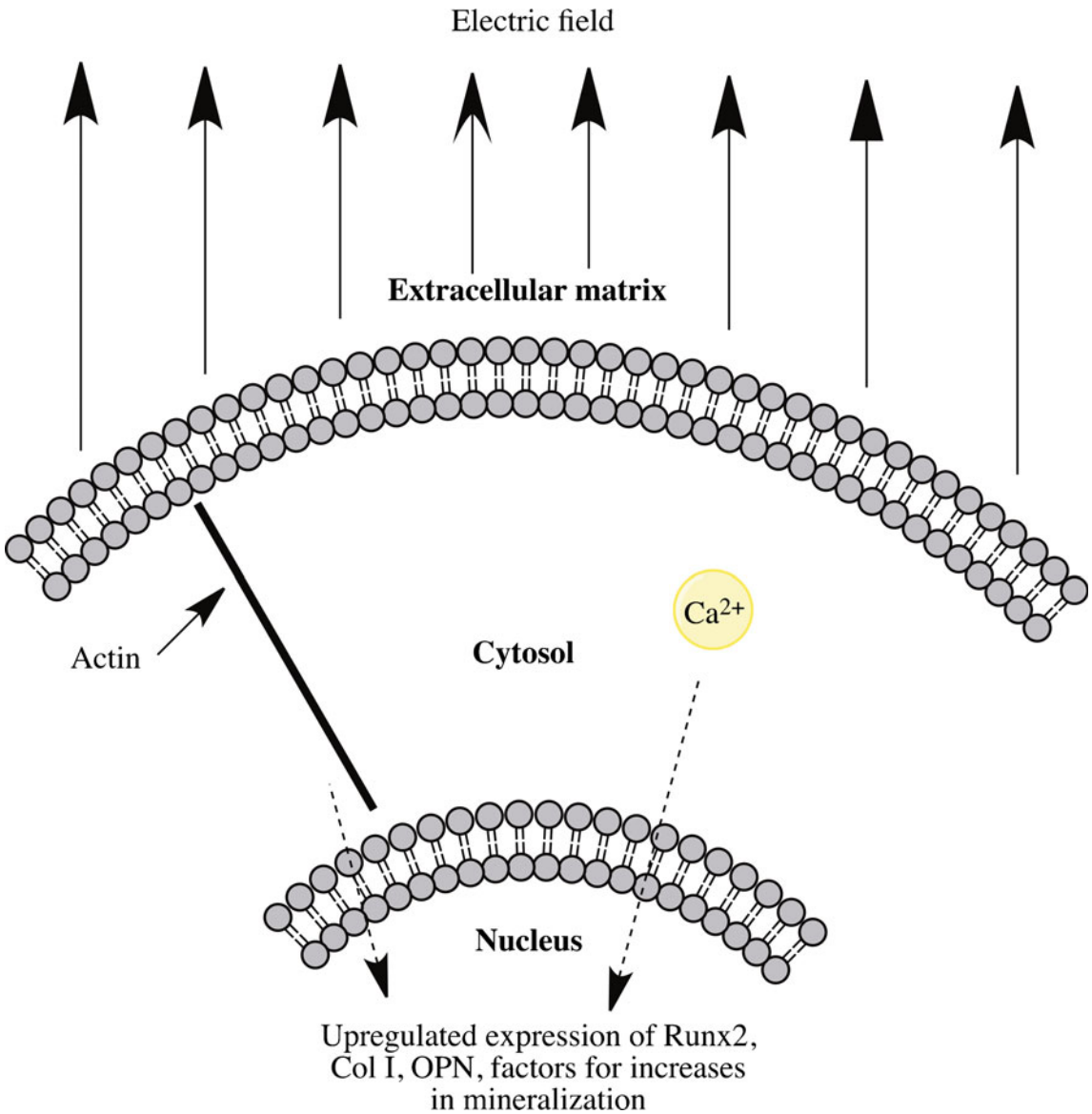

Fig. 5 This schematic illustrates mechanisms proposed for stimulation of ASCs to bone phenotype using application of electrical fields. Electrical stimulation is associated with increased calcium (that can activate ERK 1/2) and remodeling of the actin cytoskeleton. A mechanism based on actin remodeling via the protein ROCK was suspected based on similarity between response to electrical stimulation and response to flow stimulation by bone marrow derived mesenchymal stem cells. However, interrupting the action of ROCK on actin did not inhibit electrically stimulated osteogenesis. A possible explanation is alternate pathways of electrically inducing osteogenesis involving calcium signaling

scaffold (Liu et al. 2008). This group assessed osteogenic capacity by measuring osteocalcin deposition and by real-time PCR analysis for expression of osteogenic marker genes alkaline phosphatase (ALP) and osteocalcin (OCN). Surprisingly, osteogenic differentiation was observed in growth medium even without the addition of chemical osteogenic factors. This result indicates the substrate itself has the capacity to induce osteogenic differentiation. However, the examination of differentiation continued for only 10 days after induction, while differentiation is usually assessed at 28 days to indicate stability (Hattori et al. 2006; Marino et al. 2010). Following this work, Marino and colleagues showed that β-TCP scaffold drives ASCs to osteogenic differentiation that is stable even after 28 days. The level of osteogenesis was evaluated by alkaline phosphatase activity and an ELISA method quantification of expression of the osteogenic markers OCN and osteopontin (OPN). β-TCP and akermanite are examples of new, designed bioceramics that have the demonstrated ability to induce osteogenesis in ASCs without chemical supplements.

Substrate stimulus studies promote the concept of using scaffolds composed of designed, tunable bioceramics

Table 2 Studies reviewed using substrate stimulus

Authors	Key parameters				Suggested mechanism	Conclusion
	Mechanical osteoinductive component	Soluble osteoinductive component	Plating density	Assessment of osteogenesis		
Hattori et al. (2006)	Scaffold composition: β-TCP disks Scaffold size: 11 mm diameter, 2 mm thick Pore size: 100–400 μm pores Porosity: average void volume 75 %	Growth medium: DMEM, 15 % FBS, used with: Osteogenic factors: 50 μM ascorbate-2-phosphate,10 mM β-glycerophosphate,0.1 μM dexamethasone	2×10^5 cells/scaffold	In vitro: ALP activity; EIA measure of OCN secretion; SEM imaging of cells penetrating scaffold after 4 weeks and 8 weeks In vivo: OCN staining after 2 weeks chemical induction and 8 weeks mouse subcutaneous implantation	None	AT-MSCs, as BM-MSCs acquire a more osteogenic phenotype in vitro when grown on β-TCP scaffold than when grown on plastic
Kakudo et al. (2008)	Scaffold composition: honeycomb collagen Scaffold size: 5 mm diameter, 3 mm thick circles Pore size: 100–200 μm pores	Growth medium: DMEM, 10 % FBS, used with: Osteogenic factors: 82 μg/mL ascorbate-2-phosphate, 10 mM β-glycerophosphate, 10 nM dexamethasone	1×10^6 cells/scaffold	In vitro: ALP activity; Alizarin Red staining; qRT-PCR for Runx2; immunostaining for OCN; SEM observation after 14 days	None	Significant positive stains of sample in osteogenic medium on honeycomb collagen scaffold suggest this is a useful scaffold
Liu et al. (2008)	Scaffold composition: akermanite or β-TCP disks Scaffold size: 10 mm diameter, 0.8 mm thick	Growth medium: DMEM, 10 % FBS. Alone or used with: Osteogenic factors: 50 μg/ml ascorbic acid-2-phosphate, 10 mM β-glycerophosphate, 0.1 M dexamethasone	2×10^4 cells/cm^2	In vitro: ALP activity; qRT-PCR for ColI, ALP, Runx2, OCN. Elisa for OCN, after up to 10 days	Response to ions from the ceramics	Osteogenic differentiation of AT-MSCs could be enhanced on akermanite compared with β-TCP when culture extended to ~10 days, even without differentiation media
Marino et al. (2010)	Scaffold composition: β-TCP dishes Scaffold size: 10 mm diameter, 2 mm thick Pore size: 200–500 μm pores Porosity: 82 % average void volume	Growth medium: DMEM-Ham's F12, 5 % FBS. Alone or used with: Osteogenic factors: 50 mg/ml sodium 2-phosphate ascorbate,10 mM β-glycerophosphate, 20 nM dexamethasone	5×10^3 cells/cm^2	In vitro: ALP activity; EIA kit for OCN and OPN, up to 28 days	Altered behavior of focal adhesions on calcium phosphate surfaces	AT-MSC acquire sustained osteogenic phenotype when grown on β-TCP without differentiation media

AT-MSC adipose tissue-derived mesenchymal stem cells, *BM-MSCs* bone marrow-derived mesenchymal stem cells, *EIA* enzyme immunoassay, *OCN* osteocalcin, *SEM* scanning electron microscopy

capable of inducing osteogenesis in growth medium without chemical induction factors. With regard to pore size and porosity, 100–500 μm pore diameter and about 80 % average void volume were proposed as appropriate scaffold material parameters to optimize generation of bone (Marino et al. 2010; Hao et al. 2010). 3D TCP scaffold was reported to promote osteogenesis better than 2D TCP scaffolds of the same composition (Marino et al. 2010). To further define optimal scaffold composition and material parameters, more data is required on the relative advantages of composite and 3D scaffolds, seeding efficiency, and bone healing when using scaffolds with a range of pore sizes, porosity and nanoscale topography.

Active stimulus

As classically described in Wolff's law, the bone of the skeleton regularly adapts to the mechanical forces imposed by usage (Frost 1990). Shear stress is imposed on bone cells during exercise (such as walking). Cycles of loading and unloading cause the compression and relaxation of the extracellular matrix (ECM), which in turn impose strain on cells in bone and lead to pulses of fluid flow through bone. The circulatory system also produces pulsatile or oscillating fluid flow acting on bone cells, imposing shear stress. Studies (including microgravity experiments) have demonstrated that mechanical forces are important for maintaining bone (Bodle et al. 2011). Clinically, tensile strain is used for bone engineering in distraction osteogenesis, a surgical procedure used to grow bone by creating a fracture between two bone segments, then moving the segments slowly apart from each other. Electromagnetic stimulation is under investigation for therapeutic stimulation of bone repair (McCullen et al. 2010b; Rose and Bryan-Frankson 2008; Giardino et al. 2009; Tepper et al. 2005). Active stimulus of bone formation, using continuous application of strain, fluid shear or electromagnetic energy, is increasingly popular to supplement standard in vitro induction of osteogenesis with soluble small molecules. Tables 3, 4, and 5 (below) highlight studies on the application of strain, fluid shear stress, and electromagnetic fields to stimulate osteogenesis. These studies describe strain magnitude, nature of fluid flow (pulsatile vs. continuous) and frequency of electrical stimulation as key variables in actively stimulated bone healing using ASCs. These studies also importantly highlight differences in physiological responses of BMSCs and ASCs.

Tensile strain

Studies using tensile strain found strain magnitude was related to inhibition of adipogenesis, a process balanced against osteogenesis, while insertion of interruptions (rests) in strain application showed no significant effect. Table 3 shows an overview of studies using tensile strain stimulus for osteogenic induction of ASCs. One study compared effects of uniaxial, continuous and interrupted strain for 4 h/day for up to 14 days (Hanson et al. 2009). Similarly enhanced osteogenesis was observed with both types of strain compared with static culture, judging by measurement of calcium deposition. Huang and colleagues applied equibiaxial cyclic tensile strain to mouse ASCs. They reported the application of strain significantly reduced age-related increase in adipogenesis in a magnitude-dependent manner, as assessed by formation of oil droplets and expression of the adipogenic marker Bglap1 (Huang et al. 2010). Importantly, lack of sensitivity to interruptions of applied strain distinguishes ASCs from BMSCs (Hanson et al. 2009); strain modification of age-related increase in adipogenesis has important implications for osteogenesis since these processes may balance each other (Luu et al. 2009; Tokuzawa et al. 2010).

Fluid flow

Application of continuous flow and pulsating fluid flow (PFF) were each reported to increase osteogenic differentiation of ASCs as compared with static culture; best osteogenic induction was seen with PFF. Table 4 overviews studies using flow stimulus of osteogenesis in ASCs. In a study by Tjabringa and colleagues, human ASCs were seeded on poly (L-lysine) hydrobromide glass slides, cultured overnight, and then subjected to 1 h of PFF (Tjabringa et al. 2006). Parameters were chosen according to reports suggesting that shear stress on the order of 0.8–3 Pa influences bone remodeling in vivo (Rubin et al. 2006). Tjabringa and colleagues stated that 3 h after PFF application, gene expression of Runx2 was increased with respect to static control. However, expression of OPN was not increased at this timepoint. Increased Runx2 expression is an indicator of early osteogenesis and OPN expression is an indicator of late osteogenesis. So, the results of this study suggest that PFF may result in enhanced early stage, but not late stage, osteogenic differentiation. The study also monitored production of NO and expression of Cox-2, factors important in the response of bone cells to fluid shear. NO production was reported to have significantly increased after 60 min of PFF treatment. Cox-2 was significantly increased in cultures subject to PFF for 3 or 6 h. For comparison with other work in this field, assessment of osteogenic capacity at later time points would be helpful. Fröhlich and colleagues, using continuous flow to promote ASC osteogenesis in 3D bone matrix, assessed indicators of osteogenic capacity at a later time point to allow for osteogenic maturity. Assessment of osteogenesis was performed after 2 and 5 weeks of culture, by histology and immunohistochemistry for bone-cell-type

Table 3 Studies reviewed using cyclic tensile strain stimulus

Authors	Key parameters					Suggested mechanism	Conclusion
	Mechanical osteoinductive component	Soluble osteoinductive component	Plating density	Substrate	Assessment of osteogenesis		
Wall et al. (2007)	Amplitude: 10 % cyclic uniaxial tensile strain (Tissue Train Flexcell Strain Unit, FX4000) Frequency: 1 Hz Duration: 4 h/day for 14 days	Growth medium: α-MEM, 10 % FBS. Alone or with: Osteogenic factors: 50 μM ascorbic acid, 10 mM β-glycerophosphate, 0.1 μM dexamethasone	5×10^3–1×10^4 cells cm^2, grown to 100 % confluency before osteogenic induction or 60,000 cells/200 μl gel/ 35 mm well	3D culture in collagen I gel or 2D culture, on Tissue Train collagen I coated six-well culture plates	In vitro: Alizarin Red Staining after 14 days in osteogenic medium	Strain upregulated palladin that stabilizes a protein Eps8 that in turn affects actin organization,which has been shown to influence differentiation	Palladin expression is upregulated with chemically induced osteogenesis and tensile strain
Hanson et al. (2009)	Amplitude: 10 % cyclic uniaxial tensile strain (Flexcell Tension Plus system) Frequency: 1 Hz Duration: 4 h/day for 14 days Variation: 10s rest inserted after each 1 s strain cycle	Growth medium: α-MEM, 10 % FBS, used with: Osteogenic factors: 50 μM ascorbic acid, 10 mM, β-glycerophosphate, 0.1 μM dexamethasone	5.2×10^3 cells/ cm^2 grown to 100 % confluency (~5 days) before osteogenic induction	2D culture on collagen-I coated Bioflex culture plates (plate stiffness ~1.6 GPa, contrasted with 2.6 GPa for standard polystyrene)	In vitro: quantification of calcium accretion up to 14 days after induction	Explaining response to substrate elasticity - nonmuscle myosin II isoforms of focal adhesions enabling cells to feel their microenvironment through actin-myosin contractions	Tensile strain enhances osteogenic differentiation
Huang et al. (2010)	Amplitude: 0.5 %, 2 % or 10 % cyclic equibiaxial tensile strain applied as sinusoidal wave (Flexcell 4000 Ttensile system) Frequency: 0.5 Hz Duration: 48 h	Growth medium: α-MEM, 10 % FCS, used with: Osteogenic factors: 0.05 mM L-ascorbic acid-2-phosphate, 10 mM sodium β-glycerophosphate, 100 nM dexamethasone	2.8×10^4 cells/ cm^2, cultured for 16 h after plating before application of strain	2D culture on collagen I-coated flexible-bottom plate (Flexwell)	In vitro: Alizarin Red or Von Kossa Staining after up to 21 days	Mechanical stretching inhibits PPARγsignaling through Wnt pathways	Proliferative capacity is reduced with age, but restored with tensile strain stimulation

Table 4 Studies reviewed using flow stimulus

Authors	Key parameters					Suggested mechanism	Conclusion
	Mechanical osteoinductive component	Soluble osteoinductive component	Plating density	Substrate	Assessment of osteogenesis		
Tjabringa et al. (2006)	Pulsating fluid flow, 5-Hz pulse Mean shear stress: 0.6 Pa Pulse amplitude: 0.3 Pa Peak shear stress: 8.4 Pa/s	Growth medium: DMEM with 2 % FBS Osteogenic factors: Spermine at 1 or 10 μM (acting as inhibitor of flow induced bone-like response)	1.2×10^4 cells/cm^2	Polylysine-coated glass	In vitro: measurement of NO production; real-time PCR for expression of COX-1 and COX-2, runx2 and osteopontin within several hours	SSAT, involved with regulation of polyamine catabolism, is sensitive to PFF. polyamines were found to decrease PFF-induced NO production and COX-2 gene expression in human AT-MSCs → polymines are involved in AT-MSC response to mechanical loading. Polyamine increased expression of SSAT and a PMF-1, which regulates SSAT - >feedback control. MAPK extracellular signal-regulated kinase (ERK)1/2 also implicated as having a role.	Polyamines mediate mechanically induced upregulation of NO activity and *cox-2* gene expression
Fröhlich et al. (2010)	Continuous flow at a rate of 1.8 mL/min (corresponding to interstitial flow velocity of 400 μm/s through the scaffolds)	Growth medium: DMEM, 10 % FBS, with Osteogenic factors: 0.1 mM ascorbic acid-2-phosphate,10 mM sodium-β-glycerophosphate, 100 nM dexamethasone. Control growth medium was supplemented with 1 ng/mL basic fibroblast growth factor	5×10^3 cells/cm^2	3D culture in decellularized bone matrix scaffold	In vitro: immunostaining forCol I, BSP and osteopontin; SEM detection of mineralization after up to 5 weeks	Improved distribution and nutrient-wasteexchanges with perfusion	Perfusion with osteogenic medium enhances expression of bone specific markers

Table 5 Studies reviewed using electrical stimulus

Authors	Key parameters					Suggested mechanism	Conclusion
	Mechanical osteoinductive component	Soluble osteoinductive component	Plating density	Substrate	Assessment of osteogenesis		
Hammerick et al. (2010)	DC electric field Frequency: 50 Hz Amplitude: 6 V/cm peak-to-peak Duration: 6 h/day for 7 and 21 days	Growth medium: DMEM, 10 % FBS, with Osteogenic factors: 100 mg/mL ascorbic acid, 10 mM β-glycerophosphate	10^4 cells/cm^2	Tissue culture plastic	In vitro: qRT-PCR for expression of Alp, Opn, Col I, Runx2 and osteoclacin. Alp activity, intracellular calcium imaging after up to 21 days post-induction	One hint for mechanism lies in the similarity between osteoblasts in response to the oscillatory fluid flow and mASCs in response to the electric fields, both show features including an elicited calcium response and upregulation in Runx2 and OPN (citing a study by You et al, 2001). Rho GTPases, ROCK, cAMP mentioned, voltage-gated ion channel ;) ROS considered but not found significantly changed	DC field exposure increases cytoskeletal tension and, independently, stimulates osteogenesis
McCullen et al. (2010b)	AC electric fieldFrequency: 1 HzAmplitude: 1, 3, and 5 V/cmDuration: 4 h/day for 14 days	Growth medium: α-MEM, 10 % FBS, 2 mM L-glutamine with Osteogenic factors: 50 μM ascorbic acid, 10 mM β-glycerophosphate,0.1 μM dexamethasone	2×10^5 cells/cm^2	Glass slides	In vitro: Alizarin Red staining after up to 14 days	hASCs respond to electrical stimulation by increasing intracellular calcium (from intracellular stores, or opening ion channels); actin cytoskeleton rearrangement making cells more like mature osteoblasts	AC field exposure increases calcium accretion

markers collagen I (Col I), bone sialoprotein (BSP), and OPN. Osteogenesis was additionally evaluated using SEM and microcomputed tomography to image cell distribution and formation of mineralized matrix (Fröhlich et al. 2010). Pulsating fluid flow was shown to induce bone cell-like response from ASCs and constant flow was shown to promote osteogenesis through better distribution of seeded cells and enhanced exchange of nutrients in three-dimensional scaffolds.

Electromagnetic stimulation

Application of both direct current (DC) and alternating current (AC) were observed to enhance osteogenesis in ASCs. Table 5 summarizes parameters of studies using electromagnetic fields to induce ASCs towards bone formation. Hammerick and colleagues explained choice of frequency citing commercial therapeutic stimulation systems that have shown osteogenic effects with frequencies between 2 and 123 Hz (Hammerick et al. 2010). Application of electromagnetic field resulted in significantly increased osteogenic capacity as indicated by alkaline phosphatase activity and expression of markers ALP, OPN, Col I, and Runx2 at 21 days following induction. The cytoskeleton, the network of protein within cells that provide them structure, showed increased tension with application of the electric field (Hammerick et al. 2010). Loboa and colleagues applied AC electric fields to human ASCs (McCullen et al. 2010b). Choice of electric parameters was explained by reference to previous work and observations made in preliminary experiments. Application of AC field improved osteogenesis as estimated by increased intracellular calcium and Alizarin Red S staining up to 14 days after induction. A range of electromagnetic stimulation has been shown effective in improving osteogenic stimulation of ASCs; understanding ASC response to electromagnetic stimulation informs better tests of osteogenesis in vitro and opens possibilities for driving ASCs to therapeutic bone formation in clinical application.

Active signaling in the form of continuously applied tensile strain, fluid flow, and electromagnetic fields were shown to promote osteogenic phenotype in ASCs in vitro when used in combination with a chemical stimulus. Application of tensile strain, shear stress, and electromagnetic field application are all options added to the repertoire of methods of enhancing osteogenesis of ASCs in vitro for bone tissue engineering. These methods can improve accuracy of in vitro assays used to predict ASCs performance in bone healing. They may also be used to better prepare ASCs to function well in vivo. In the case of electromagnetic fields, which can be applied externally (Lendeckel et al. 2004; Mesimäki et al. 2009), in vitro optimization of parameters can inform choice of parameters for post-implantation therapy. Shear stress imposed by fluid flow for several hours stimulated bone cell-like response from ASCs. Tensile strain and electromagnetic field stimuli were applied for several hours per day. Substrate-stimulated osteogenesis was assessed by standard stem cell in vitro differentiation assays which require a couple of weeks of culture to generate results. The short period of stimulus required to achieve a bone-like response from ASCs is a potential advantage for active over substrate stimulus. However, as contrasted with substrate-induced osteogenesis, actively induced osteogenesis was consistently produced in combination with a traditional soluble chemical stimulus. The only exception was the study of Tjabringa and colleagues, and this looked only at short-term response (Tjabringa et al. 2006). Another point for improvement in the active signaling studies is that these studies had not been extended to examine in vivo bone formation, and a couple tested cell lines from only two donors despite reported significant variability between donors. To further define optimal active stimulus parameters, more data are required on varied parameters of active stimulus; for instance, duration of stimulus, whether the strain is applied along one axis (uniaxial) or two (biaxial), magnitude of shear stress, and interaction of different active stimuli and scaffolds that are osteoinductive.

Consideration of apparent conflicts in findings

The effect of mechanical stimulus on cell behavior is affected by intrinsic properties of the cell and properties of the ECM as well as extrinsic forces acting on the cell (Chou et al. 2009; Dulgar-Tulloch et al. 2009; Jaalouk and Lammerding 2009; McCullen et al. 2010b). Even when considering strategies for the rather specific purpose of bone engineering with cells derived from human fat, there are several parameters for influencing the osteogenic differentiation; for instance, media formulation (Lindroos et al. 2010; Lund et al. 2009), oxygen tension (He et al. 2010), harvest location, and sex of donor (Aksu et al. 2008) have all been reported to have an effect on the osteogenic potential of ASCs. The method of isolation (Bodle et al. 2011), choice of chemical osteoinduction formula (Kroeze et al. 2011), and method chosen for characterizing the osteogenic response may help to explain the observed difficulty developing a unified view point. Because of the dynamic nature of the osteogenic process, timing of assessment of osteogenic differentiation is an important factor. Certain conditions of osteogenesis may lead to slow osteogenic maturation, encouraging researchers to investigate early markers such as alkaline phosphatase activity rather than later ones such as Alizarin Red S and von Kossa staining for mineralization. Even the same factors in different contexts may have different effects; for example, one study reports a spectrum of opposite effects of Wnt signals depending level of differentiation

(Quarto et al. 2010). Communication between cells can modify the osteogenic response (Lu et al. 2011). Finally, osteogenic indicators of in vitro assays, such as NO production, are not equivalent to in vivo bone healing, but only signs of potential for such healing. Practical limitations restrict the number of variables examined in every experiment. The most important issue here is to select the appropriate model or models—simple enough to control and understand, but with sufficient complexity to address the experimental question—then build on results. Studies highlight differences in osteogenic response to biophysical stimuli related to choice of species and cell type, and describe some conditions in which such differences could be reduced.

Animal models used during medical research should react to a treatment in a way that resembles human physiology; with regard to the response to biophysical stimuli, notable variation is seen amongst different species. In this review, all but two studies used human ASCs. A study by Huang and colleagues, using mouse ASCs, was included because it uniquely uses ASCs in a disease-correlated state (age-related reduction of proliferation and differentiation ability) (Huang et al. 2010). Hammerick and colleagues also used mouse ASCs. This study was included as providing unique mechanistic insight into the electrical stimulation of osteogenesis (Hammerick et al. 2010). In general, the comparison of stem cell behavior between species is not straightforward. For instance, goat stem cells have been observed to take more time for mineralization after chemical osteogenic induction than human stem cells (Zandieh-Doulabi B, personal communication). Tjabringa and colleagues (Tjabringa et al. 2006) highlighted species differences in their group's study of PFF-induced osteogenesis: in goat adipose-derived stem cells, only cells stimulated towards osteogenesis showed bone-cell like response to PFF. However, human ASCs responded to PFF even without chemical stimulation towards osteoblast phenotype. NO production was delayed in human ASCs with respect to goat ASCs; human cells required 60 min of PFF stimulation for NO production while goat cells produced NO after only 5 min PFF. The nature of chemical and mechanical stimuli required for osteogenic response, and timing of this response, has been shown to vary amongst ASCs from different species, confounding the use of animal models for human ASCs physiology.

Response to mechanical stimuli varies even between BMSCs and ASCs from the same species. One notable difference among studies involves the role of actin cytoskeleton rearrangement. Mechanically induced osteogenesis stimulated actin cytoskeleton rearrangement in both human ASCs and BMSCs. However, the role of actin rearrangement is different. Actin rearrangement was necessary for flow and elasticity-determined osteogenesis in studies of BMSCs (Engler et al. 2006; Arnsdorf et al. 2009a, b). By contrast, disruption of actin cytoskeleton rearrangement did not affect osteogenesis in ASCs associated with biophysical stimulus (Hammerick et al. 2010; Wall et al. 2007). Understanding of osteogenesis in BMSCs cannot be directly used to predict response in ASCs.

Even human ASCs from different donors vary in sensitivity to stimuli and osteogenic capacity, but such variation can be reduced. McCullen et al., observing enhanced osteogenesis and intracellular calcium activity in ASCs stimulated with AC fields, found notable differences amongst cell lines in terms of calcium signal increases. These researchers reported a trend correlating higher plating densities with more consistent cellular response (McCullen et al. 2010b). Hanson and colleagues observed less variation in mineralization by two cell lines when cells were grown on flexplates necessary for strain application. This effect was attributed to the influence of substrate elasticity (Hanson et al. 2009). Inter-individual variation in osteogenic response was observed, but higher cell density and more elastic substrate were noted to reduce this variation.

Differences in ASCs osteogenic response due to cell source, cell type, and osteogenic strategy are an important consideration for bone engineering, as are potential methods for reducing these differences. One potential strategy for handling the differences in ASC osteogenic response is to tune the individual osteogenic stimuli. Understanding the individual effects of different stimuli is difficult, since a cell is always subject to one influence in the presence of others. For instance, applied strain may modify elastic modulus of cell substrate (McCullen et al. 2010b; Joshi and Webb 2008). For best comparison of results from studies in different laboratories, researchers should establish some standard induction schemes that can be logically adapted to suit individual needs (Kroeze et al. 2011). There is a need for a multiparametric and integrative approach with the help of assessments of in vitro osteogenic phenotype at the molecular level (increased expression of Runx2 mRNA, etc.), bone-like response to mechanical stimulus (e.g., NO production), and in vivo therapeutic bone formation. Each method provides the best results in different situations; there are methods that work better with a specific method of osteogenic induction or at a given time point after osteogenic induction. Some assessment methods show results instantly while others require weeks for availability of more definitive results. What understanding is gained from these measurements of osteogenesis will rely on our ability to integrate results across different biological levels and build logical models that can describe the biological basis for osteogenic response.

Hypotheses of mechanism

The growing promise of clinical application for stem cells, especially ASCs (Gonzalez-Rey et al. 2010; Ra et al. 2011;

Casteilla et al. 2011; Lendeckel et al. 2004; Mesimäki et al. 2009), motivates an interest to explain cellular processes underlying lineage commitment. A better understanding of how these ASCs respond to various conditions will allow better prediction and control in bone tissue engineering applications. One interesting issue is the integration of diverse signals to generate a coordinated response. At some level within the cell, the signals from different stimuli overlap. Stem cell "reprogramming," that is, overriding one differentiation signal with another, has been demonstrated (Engler et al. 2006; Takahashi and Yamanaka 2006). Studies of overlap in osteogenic signaling pathways open opportunities for bone engineering "reprogramming," enhancing a desired signal or overriding a signal that is undesired, for instance as the effect of disease.

The role of the connection between ECM and nucleus in generating regulation from structural changes: integrins, focal adhesions and actin cytoskeleton

Scaffolds affect osteogenesis by altering adhesion, which in turn may alter gene function directly through the cytoskeleton, or through indirect mechanisms such as G-proteins or ion channels. More reports in literature are finding significant roles for the cytoskeleton in differentiation of stem cells (Engler et al. 2006; McBeath et al. 2004). Complicating interpretation of cytoskeletal changes is the fact that, in addition to transmitting signals from the extracellular environment, the cytoskeleton and associated factors are also tools the cell uses to react to its environment (McCullen et al. 2010a). For instance, an increase in actin stress fiber formation and assembly is a typical reaction to application of tensile strain (Jaalouk and Lammerding, 2009) or increased matrix stiffness (Wells and Discher 2008). However, a stem cell experiencing osteogenesis stimulated by chemicals will achieve the mechanical properties of a bone cell by changing the arrangement of its actin from thick bundles crossing the cytoplasm to a thin meshwork filling the cell (Titushkin and Cho 2007). In hypotheses of direct mechanisms for ASC osteogenesis induced by biophysical factors, adhesion interaction is seen as an important first step, with downstream possibilities less defined.

Biophysical factors can have a basic role in enhancing osteogenesis by improving adhesion of osteogenic cells. Fröhlich and colleagues tested flow as osteogenic stimulus with a decellularized bone scaffold. With osteogenic medium, they found enhanced expression of bone specific markers in perfusion culture as compared with static culture (Fröhlich et al. 2010). With perfusion, the three proteins used as osteogenic markers were uniformly distributed through the construct. In static culture they were only at the outer regions. So, improved osteogenesis was attributed to better distribution of cells and nutrients with flow, rather than to scaffold stimulus or specifically flow-induced osteogenesis. Marino and colleagues saw increased phosphorylation of focal adhesion kinase (FAK) at tyrosine 397 in scaffold-induced osteogenesis. This activation was interpreted as a sign of healthy adhesion; the authors also noted the importance of FAK activation as a tool for interpreting extracellular signals (Marino et al. 2010). Importantly, FAK phosphorylation at tryosine 397 has previously been shown to play a role in mechanically stimulated differentiation (Teo et al. 2010; Rubin et al. 2006). This hypothesis of substrate-induced ASC osteogenesis is illustrated in Fig. 2. One prospectively interesting study would be to disrupt this FAK activation to investigate whether scaffold-induced osteogenesis occurs independently.

Other factors may contribute to stimulating ASC osteogenesis through direct connections linking ECM and nucleus. For instance, treatments or surface modifications may be used to alter growth surfaces so that they favor attachment of these osteogenic cells, contributing to bone formation. ECM also presents chemical cues, because a lot of growth factors are stored in ECM. Additionally, nanoscale topographical features in growth substrates influence stem cell behavior (Teo et al. 2010). Finally, elasticity has a plausible effect on osteogenesis of ASC (Hanson et al. 2009; Engler et al. 2006; McCullen et al. 2010b; Joshi and Webb 2008).

Indirect and chemical mechanisms: receptors, ion channels, G-proteins

Indirect mechanisms may transmit mechanical signals to the nucleus where gene regulation is accomplished. Indirect mechanisms include calcium signaling, G-proteins, polyamines, NO, and Cox-2.

Stimulation by scaffold ions may drive scaffold-induced osteogenesis in ASCs, as illustrated in Fig. 2. In the case of β-TCP, phosphate and calcium ions are released, while akermanite dissolves into silicon, calcium and magnesium ions. Calcium ions could enter a cell through a calcium receptor interacting with a G protein. Calcium ions have been shown to stimulate proliferation of osteoblasts, and magnesium ions have been associated with increased mineralization (Liu et al. 2008). McCullen and colleagues also showed ionic calcium enhanced mineralization by human ASCs (McCullen et al. 2010a).

Strain-induced ASC osteogenesis likely occurs by indirect mechanisms. Mechanistic studies reported osteogenesis induced by stimulation occurred independently of associated cytoskeletal changes (Wall et al. 2007; Huang et al. 2010). In a study by Wall and colleagues, results showed upregulation of palladin, an actin-associated protein, was correlated with osteogenesis induced by cyclic strain and osteogenic medium. However, inhibiting palladin expression decreased formation of actin stress fibers without

interfering with osteogenesis as assessed by Alizarin Red S staining. So, some indirect mechanism, independent of actin cytoskeleton, must be responsible for strain-induced osteogenesis. A schematic of this mechanistic study is included in Fig. 3. This figure illustrates an alternate pathway for strain-induced osteogenesis proposed by Huang and colleagues. This group hypothesized that strain increases bone-to-adipose ratio via Wnt pathways.

Electrically stimulated ASC osteogenesis likely also occurs by indirect mechanisms. McCullen and colleagues observed higher intracellular calcium activity along with enhanced osteogenesis in ASCs stimulated with AC fields (McCullen et al. 2010b). These researchers suggest application of an electromagnetic field may serve to enhance chemically induced osteogenesis through affecting the cytoskeleton, a mechanism illustrated in Fig. 5. Hammerick and colleagues employed an electromagnetic and chemical stimulus of osteogenesis of ASCs cultured on a plastic substrate. Like McCullen and colleagues, they found electromagnetic stimulation affected actin cytoskeleton and significantly enhanced osteogenesis (Hammerick et al. 2010). However, when these researchers inhibited rho-associated protein kinase (ROCK), significantly decreasing cytoskeletal tension, osteogenic markers OPN, Col I and Runx2 were upregulated even more with electromagnetic stimulation as compared to static control (Hammerick et al. 2010). This result suggests that electrically induced ASC osteogenesis is correlated with, but not dependent upon actin rearrangement. This group investigated the possibility that their electrically induced gene expression changes were effected through oxidative stress but found no evidence of this. Figure 5 indicates a proposed alternative mechanism, intracellular calcium stimulating osteogenesis. Higher intracellular calcium activity may influence mechanically induced osteogenesis through a different pathway, for instance one involving PKC and ERK 1/2 implicated fluid shear stress osteogenic activation (Liu et al. 2010). Another possible mechanism involves voltage-gated channels.

The process of flow stimulation of ASC osteogenesis is explained by an indirect mechanism involving polyamines, the enzyme Cox-2 and NO. A study by Tjabringa and colleagues showed PFF led to increased gene expression of spermidine/spermine N (1)-acetyltransferase (SSAT), an enzyme associated with polyamine activity (Tjabringa et al. 2006). These researchers showed that 30-min exposure to polyamine spermine inhibited flow-induced NO-production and Cox-2 expression. The results of this experiment imply polyamines play a role in modulating flow-induced ASC osteogenesis. Tjabringa and colleagues suggest an ERK 1/2 pathway may be involved, downstream of NO production, in PFF activation of ASCs osteogenesis. This proposed mechanism is illustrated in Fig. 4. An alternate proposed pathway also shown here was highlighted in a review by Liu et al. (2010). Integrin$\alpha5\beta1$, upregulated in ASCs with applied fluid shear stress, has been separately identified as important in promoting osteogenesis through ERK 1/2 activation (Liu et al. 2010). A prospective interesting follow-up study would involve investigation of ERK 1/2 activation. If ERK 1/2 activation was observed with PFF, this follow-up study should block this activation to examine whether FSS induced ASC osteogenesis still proceeds.

Several hypotheses of indirect mechanisms for biophysical osteogenic stimuli were not directly investigated in the studies reviewed, and a few examples are listed here. ASCs secrete osteoinductive growth factors, which may contribute to bone formation by recruiting host bone-forming cells or stimulating angiogenesis when implanted in vivo (Hao et al. 2010; Scherberich et al. 2010). Stretch-activated cation channels interpret mechanical strain in osteoblasts (Kearney et al. 2010). One hypothesis is that forces acting on cells change protein conformation, exposing binding sites in a functionally relevant way (Teo et al. 2010). Paracrine signaling from osteoblasts grown on films with nano-scale hydroxyapatite were able to induce osteogenesis in co-cultured ASCs through a pathway involving BMP2 (Lu et al. 2011). Actin filaments linked to ECM by integrins are connected to the nucleus at the other end. Elements of cytoskeleton bridging actin fibers to the nuclear membrane, including lamin proteins, also have an important role in translating biophysical stimuli of osteogenesis (Teo et al. 2010).

Pathways common and distinct to different modes of osteogenic stimulus, other cell types – options for 'reprogramming'

The overarching goal of ASCs bone engineering research is to prepare these cells in vitro to support bone healing in vivo, not to make perfect bone in vitro. For example, bioceramics are mechanically similar to bone, but in clinical application of ASCs their osteoinductive ability has been of greater use than their strength (Mesimäki et al. 2009). This review has elaborated on how substrates, electromagnetic fields, strain, and fluid flow can be applied in vitro to ASCs for bone engineering research. What happens after implantation is implant-extrinsic, except stimuli of substrate used to carry transplanted cells and electromagnetic stimuli that may be delivered after implantation (Tepper et al. 2005). Biophysical forces applied in vitro are one of many types of stimuli contributing to the regulation of ASCs osteogenesis in vivo. Studies show biophysical stimuli used for ASCs osteogenesis activated multiple pathways; researchers disrupted one actin cytoskeleton effect associated with osteogenesis, and were surprised to observe ASC osteogenesis proceed uninterrupted (Wall et al. 2007; Hammerick et al. 2010).

Informed manipulation of biophysical osteogenic induction might be used for ASCs osteogenesis "reprogramming,"

that is, override one differentiation signal with another to promote a desired outcome in an in vivo environment not naturally suited for that outcome. This has previously been accomplished in other contexts (Engler et al. 2006; Takahashi and Yamanaka 2006). Stem cell reprogramming should act through a "hub," a point of convergence for a number of signaling pathways. Osteogenic transcription factor Runx2 is a hub through which induction of osteoblast differentiation may be regulated to maintain proper function of the cell, tissue, and organism (Franceschi and Xiao 2003). ERK is another hub activated by mechanical stimuli; ERK activation is important in determining osteoblast survival, proliferation, and differentiation (Jessop et al. 2002). As ASC osteogenesis is sensitive to multiple influences and activated by multiple pathways, focusing on individual genes or pathways may prove too narrow for comprehensive understanding of effects relevant to this process. Biological network analysis, particularly incorporating influences of and response of hubs Runx2 and ERK, will allow integration of literature to better characterize contexts under which ASCs will generate an osteogenic response to a given combination of stimuli. Furthermore, biological network analysis will inform hypotheses on different ways engineered ASCs may better support bone healing, e.g., through enhancement of processes such as angiogenesis (Shoji et al. 2010).

Concluding remarks

Manipulation of the physical microenvironment of ASCs, using stimulus from substrate or applied strain, flow, or electromagnetic fields, expands the repertoire of tools available for bone tissue engineering with these cells, increasing possibilities for adaptation to clinical translation. This review examined some examples of how physical features and chemical properties of surfaces, tensile strain, fluid flow, and electromagnetic stimulation affect osteogenic differentiation of human ASCs. For best understanding and control of ASC osteogenesis stimulated by biophysical factors, we should first support development of appropriate consensus protocols. As scientific publication relies on novelty, adopting standard protocols may be slow, so standards should first define guidelines for analyzing data and defining end phenotypes, then protocols for isolation and osteogenic stimulation may be standardized. Next, we should support the development of models that integrate information across different studies to provide a cohesive explanation of the cellular processes underlying ASC osteogenesis. Finally, we should continue directed exploration of previously unconsidered influences on ASC osteogenesis, such as non-soluble chemicals, nanotopography, and elasticity in growth substrates. We should also consider explanations for unique mechanistic phenomena in ASC osteogenesis, for instance, defining the role of the Wnt pathways proposed as an explanation for the fact that flow- and electrically-stimulated ASC osteogenesis are independent of actin rearrangement. A better understanding of ASC osteogenesis as stimulated by biophysical factors illuminates a path for better general understanding of stem cell differentiation by biophysical factors. Recently, growth surfaces have been shown to support high survival rate of mesenchymal stem cells for more than 10 days without refrigeration (Gorodetsky et al. 2011). Artificial stem cell niches generated by modulating physical microenvironment could even soon replace soluble factors for induction of differentiation, making it easier for tissue engineering and stem cell biology to be controlled in the resource-limited settings of developing countries, away from expensive laboratories and stringent good manufacturing practice.

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References

Aksu AE, Rubin JP, Dudas JR, Marra KG (2008) Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Ann Plast Surg 60(3):306–322

Arnsdorf EJ, Tummala P, Jacobs CR (2009a) Non-canonical Wnt signaling and N-cadherin related beta-catenin signaling play a role in mechanically induced osteogenic cell fate. PLoS One 4 (4):e5388

Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR (2009b) Mechanically induced osteogenic differentiation–the role of RhoA, ROCKII and cytoskeletal dynamics. J Cell Sci 122(Pt 4):546–553

Bodle JC, Hanson AD, Loboa EG (2011) Adipose-derived stem cells in functional bone tissue engineering: lessons from bone mechanobiology. Tissue Eng Part B Rev 17(3):195–211

Burg KJPS, Kellam JF (2000) Biomaterial developments for bone tissue engineering. Biomaterials 21(23):2347–2359

Calori GM, Mazza E, Colombo M, Ripamonti C (2011) The use of bone-graft substitutes in large bone defects: any specific needs? Injury 42(Suppl 2):S56–S63

Casteilla L, Planat-Benard V, Laharrague P, Cousin B (2011) Adipose-derived stromal cells: their identity and uses in clinical trials, an update. World J Stem Cells 3(4):25–33

Chou SY, Cheng CM, LeDuc PR (2009) Composite polymer systems with control of local substrate elasticity and their effect on cytoskeletal and morphological characteristics of adherent cells. Biomaterials 30(18):3136–3142

Dulgar-Tulloch AJ, Bizios R, Siegel RW (2009) Human mesenchymal stem cell adhesion and proliferation in response to ceramic chemistry and nanoscale topography. J Biomed Mater Res A 90 (2):586–594

Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126(4):677–689

Franceschi RT, Xiao G (2003) Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. J Cell Biochem 88(3):446–454

Fröhlich MGW, Marolt D, Gimble JM, Kregar-Velikonja N, Vunjak-Novakovic G (2010) Bone grafts engineered from human adipose-derived stem cells in perfusion bioreactor culture. Tissue Eng Part A 16(1):179–189

Frost H (1990) Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. Anat Rec 226(4):403–413

Giardino et al. (2009) Electromagnetic field stimulator device for anatomic biophysical chondroprotection. U.S. Patent No. 7,566,295 B2, investors, Jul. 28

Gonzalez-Rey E, Gonzalez MA, Varela N, O'Valle F, Hernandez-Cortes P, Rico L, Büscher D, Delgado M (2010) Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. Ann Rheum Dis 69(1):241–248

Gorodetsky R, Levdansky L, Gaberman E, Gurevitch O, Lubzens E, McBride WH (2011) Fibrin microbeads loaded with mesenchymal cells support their long-term survival while sealed at room temperature. Tissue Eng Part C Methods 17(7):745–755

Haimi S, Moimas L, Pirhonen E, Lindroos B, Huhtala H, Raty S, Kuokkanen H, Sandor GK, Miettinen S, Suuronen R (2009) Calcium phosphate surface treatment of bioactive glass causes a delay in early osteogenic differentiation of adipose stem cells. J Biomed Mater Res A 91(2):540–547

Hammerick KEJA, Huang Z, Prinz FB, Longaker MT (2010) Pulsed direct current electric fields enhance osteogenesis in adipose-derived stromal cells. Tissue Eng Part A 16(3):917–931

Hanson AD, Marvel SW, Bernacki SH, Banes AJ, van Aalst J, Loboa EG (2009) Osteogenic effects of rest inserted and continuous cyclic tensile strain on hASC lines with disparate osteodifferentiation capabilities. Ann Biomed Eng 37(5):955–965

Hao W, Pang L, Jiang M, Lv R, Xiong Z, Hu YY (2010) Skeletal repair in rabbits using a novel biomimetic composite based on adipose-derived stem cells encapsulated in collagen I gel with PLGA-beta-TCP scaffold. J Orthop Res 28(2):252–257

Hattori H, Masuoka K, Sato M, Ishihara M, Asazuma T, Takase B, Kikuchi M, Nemoto K (2006) Bone formation using human adipose tissue-derived stromal cells and a biodegradable scaffold. J Biomed Mater Res B Appl Biomater 76(1):230–239

He J, Genetos DC, Yellowley CE, Leach JK (2010) Oxygen tension differentially influences osteogenic differentiation of human adipose stem cells in 2D and 3D cultures. J Cell Biochem 110(1):87–96

Huang SC, Wu TC, Yu HC, Chen MR, Liu CM, Chiang WS, Lin KM (2010) Mechanical strain modulates age-related changes in the proliferation and differentiation of mouse adipose-derived stromal cells. BMC Cell Biol 11:18–31

Jaalouk DE, Lammerding J (2009) Mechanotransduction gone awry. Nat Rev Mol Cell Biol 10(1):63–73

Jessop HLRS, Pitsillides AA, Lanyon LE (2002) Mechanical strain and fluid movement both activate extracellular regulated kinase (ERK) in osteoblast-like cells but via different signaling pathways. Bone 31(1):186–194

Joshi SD, Webb K (2008) Variation of cyclic strain parameters regulates development of elastic modulus in fibroblast/substrate constructs. J Orthop Res 26(8):1105–1113

Kakudo N, Shimotsuma A, Miyake S, Kushida S, Kusumoto K (2008) Bone tissue engineering using human adipose-derived stem cells and honeycomb collagen scaffold. J Biomed Mater Res A 84 (1):191–197

Kearney EM, Farrell E, Prendergast PJ, Campbell VA (2010) Tensile strain as a regulator of mesenchymal stem cell osteogenesis. Ann Biomed Eng 38(5):1767–1779

Kim B-S, Park I-K, Hoshiba T, Jiang H-L, Choi Y-J, Akaike T, Cho C-S (2011) Design of artificial extracellular matrices for tissue engineering. Prog Polym Sci 36(2):238–268

Kroeze RJ, Knippenberg M, Helder MN (2011) Osteogenic differentiation strategies for adipose-derived mesenchymal stem cells. Methods Mol Biol 702:233–248

Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP (2004) Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. J Craniomaxillofac Surg 32(6):370–373

Levi B, Longaker MT (2011) Concise review: adipose-derived stromal cells for skeletal regenerative medicine. Stem Cells 29(4):576–582

Lindroos B, Aho KL, Kuokkanen H, Raty S, Huhtala H, Lemponen R, Yli-Harja O, Suuronen R, Miettinen S (2010) Differential gene expression in adipose stem cells cultured in allogeneic human serum versus fetal bovine serum. Tissue Eng Part A 16(7):2281–2294

Liu L, Yuan W, Wang J (2010) Mechanisms for osteogenic differentiation of human mesenchymal stem cells induced by fluid shear stress. Biomech Model Mechanobiol 9(6):659–670

Liu Q, Cen L, Yin S, Chen L, Liu G, Chang J, Cui L (2008) A comparative study of proliferation and osteogenic differentiation of adipose-derived stem cells on akermanite and beta-TCP ceramics. Biomaterials 29(36):4792–4799

Lu Z, Roohani-Esfahani SI, Kwok PC, Zreiqat H (2011) Osteoblasts on rod shaped hydroxyapatite nanoparticles incorporated PCL film provide an optimal osteogenic niche for stem cell differentiation. Tissue Eng Part A 17(11–12):1651–1661

Lund P, Pilgaard L, Duroux M, Fink T, Zachar V (2009) Effect of growth media and serum replacements on the proliferation and differentiation of adipose-derived stem cells. Cytotherapy 11 (2):189–197

Luu YK, Capilla E, Rosen CJ, Gilsanz V, Pessin JE, Judex S, Rubin CT (2009) Mechanical stimulation of mesenchymal stem cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced obesity. J Bone Miner Res 24(1):50–61

Marino G, Rosso F, Cafiero G, Tortora C, Moraci M, Barbarisi M, Barbarisi A (2010) Beta-tricalcium phosphate 3D scaffold promote alone osteogenic differentiation of human adipose stem cells: in vitro study. J Mater Sci Mater Med 21(1):353–363

McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS (2004) Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 6(4):483–495

McCullen SD, Haslauer CM, Loboa EG (2010a) Musculoskeletal mechanobiology: interpretation by external force and engineered substratum. J Biomech 43(1):119–127

McCullen SD, McQuilling JP, Grossfeld RM, Lubischer JL, Clarke LI, Loboa EG (2010b) Application of low-frequency alternating current electric fields via interdigitated electrodes: effects on cellular viability, cytoplasmic calcium, and osteogenic differentiation of human adipose-derived stem cells. Tissue Eng Part C Methods 16 (6):1377–1386

Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R (2009) Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg 38(3):201–209

Nandi SKRS, Mukherjee P, Kundu B, De DK, Basu D (2010) Orthopaedic applications of bone graft & graft substitutes. Indian J Med Res 132:15–30

Quarto N, Behr B, Longaker MT (2010) Opposite spectrum of activity of canonical Wnt signaling in the osteogenic context of undifferentiated and differentiated mesenchymal cells: implications for tissue engineering. Tissue Eng Part A 16(10):3185–3197

Ra JC, Kang SK, Shin IS, Park HG, Joo SA, Kim JG, Kang BC, Lee YS, Nakama K, Piao M, Sohl B, Kurtz A (2011) Stem cell treatment for patients with autoimmune disease by systemic

infusion of culture-expanded autologous adipose tissue derived mesenchymal stem cells. J Transl Med 21(9):181–191

Reed CR, Han L, Andrady A, Caballero M, Jack MC, Collins JB, Saba SC, Loboa EG, Cairns BA, van Aalst JA (2009) Composite tissue engineering on polycaprolactone nanofiber scaffolds. Ann Plast Surg 62(5):505–512

Rose R, Bryan-Frankson B (2008) Is there still a role for pulsed electromagnetic field in the treatment of delayed unions and nonunions? Internet J Orthop Surg 10(1)

Rubin J, Rubin C, Jacobs CR (2006) Molecular pathways mediating mechanical signaling in bone. Gene 367:1–16

Scherberich A, Muller AM, Schäfer DJ, Banfi A, Martin I (2010) Adipose tissue-derived progenitors for engineering osteogenic and vasculogenic grafts. J Cell Physiol 225(2):348–3

Sefcik LS, Neal RA, Kaszuba SN, Parker AM, Katz AJ, Ogle RC, Botchwey EA (2008) Collagen nanofibres are a biomimetic substrate for the serum-free osteogenic differentiation of human adipose stem cells. J Tissue Eng Regen Med 2(4):210–220

Shoji T, Ii M, Mifune Y, Matsumoto T, Kawamoto A, Kwon SM, Kuroda T, Kuroda R, Kurosaka M, Asahara T (2010) Local transplantation of human multipotent adipose-derived stem cells accelerates fracture healing via enhanced osteogenesis and angiogenesis. Lab Invest 90(4):637–649

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676

Teo BKAS, Chan LY, Yim EK (2010) Nanotopography/mechanical induction of stem-cell differentiation. Meth Cell Biol 98:241–294

Tepper et al., inventors; 2005 Jan. 13. Combined tissue/bone growth stimulator and external fixation device. U.S. Patent 6,678,562 B1.

Titushkin I, Cho M (2007) Modulation of cellular mechanics during osteogenic differentiation of human mesenchymal stem cells. Biophys J 93(10):3693–3702

Tjabringa GS, Vezeridis PS, Zandieh-Doulabi B, Helder MN, Wuisman PI, Klein-Nulend J (2006) Polyamines modulate nitric oxide production and COX-2 gene expression in response to mechanical loading in human adipose tissue-derived mesenchymal stem cells. Stem Cells 24(10):2262–2269

Tokuzawa Y, Yagi K, Yamashita Y, Nakachi Y, Nikaido I, Bono H, Ninomiya Y, Kanesaki-Yatsuka Y, Akita M, Motegi H, Wakana S, Noda T, Sablitzky F, Arai S, Kurokawa R, Fukuda T, Katagiri T, Schönbach C, Suda T, Mizuno Y, Okazaki Y (2010) Id4, a new candidate gene for senile osteoporosis, acts as a molecular switch promoting osteoblast differentiation. PLoS Genetics 6(7):e1001019 1–15

Wall ME, Rachlin A, Otey CA, Loboa EG (2007) Human adipose-derived adult stem cells upregulate palladin during osteogenesis and in response to cyclic tensile strain. Am J Physiol Cell Physiol 293(5):C1532–C1538

Wells RG, Discher DE (2008) Matrix elasticity, cytoskeletal tension, and TGF-beta: the insoluble and soluble meet. Sci Signal 1(10): pe13 1–3