Honing Cell and Tissue Culture Conditions for Bone and Cartilage Tissue Engineering

Johnny Lam,^{1,3} Esther J. Lee,^{1,3} Elisa C. Clark,¹ and Antonios G. Mikos²

¹Department of Bioengineering, Rice University, Houston, Texas 77251

²Department of Chemical and Biomolecular Engineering, Rice University, Houston, Texas 77251

Correspondence: mikos@rice.edu

An avenue of tremendous interest and need in health care encompasses the regeneration of bone and cartilage. Over the years, numerous tissue engineering strategies have contributed substantial progress toward the realization of clinically relevant therapies. Cell and tissue culture protocols, however, show many variations that make experimental results among different publications challenging to compare. This collection surveys prevalent cell sources, soluble factors, culture medium formulations, environmental factors, and genetic modification approaches in the literature. The intent of consolidating this information is to provide a starting resource for scientists considering how to optimize the parameters for cell differentiation and tissue culture procedures within the context of bone and cartilage tissue engineering.

The superb elasticity and tensile strength of bone can be ascribed to its structure and composition. The skeleton consists of dense cortical bone and highly porous cancellous bone (Buckwalter et al. 1995). Disorganized collagen fibers comprising immature woven bone eventually gain spatial alignment following calcification to generate lamellar bone (Huttenlocker et al. 2013). Osseous tissue and periosteum (outer fibrous membrane) envelop a central cavity filled with marrow. Within this interior reside a variety of osteogenic and hematopoietic cell types that contribute to tissue remodeling and vascularization. Bone possesses both organic and inorganic materials, with the former comprising matrix proteins, proteogly-

cans, and collagen fibers, whereas the latter includes hydroxyapatite minerals (Lee et al. 2014). A high intrinsic capacity for regeneration of bone in both youths and adults differentiates this hard tissue from many other tissues in the human body. However, the natural course of healing may nonetheless be compromised in the event of traumatic injuries or devastating bone diseases that leave behind large unbridgeable defects, which will be further compounded if the region is load-bearing or complexly shaped. In these scenarios, additional clinical intervention is warranted to orchestrate repair.

Although autologous bone grafting represents the current clinical gold standard (Pape et al. 2010) for the treatment of large bone de-

³These authors contributed equally to this work.

Editor: Joseph P. Vacanti

Additional Perspectives on Tissue Engineering and Regenerative Medicine available at www.perspectivesinmedicine.org

Copyright © 2017 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a025734

Cite this article as *Cold Spring Harb Perspect Med* 2017;7:a025734

J. Lam et al.

fects because of its many advantages (no immune rejection risks, osteoinductive, and osteoconductive) (Campana et al. 2014), the technique still encounters significant hurdles in the form of limited donor supply and variable quality. Allograft transplantation can provide alternative options in such instances. However, the preparation of allografts is time-consuming and often compromises their mechanical integrity as well as protein bioactivity (Campana et al. 2014). To overcome these limitations, the field of bone tissue engineering provides a paradigm for the development of scaffolds that ideally possess adequate mechanical properties, support bone growth, degrade on new tissue formation, show biocompatibility, be highly porous, and integrate with the surrounding bone (Sitharaman et al. 2008). Indeed, many bone tissue engineering strategies leverage well-understood mechanisms of bone formation to facilitate repair.

Bone development and repair adhere to either intramembranous (cranial bones, clavicles, scapula) or endochondral (all other bones) pathways (Burr and Allen 2013). The first stage in bone repair typically involves hematoma formation from clotting of ruptured blood vessels, which creates a highly hypoxic and acidic local environment. An inflammatory response to the affected site precedes revascularization. A milieu of signaling molecules coaxes mesenchymal stem cells (MSCs) from the surrounding bone to aggregate at the fracture site, and these recruited cells then osteogenically or chondrogenically differentiate to generate an initial matrix for woven bone (Mehta et al. 2012). The gradual reconstitution of woven bone into lamellar bone concludes the regenerative process. A distinguishing feature between endochondral and intramembranous ossification is that the former derives new bone from a cartilage template, whereas the latter does not involve this intermediate step.

Articular cartilage, another major orthopedic aspect of the skeletal system, is a flexible connective tissue that lines the surface of bones in synovial joints throughout the body, permitting their painless and relatively frictionless articulation. The cartilage extracellular matrix is

stratified into four biochemically and architecturally distinctive layers that include the superficial zone, the middle zone, the deep zone, and the calcified zone, which together give rise to the tissue's unique viscoelastic and swelling properties; these particular attributes are responsible for reliably transmitting and distributing mechanical forces of motion to the underlying subchondral bone. Despite its elegant structure and biochemical makeup, articular cartilage is avascular, alymphatic, and aneural and, therefore, possesses a low endogenous capacity for regeneration. Furthermore, the resident cells sparsely populating the cartilage tissue, otherwise known as articular chondrocytes (ACs), maintain very low metabolic activity. Consequently, damage to articular cartilage caused by trauma or disease often requires intervention via exogenous means to stimulate repair.

Cartilage injuries present as either partial or full-thickness defects. Partial cartilage defects are characterized as chondral fissures or flaps that are limited to only the cartilage region of osteochondral tissues. If left untreated, partial cartilage defects can progressively degenerate to eventually affect the whole joint surface. Unlike partial defects, full-thickness defects penetrate beyond the chondral layer into the subchondral bone. Such osteochondral fractures often elicit bleeding via the subchondral bone and induce inflammation through the stimulation of a wound-healing response. The subsequent recruitment of biochemical cytokines and progenitor cells from the bone marrow does offer some form of tissue repair formation, and has been leveraged in microfracture surgical techniques for cartilage repair. However, the unorganized fibrocartilage that typically arises from such processes usually cannot sustain long-term function. To address such shortcomings of clinical approaches for cartilage repair, cartilage tissue engineers are pursuing promising research avenues combining progenitor cells, biochemical signals, and biocompatible materials for the development of innovative new-age solutions. Indeed, significant strides have been made over the years toward addressing the many identified challenges of engineering biomechanically suitable articular cartilage.

Bone and cartilage tissue engineering has given rise to many promising scaffolds with potential to stimulate long-lasting tissue regeneration. However, the ongoing inundation of the field with a growing number of tissue engineered bone and cartilage scaffolds complicates the selection of an ideal, optimized technology for orthopedic tissue regeneration. Furthermore, a perusal of the literature reveals major to minor differences in the cell and tissue culture conditions used between similar tissue engineering technologies toward scaffold development, thereby introducing confounding factors that make the comparison of research outcomes difficult. To coalesce these differences, the present article provides an overview of the different factors used in tissue engineering strategies toward the generation of scaffolds for bone and cartilage repair. Additionally, this review also highlights some of the advantages and disadvantages of each factor with the intent of guiding appropriate selection in future research endeavors.

CELL SOURCES

Although bone tissues maintain a high endogenous capacity for self-regeneration that is mediated by dynamic processes involving the recruitment and differentiation of host progenitor cells, the impaired healing of large bone defects caused by injury or disease warrants the development of new cell-based therapies. The majority of current cell-based approaches engendered from bone tissue engineering leverage the high natural tendency of bone marrow-derived stem cells to undergo osteogenic differentiation in vitro (Friedenstein et al. 1968; Caplan 1991; Pittenger et al. 1999). Additionally, the ability to expand these marrow-derived progenitor cells in vitro with negligible effects of their multipotency further advocates their use as an autologous cell source for bone repair and mitigates the urgency to search for other cell sources to be used in bone tissue engineering applications.

A number of emergent preclinical studies for cartilage repair in osteochondral defects increasingly rely on the regenerative capacity of

cells. Given the immediate alleviation of arthritic symptoms and newly formed cartilaginous tissues filling acute defects, it is clear that the implanted cells generally play an active therapeutic role in the repair process. However, the ephemeral nature of the restored cartilage tissue indicates that cell-based therapies for cartilage tissue defects still require extensive optimization. Indeed, the usage of marrow-derived skeletal progenitor cells for cartilage tissue engineering, unlike bone tissue engineering, is often complicated by their inability to reliably produce cartilage-specific extracellular matrix, which necessitates the search for alternative progenitor cell sources. The following section reviews a selection of promising cell sources, an important factor to consider, which is currently being investigated for new bone and cartilage tissue engineering applications.

Mesenchymal Stem Cells

Bone marrow-derived MSCs (BM-MSCs) have been most extensively investigated because of their presence in bone. They are typically isolated via one of the following approaches: (1) maintaining only the cell fraction adherent to tissue culture flasks, (2) sorting for an STRO-1 or CD105-positive cell population, or (3) sorting for a CD45 or Gly-A-negative cell population (Gronthos et al. 1994; Majumdar et al. 2000; Reyes et al. 2001; Bosnakovski et al. 2005). BM-MSCs retain their multipotency when propagated in vitro and can be effectively directed toward the osteogenic lineage through the inclusion of supplements or bioactive factors (elaborated on in subsequent sections). A wide body of literature substantiates their high osteogenic capacity both in vitro (Jaiswal et al. 1997; Nuttelman et al. 2004; Thibault et al. 2013) and in vivo (Kadiyala et al. 1997; Shin et al. 2004; Niemeyer et al. 2010b). Additionally, the literature further shows that both local and systemic delivery of BM-MSCs could contribute to orthotopic as well as heterotopic bone formation in vivo (Colnot 2011). BM-MSCs also represent a viable alternative cell source to ACs for cartilage tissue engineering. Following early reports of the successful induction of BM-MSC

J. Lam et al.

chondrogenic differentiation (Ashton et al. 1980; Friedenstein 1980; Johnstone et al. 1998), BM-MSCs have since been used for the generation of cartilage scaffolds in vitro and used in many preclinical (Mazor et al. 2014) and clinical in vivo studies (Anderson et al. 2014). Given their promise in stimulating the repair of cartilage defects (Anderson et al. 2014; Gopal et al. 2014), BM-MSCs generally represent a well-accepted alternative cell source, yet the potential of introducing donor morbidity from the bone marrow harvest procedure motivates the derivation of MSCs from other anatomical sites in the body.

Adipose-derived MSCs (AMSCs) can be readily obtained in large numbers (superseding cell numbers retrievable from bone marrow) from minimally invasive liposuction procedures or needle biopsies. AMSCs represent an attractive cell source with significant clinical value owing to their high yields from readily available lipoaspirates, fast proliferation, and negligible effect on donor morbidities (Zuk et al. 2001; Kern et al. 2006; Koga et al. 2009). A series of shaking, centrifugation, and filtration protocols can be used to extract the cell population of interest (Bunnell et al. 2008). AMSCs have been shown to undergo osteogenic differentiation when stimulated by appropriate exogenous factors (Knippenberg et al. 2006; Zeng et al. 2007) and when maintained on various types of scaffolds (Dudas et al. 2006; Yoon et al. 2007; Haimi et al. 2008; Liu et al. 2008). Despite their ability to undergo osteogenic differentiation, it has been repeatedly reported that the potential of AMSCs to form bone were inferior when compared with BM-MSCs (Liao and Chen 2014). Specifically, AMSCs, although phenotypically similar to BM-MSCs (STRO-1-positive and CD34-negative), produced less alkaline phosphatase and mineralization than BM-MSCs when assessed for osteogenic differentiation (Im et al. 2005). It was further shown that AMSCs maintained lower levels of osteogenic gene expression and were less efficient during osteogenesis when compared with BM-MSCs in vitro (Zhang et al. 2009; Shafiee et al. 2011; Vishnubalaji et al. 2012). In vivo, the implantation of AMSCs into heterotopic (Hayashi

et al. 2008; Zhang et al. 2009) and orthotopic (Niemeyer et al. 2010a) sites both resulted in inferior bone formation when compared with the implantation of BM-MSCs, although the addition of supplemental biochemical factors appeared to enhance the osteogenic potency of AMSCs orthotopically (Niemeyer et al. 2010a). Although AMSCs have been used with more relative success for the generation of chondrogenic cells that are comparable phenotypically to those derived from BM-MSCs (Yang et al. 2014), differences in ontological and anatomical origin inevitably translate into differing aptitudes for chondrogenesis (Hennig et al. 2007; Kim and Im 2009). In particular, AMSCs express a unique ligand receptor repertoire that does not respond as effectively as BM-MSCs to the same chondrogenic stimulants. However, the combination of bone morphogenetic protein 6 (BMP-6) and transforming growth factor β 3 (TGF- β 3) was able to rescue the reduced chondrogenic gene profile of AMSCs to levels comparable to that of BM-MSCs induced by TGF- β 3 alone (Hennig et al. 2007; Puetzer et al. 2010). Nevertheless, AMSCs still remain a clinically advantageous cell source because of their increased proliferative capacity and immunomodulatory effects when compared with BM-MSCs (Li et al. 2015), which should be considered when determining the primary purpose of the cellular component in cell-based therapies.

MSCs derived from synovial tissue (SMSCs) represent another promising source of progenitor cells for bone and cartilage tissue engineering. Although SMSCs have shown some capacity for osteogenic differentiation (De Bari et al. 2001; Yoshimura et al. 2007), the lack of consensus regarding their osteogenic potential (Fan et al. 2009) has deterred their usage in bone-related applications. On the contrary, a number of studies have suggested that SMSCs may actually possess higher chondrogenic potency when compared with MSCs sourced from other tissues (Sakaguchi et al. 2005). For instance, pellet cultures of SMSCs produced more ECM (Yoshimura et al. 2007) and were larger than AMSC pellets and heavier than BM-MSCs pellets that were chondrogenically induced under the same conditions (Sakaguchi et al. 2005). In

addition to their in vitro chondrogenicity, a handful of studies have since also shown their ability to stimulate cartilage repair in vivo (Koga et al. 2007, 2008). Indeed, the minimal effects of donor age, passage number, and cryopreservation on SMSC multipotency, as well as the reduced capacity of SMSCs to undergo osteogenic differentiation (Shirasawa et al. 2006; Jones and Pei 2012) make them a very suitable candidate for cartilage tissue engineering applications. However, the unique combinations of growth factors and biochemical signals required for the efficacious chondrogenic differentiation (Nishimura et al. 1999; Park et al. 2005; Fan et al. 2009) must be considered when selecting SMSCs for cell-based cartilage repair strategies.

MSCs extracted from umbilical cord blood (UCB-MSCs) have shown osteogenic potential (Kestendjieva et al. 2008). Differentiation can be induced through similar methods as for BM-MSCs. Current research largely focuses on culturing UCB-MSCs on different biomaterials, which has generated some encouraging in vivo results thus far (Jang et al. 2008; Diao et al. 2009b). Multipotent stem cells isolated from skeletal muscle may also have osteogenic potential (Wright et al. 2002; Bueno et al. 2008; Kim et al. 2008). However, further cell characterization and culture condition optimization is warranted. Osteogenesis by dental pulp stem cells extracted from both periosteal (Agata et al. 2007; Hayashi et al. 2008; Stockmann et al. 2012) and periodontal (Laino et al. 2005; de Mendonça Costa et al. 2008; Yang et al. 2008) tissues has likewise been observed with promising preliminary results. Regarding other stem-cell sources to be used in applications for cartilage repair, a subset of progenitor cells has been identified within and can be isolated from the cartilage tissue itself (O'Sullivan et al. 2011; Jiang and Tuan 2015). Specifically, chondroprogenitor cells (CPCs) isolated from the surface zone of articular cartilage express a number of MSC markers (CD105 and CD166) (Hiraoka et al. 2006; Chang et al. 2011) and form cartilaginous 3D pellets in vitro (Khan et al. 2009). Their presence in osteoarthritic (OA) cartilage (Alsalameh et al. 2004) as well as their relative resistance to hypertrophy (Fickert et al. 2004)

Bone and Cartilage Cell Culture Optimization

impart the use of debrided OA cartilage destined for disposal and make CPCs another promising cell source with significant chondrogenic potential.

Different cell types have been coaxed toward the osteogenic and chondrogenic lineages, albeit with varying degrees of success. Considerations for sourcing will depend on a host of factors, including ease of availability, the intended mechanism of therapy, and the processing or the feasibility of using autologous versus allogeneic cells.

Cell Cocultures

Because MSCs are precursors to bone-forming osteoblasts, they are most prevalently used in cell-based tissue engineering approaches. Inclusion of other cell populations, however, may impart additional benefits. Monocytes in direct contact with MSCs activate the STAT3 signaling pathway, prompting MSC differentiation into osteoblasts (Nicolaidou et al. 2012). Likewise, incorporating endothelial cells (ECs) into coculture systems has shown enhanced vascularization, along with increased proliferation, differentiation, matrix deposition, and mineralization of MSCs (Choong et al. 2006; Seebach et al. 2010; Gershovich et al. 2013). Primary ECs seem to yield more favorable osteogenic results than a transformed cell line (Guillotin et al. 2004). More mature ECs also generate greater numbers of prevascular structures compared with endothelial progenitor cells (Rouwkema et al. 2009).

Coculturing MSCs with bone cells may also recapitulate interactions in the native microenvironment. Osteoblasts drove osteogenic differentiation via the Wnt pathway when cultured indirectly with MSCs (Wang et al. 2007). However, both cell populations placed in direct contact negated this stimulatory effect. Osteoblasts and osteocytes synergistically enhanced MSC differentiation in indirect coculture, although, interestingly, osteocytes had more influence over alkaline phosphatase activity and mineralization than did osteoblasts (Birmingham et al. 2012). These collective findings were made in the absence of osteogenic culture medium.

J. Lam et al.

To improve performance, cell-based strategies for cartilage repair have recently shifted attention from AC or MSC monocultures to the usage of their cocultures for cartilage tissue formation (Hubka et al. 2014; Leijten et al. 2014). A number of studies have since been performed by our group and others (Wu et al. 2011; Acharya et al. 2012; Meretoja et al. 2012, 2013; Levorson et al. 2014) showing the superior chondrogenic ability of AC-MSCs cocultures to form cartilage tissue when compared with their individual monocultures. Using pellet (Wu et al. 2011) and 3D fiber-mesh cultures (Meretoja et al. 2014), it was shown that the coculture of ACs with MSCs stimulated the chondrogenic matrix production as well as chondrogenic (re)-differentiation of the former cell population. Such trophic effects from the MSCs could be further used to inhibit overall chondrogenic hypertrophy (Meretoja et al. 2013) and to increase coculture sensitivity to chondrogenic stimulants (Dahlin et al. 2014). These data establish the direct as well as indirect roles that MSCs can adopt when used for cartilage repair. As reviewed previously, it is well known that the anatomical origin of MSCs does significantly affect their eventual multipotent capacity to directly form mesenchymal tissues. Interestingly, it was revealed that the trophic ability of MSCs to stimulate ACs was not influenced by MSC origin (Wu et al. 2012). Hence, the importance of MSC origin should be heavily considered in the experimental design when determining whether direct mesenchymal tissue differentiation or trophic stimulation will contribute the primary mode of therapy.

Finally, cocultures of osteogenically and chondrogenically predifferentiated MSCs have been used in the context of osteochondral tissue engineering. When encapsulated separately in bilayer hydrogels, each population retained its respective phenotype in the absence of growth factors (Lam et al. 2014). Moreover, chondrogenic MSCs encouraged the proliferation of osteogenic MSCs. Recent reviews by Hubka et al. (2014) and Leijten et al. (2013) offer excellent overviews of evolving trends in the usage of direct and indirect cocultures for cartilage tissue engineering applications.

When implementing a coculture system to stimulate osteogenic and/or chondrogenic differentiation, consider whether the cell populations would thrive in direct or indirect contact. Additionally, coculture ratios and culture medium formulations that accommodate both cell types will require optimization.

Culture Medium

Despite the little attention given to the culture media compositions used for general MSC expansion and subsequent differentiation, growing evidence suggests that even nuances in media composition can significantly influence the biological properties and differentiation potential of MSCs (Sotiropoulou et al. 2006; Apel et al. 2009; Dexheimer et al. 2012). Indeed, osteogenic (OM) and chondrogenic (CGM) induction medium encompasses an extensive array of formulations. Maniopoulos and colleagues provided the original recipe for OM, consisting of α minimal essential medium (α -MEM), 15% fetal bovine serum (FBS), and antibiotics/antifungals, along with supplements 50 μ g/ml ascorbic acid (Asc), 10 mM β glycerophosphate (β -GP), and 10^{-8} M dexamethasone (Dex) (Maniopoulos et al. 1988). Although many subsequent works still largely adhere to the aforementioned composition, the basal medium has been interchanged with Dulbecco's modified eagle medium (DMEM) (Jaiswal et al. 1997), DMEM/F12 (Venugopal et al. 2013), and MSC-defined growth medium (MSCGM) (Hildebrandt et al. 2009), among others. The batch of FBS remains paramount, as interlot variability may impinge on the consistency and comparability of experimental results (Wappler et al. 2013). Quality assurance can be achieved through laborious and expensive pretesting procedures. The base medium composition used to induce MSC chondrogenesis typically comprise serum-free DMEM supplemented with 10^{-8} to 10^{-7} M Dex, 50 g/L Asc, and ITS⁺ (6.25 μ g/mL insulin, 6.25 μ g/mL transferrin, 6.25 ng/mL selenious acid, 1.25 mg/mL bovine serum albumin, and 5.35 μ g/mL linoleic acid) (Johnstone et al. 1998). 10 ng/mL of chondrogenic growth factors, such as TGF- β 1 or TGF- β 3 (Johnstone

et al. 1998; Puetzer et al. 2010), are often also added to the chondrogenic medium to effectively stimulate chondrogenic differentiation.

Regarding supplements, Asc aids in metabolizing hydroxyproline and hydroxylysine found in collagen, which constitutes a major organic component of bone. These hydroxylated amino acids ensure ECM stability and proper collagen fiber cross-linking (Packer 1997). Because of the challenges of using Asc under standard culture conditions (neutral pH, 37°C), its more stable derivative Asc 2-phosphate is generally substituted (Takamizawa et al. 2004). β -GP offers organic phosphates that contribute to the formation of bone mineral and is usually not supplemented in pure CGMs. However, a reduction of β -GP may be necessary based on cell type to avoid dystrophic mineralization (Langenbach and Handschel 2013). The synthetic glucocorticoid Dex facilitates in vitro osteogenic differentiation of marrow stromal cells, typically within a concentration range of 10^{-9} to 10^{-7} M (Jaiswal et al. 1997). Recently, efforts have been made to tailor components of osteogenic medium within physiologically relevant levels. Honda and colleagues used computational algorithms to design cocktails that more rapidly initiated human and murine MSC mineralization in vitro (Honda et al. 2013). Further integration of bioinformatics with tissue engineering could potentially accelerate optimal culture medium formulations.

Although more focus has been given toward optimizing the composition of OM and CGM for maximizing osteogenic and chondrogenic differentiation, respectively, the biochemical constituents of general culture media used for cell expansion can also have a profound effect on MSC multipotency and differentiation potential. For instance, it was shown that the expansion of MSCs with high-glucose DMEM-based media (when compared with low-glucose) formulations may induce premature senescence (Chang et al. 2015) and steer their differentiation potential toward osteogenic (Li et al. 2007) or adipogenic lineages (Aguiari et al. 2008). In a recent study comparing four commonly used general expansion media, it was shown that media composition significantly influenced growth

characteristics and that the addition of proliferative growth factors during expansion affected MSC chondrogenic potential (Hagmann et al. 2013). Indeed, the addition of fibroblast growth factor 2 (FGF-2) enhanced the proliferation rate of MSCs without adversely affecting their multipotentiality and allowed the cells to undergo chondrogenesis more effectively as indicated by increased proteoglycan production (Tsumumi et al. 2001; Bianchi et al. 2003; Solchaga et al. 2005). Furthermore, individual nutrients can have varying magnitudes of effect on the chondrogenic differentiation of MSCs and may need to be optimized (Cigan et al. 2013).

Conditioned medium (CM) containing secretions from cell populations of interest may also be leveraged. Enhanced osteogenic differentiation has been observed for different species of MSCs, driven by CM derived from osteocytes (Heino et al. 2004), osteoclasts (Kreja et al. 2010), human fetal osteoblasts (Ye et al. 2007), HT-39 breast cancer cells (Bunyaratavej et al. 2000), or chondrocytes (Nurminskaya et al. 2003), among others. With regard to chondrogenic differentiation, the treatment of MSCs with CM from ACs also proved sufficient in stimulating chondrogenic differentiation of the former cell population (Hwang et al. 2011). Because the stimulatory factors in CM are not being continually produced as in cell cocultures, they may be less stable and thus not conducive for lengthier in vitro culture durations.

Soluble Factors

Culture medium can also be augmented with growth factors to stimulate osteogenic and/or chondrogenic differentiation in vitro. Bone morphogenetic proteins (BMPs), constituting a subset of the TGF- β superfamily, are potent drivers of osteogenesis. The most prominent members include BMP-2, 4, 6, 7, and 9 (Luu et al. 2007). In addition to their ability to stimulate osteogenic differentiation, several BMP molecules, such as BMP-2, 4, 6, and 7, also carry the capacity to stimulate the chondrogenic differentiation of MSCs (Lam et al. 2015). Indeed, BMP-2 plays essential roles in stimulating chondrogenic processes via Sox9 expression and reg-

J. Lam et al.

ulating the development of cartilage tissues (Santo et al. 2013). A handful of other bioactive factors contribute to different facets of bone and cartilage repair. Tumor necrosis factor α (TNF- α) helps mobilize MSCs to the site of injury and influences osteoclast function (Dimitriou et al. 2005). Additionally, it has been suggested that the chemokine stromal derived factor 1 (SDF-1) is capable of inducing MSC migration and homing to cartilage defect sites to enhance tissue repair (Sukegawa et al. 2012; Zhang et al. 2013). Vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), and FGF have been implicated in the formation of new vasculature to promote nutrient/waste transport throughout bone tissue (Vo et al. 2012). The three TGF- β isoforms β 1, β 2, and β 3 primarily regulate the proliferation and matrix production of differentiated cells (Pneumatics et al. 2010). The TGF- β isoforms have been extensively used particularly for cartilage tissue engineering applications for their ability to robustly stimulate MSC chondrogenic differentiation and enhance cartilaginous matrix production (Massagué 1990; Hunziker et al. 2001). However, the differential effects of each isoform on chondrogenesis (Chimal-Monroy and Diaz de Leon 1997) need to be considered when selecting the appropriate growth factor for developing cell-based cartilage therapies.

Growth factors may be assessed individually or in combination to elucidate potential synergism. The latter involves codelivering or sequentially administering select bioactive factors. Multiple combinations for effectively stimulating osteogenic differentiation have been explored to date, including BMP-2 and VEGF (Young et al. 2009), BMP-2 and IGF-1 (Kim et al. 2012), BMP-2 and BMP-7 (Basmanav et al. 2008), and PDGF and BMP-2 (Kim et al. 2013b). The controlled delivery of growth factors in combination for cartilage repair, such as IGF-1 with TGF- β 1 (Holland et al. 2005) or TGF- β 3 (Kim et al. 2013a), TGF- β 2 with BMP7 (Lim et al. 2010), and SDF-1 α with TGF- β 1 (Chim et al. 2012), has also met relative success at inducing cartilage tissue formation *in vivo*. The effects of temporal signaling patterns, how-

ever, cannot be understated; for example, initial dosing with BMP-2 followed by dual release of BMP-2 and IGF-1 more effectively promoted osteogenic behavior than simultaneously administering both factors over the entire duration (Raiche and Puleo 2004). In addition, the transient exposure of bovine chondrocytes encapsulated in alginate hydrogels to TGF- β 3 for the 2 weeks of an 8-week culture showed enhanced glycosaminoglycan deposition and compressive moduli when compared with continuous TGF- β 3 exposure over the entire culture duration (Byers et al. 2008). Delineating the correlation between concentration and timing will be crucial to leveraging innovative growth factor delivery strategies. Furthermore, given that supraphysiological doses are often used, current endeavors should strive to lower these amounts without compromising therapeutic efficacy.

Because of the high costs associated with growth factors, a more economical alternative may entail synthetic peptides that emulate their functions (BMP-2 [Seol et al. 2006], VEGF [D'Andrea et al. 2005], and FGF-2 [Lin et al. 2006]). These sequences can furthermore be spatially arranged on biomaterials (Samorezov and Alsberg 2015). A third option involves genetically encoding cells to express desired soluble factors, as detailed in a subsequent section.

ENVIRONMENTAL FACTORS

In the design of most tissue engineering applications, biochemical parameters are commonly used as a means of altering cell behavior. However, considerable attention must also be given to the design of physical and environmental parameters as they have been shown to impart significant influence over cell phenotype (Brandl et al. 2007; Lutolf et al. 2009). This is especially important when developing tissue engineered constructs to fulfill both the biological as well as mechanical roles of the tissues to be supplanted such as bone and cartilage.

It is well known that mammalian cells cultured in 2D behave differently when compared with cultures grown in a 3D environment. On tissue culture polystyrene (2D), expanding cells

are polarized in such a way that only a portion of the cell membrane is interacting with the material, thereby resulting in an unnatural phenotypic response (Gieni and Hendzel 2008; Tibbitt and Anseth 2009). Within a 3D environment, biochemical and mechanical cues are presented in a manner that recapitulates physiological conditions, which would result in more biologically relevant cell–matrix interactions (Cukierman et al. 2001). As an example, ACs expanded in 2D culture undergo dedifferentiation (Mertotja et al. 2014) but maintain their phenotype when grown in 3D culture (Caron et al. 2012). Indeed, a variety of biomaterials have been developed to provide the proper 3D environmental context for culturing bone and cartilage cells. The reader is directed to comprehensive reviews by Dawson et al. (2008), Tibbitt and Anseth (2009), and Lee et al. (2014) for more information regarding such biomaterials.

The substrate stiffness can also play a significant role in influencing phenotypic fate (Discher et al. 2005). Discher and colleagues showed in their seminal work (Engler et al. 2006) that MSCs specify lineage commitment according to the elasticity of the interacting matrix and that the matrix elasticity effect can overcome the effects of soluble inductive factors. Specifically, MSCs underwent neurogenic differentiation on soft matrices (elastic modulus of 0.1–1 kPa) but adopted myogenic or osteogenic phenotypes on stiffer matrices (elastic modulus of 25–40 kPa) (Engler et al. 2006). In another study, MSCs grown on soft matrices (elastic modulus of ~1 kPa) underwent both chondrogenic and adipogenic differentiation. However, the addition of TGF- β 1 to these MSCs enhanced chondrogenesis but suppressed adipogenesis; such an effect was not observed when TGF- β 1 was added to MSCs undergoing myogenesis on stiffer substrates (elastic modulus of ~15 kPa) (Park et al. 2011). Together, these results show the ability to use matrix stiffness as a tool to influence cell phenotype for bone and cartilage tissue engineering as well as illustrate the complexity of mechanobiology that is beyond the scope of this article. Excellent reviews on this subject are available (Discher et al. 2005, 2009).

Bone and Cartilage Cell Culture Optimization

Hypoxia, or low-oxygen tension, is another environmental factor that can significantly affect the phenotypic fate of MSCs. When exposed to hypoxic conditions, which recapitulate the low-oxygen tension present in avascular cartilage tissues, MSCs undergo chondrogenic differentiation as mediated by the transcription factor hypoxia-inducible factor 1 α (HIF-1 α) (Kanichai et al. 2008). Interestingly, Leijten et al. (2014) showed that the chondrogenic conditioning of MSCs in hypoxia, but not normoxia, directed the MSCs toward a stable articular cartilage phenotype as indicated by the expression of key articular cartilage markers at both the gene and protein levels. With this knowledge, hypoxia as well as the exogenous stimulation of HIF-1 α can both be used as tools in cartilage tissue engineering applications to effectively stimulate MSC chondrogenesis (Duval et al. 2012).

GENETICALLY ENGINEERED CELLS

Administering raw growth factors involves a finite amount of protein with limited bioactivity and, therefore, supraphysiological doses are often necessary. Biomaterials modulate the release kinetics of these molecules to some extent, although initial bursts and eventual tapering still occur. Hence, genetic approaches that capitalize on the cells' own machinery to secrete therapeutically relevant factors present an attractive avenue.

Various strategies, broadly categorized as nonviral or viral, exist to introduce foreign genetic material into cells. Nonviral methods can be further classified into chemical or physical approaches; the former encompasses lipids and polymers, whereas the latter includes techniques such as electroporation, magnetofection, ultrasound, and DNA bombardment (Nayerossadat et al. 2012). Gene expression by nonviral means tends to be transient, and transfection efficiency remains significantly lower than with viruses (retrovirus, adenovirus, adenoassociated virus, herpes virus, baculovirus) (Zhang and Godbey 2006). Viral-mediated transduction permits long-term expression and potentially genomic integration, yet there

J. Lam et al.

are typically size restrictions on the genetic cargo (Kofron and Laurencin 2006). Protocol usage will vary by cell type, the genetic components to be delivered, and desired temporal expression patterns.

Nonviral and viral vectors alike can be merged with biomaterial scaffolds, yielding gene-activated matrices (GAMs). Cells seeded on these constructs may undergo transfection, express the protein of interest, and undergo processes including differentiation. Human MSCs cultured on collagen-nanohydroxyapatite scaffolds dispersed with polyplexes containing ephrinB2 DNA showed enhanced osteogenesis and mineralization (Tierney et al. 2013). Diao et al. (2009a) combined chitosan/gelatin scaffolds with TGF- β 1 plasmids, which promoted chondrogenesis of rabbit MSCs, as observed by up-regulated collagen II and aggrecan. Another study capitalized on bilayered oligo(poly(ethylene glycol) fumarate)/carboxymethyl cellulose hydrogels to nonvirally deliver DNA encoding the SOX trio and Runx2 to rat osteochondral defects. This co-delivery approach targeting host cells led to better tissue infiltration and improved bone formation but not that of cartilage (Needham et al. 2014).

Oftentimes, the desired bioactive factor is constitutively expressed, resulting in unregulated dose and duration. Cell differentiation and tissue formation, however, follow intricate spatiotemporal patterns and demand a high level of precision. In this regard, chemical inducers, such as antibiotics, amino acids, vitamins, or food additives, provide a layer of transcriptional control for ex vivo-modified cells (Lee et al. 2014). An earlier work used a doxycycline-inducible system to regulate BMP-2 expression in murine MSCs. Following implantation in critical-size segmental defects, these genetically modified cells enhanced bone repair, although several instances of bone overgrowth were observed (Moutsatsos et al. 2001). Similarly, Koh et al. (2006) transduced murine fibroblasts with a rapalog/rapamycin-inducible BMP-2 expression system, then showed bone formation in both subcutaneous and critical-sized calvarial defect models in mice. Pertaining to cartilage

tissue engineering, a tetracycline-based system has been used to overexpress Sox9 in primary chondrocytes for phenotype maintenance (Yao et al. 2014). Another study also leveraged doxycycline induction to tune interleukin (IL)-1Ra expression in human MSCs, thereby alleviating proinflammatory responses and stimulating regeneration of articular cartilage (Glass et al. 2014).

In consideration of eventual clinical translation, small molecule ligands tend to diffuse non-specifically and do not permit precise temporal control as a result of gradual clearance from the body. Some compounds may also be naturally present in the body at levels that could unintentionally trigger expression, whereas other chemical inducers, especially antibiotics, might be toxic or cause resistance in patients when dosed in significant quantities over prolonged periods. For greater stringency in gene regulation, other possible stimuli including ultrasound, heat, or light can be investigated.

CONCLUDING REMARKS

Indeed, fine-tuning the various parameters for cell and tissue culture-based strategies in bone and cartilage tissue engineering represents a nontrivial endeavor. Tables 1 and 2 highlight different conditions used in recent publications. Surveying the available resources and carefully identifying the downstream application will aid in selection of the appropriate cell source or several for coculture systems. Based on the given cell population(s), culture medium and other environmental adjustments can be formulated accordingly. Specific soluble factors may be incorporated to enhance osteogenesis or chondrogenesis, either as supplements or encoded genetically within the cells themselves. The techniques range significantly in terms of labor intensiveness, and scientists should discern the best course of action to achieve their research aims. Although no comprehensive guidebook exists for cell and tissue culture optimization, the collective material described herein points to various strategies that have been attempted and where one might proceed from these paths.

Bone and Cartilage Cell Culture Optimization

Table 1. Conditions used to promote osteogenic differentiation

References	Cell source	Osteogenic medium composition	Soluble factors	Environmental factors
Correia et al. 2012	AMSCs (human)	Low-glucose DMEM, 10% FBS, 50 μ g/mL Asc-2-phosphate, 10 mM β -GP, 10 mM HEPES, 100 nM Dex	1 ng/mL bFGF	3D (silk fibroin scaffold) in vitro and in vivo
Brennan et al. 2015	BM-MSCs (human)	α -MEM, 8% human platelet lysate, 1 IU/mL heparin, 250 μ M Asc, 10 mM β -GP, 100 nM Dex	N/A	2D (culture plastic) and 3D (PCL scaffold) in vitro
Castren et al. 2015	BM-MSCs (human)	Low glucose DMEM, 2 mM L-glutamine, 2% human AB-plasma, 0.5% human platelet lysate, 40 IU/mL heparin, 200 μ M Asc-2-phosphate, 10 mM β -GP, 100 nM Dex	N/A	2D (culture plastic) and 3D (type I collagen sponge) in vitro
Duttenhoefer et al. 2015	BM-MSCs (human)	Iscove's modified Dulbecco's medium, 10% FCS, 0.1 mM Asc, 10 mM β -GP, 10 nM Dex	5 ng/mL bFGF	2D (culture plastic) in vitro
Faia-Torres et al. 2014	BM-MSCs (human)	α -MEM, 10% FBS, 50 mM Asc-2-phosphate, 2 mM β -GP, 10 nM 1,25-dihydroxyvitamin D ₃	1 ng/mL FGF-2	2D (PCL membrane) in vitro; surface roughness
Hoch et al. 2015	BM-MSCs (human)	α -MEM, 10% FBS, 50 μ g/mL Asc-2-phosphate, 10 mM β -GP, 10 nM Dex	N/A	2D (culture plastic) in vitro; 3D (alginate hydrogel) in vivo
Nayak et al. 2010	BM-MSCs (human)	DMEM, 10% FBS, 1% nonessential amino acids, 50 μ g/mL Asc, 10 mM β -GP, 1 μ M Dex	N/A	2D (multiwalled carbon-nanotube film) in vitro
Cha et al. 2016	BM-MSCs (mouse)	DMEM, 10% FBS, 1% glutamax, 0.2 mM Asc, 10 mM glycerol 2-phosphate	Tauroursodeoxycholic acid	2D (culture plastic) in vitro; 3D (type I collagen sponge) in vivo
Watson et al. 2015	BM-MSCs (rat)	α -MEM, 10% FBS, 50 mg/L Asc, 10 mM β -GP, 100 nM Dex	N/A	3D (poly(NiPAAM)-based hydrogel) in vitro and in vivo
Shen et al. 2015	UC-MSCs (human)	DMEM/F12 (50/50), 10% FBS	N/A	2D (culture plastic) and 3D (methylcellulose-based scaffold) in vitro; matrix stiffness

DMEM, Dulbecco's modified eagle medium; Dex, dexamethasone; AMSC, adipose-derived mesenchymal stem cell; bFGF, basic fibroblast growth factor; β -GP, β glycerophosphate; α MEM, α minimal essential medium; FBS, fetal bovine serum; BM-MSC, bone marrow-derived mesenchymal stem cell; PCL, polycaprolactone; UC-MSC, umbilical cord mesenchymal stem cell; FGF-2, fibroblast growth factor 2.

J. Lam et al.

Table 2. Conditions used to promote chondrogenic differentiation

References	Cell source	Chondrogenic medium composition	Soluble factors	Environmental factors
Mellor et al. 2015	ASCs (human)	DMEM, 1% Dex, 1% ITS ⁺ , 1% Asc, FBS (0%–10%)	10 ng/mL BMP6, 10 ng/mL TGF-β1 (varied)	3D (pellet culture and electrospun PLA stacked scaffolds) in vitro
Santo et al. 2015	ASCs (human)	DMEM, 1 mM Dex, 17 mM L-Asc, 35 mM L-proline, ITS liquid media supplement, 10% FBS	10 ng/mL TGF-β1	3D (platelet lysate chitosan/chondroitin sulfate nanoparticle hydrogels) in vitro
Babur et al. 2015	BM-MSCs (human)	High-glucose DMEM, 10 ⁻⁷ M Dex, 200 μM Asc-2-phosphate, 40 μg/mL L-proline, 1% ITS-X	10 ng/mL TGF-β1	3D (micropellets in multiwell plates, later stacked to form osteochondral structure) in vitro
Futrega et al. 2015	BM-MSCs (human)	High-glucose DMEM, 1 × glutamax, 100 nM Dex, 200 μM Asc-2-phosphate, 40 μg/mL L-proline, 1% ITS-X	10 ng/mL TGF-β1	3D (microwell mesh aggregates) in vitro
Luo et al. 2015	BM-MSCs (human)	0.1 μM Dex, 50 μg/mL L-Asc, 2.5% ITS ⁺ , 40 μg/mL L-proline	10 ng/mL TGF-β3, and/or 20 ng/mL MGF	2D (96-well plate, transwell plate assays) in vitro
Occhetta et al. 2015	BM-MSCs (human)	DMEM, 100 mM HEPES buffer, 0.29 mg/mL L-glutamate, 0.1 mM Asc-2-phosphate, ITS ⁺ 1.5 μg/mL human serum albumin, 4.7 μg/mL linoleic acid, 10 ⁻⁷ M Dex	1 ng/mL TGF-β3	3D (microfluidic perfused system) in vitro
Parmar et al. 2015	BM-MSCs (human)	4.5 g/L DMEM, 0.1 mM Dex, 50 μg/mL L-proline, 50 μg/mL Asc-2-phosphate, 1 × ITS premix	20 ng/mL TGF-β3	3D (encapsulated in peptide-modifiable collagen-like hydrogels) in vitro
Rey-Rico et al. 2014	BM-MSCs (human)	DMEM, 0.1 μM Dex, 50 μg/mL Asc, 40 μg/mL proline, 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 μg/mL selenacious acid, 1.25 mg/mL BSA, 5.55 μg/mL linoleic acid	10 ng/mL TGF-β1 (in media and released from scaffold)	3D (pellet culture in the presence of TGF-β1-releasing poly(ethylene oxide)-terephthalate/poly(butylene terephthalate) scaffolds—not grown on scaffolds) in vitro
Steinmetz et al. 2015	BM-MSCs (human)	High-glucose DMEM, 1 mL/100 mL media ITS ⁺ premix, 100 nM Dex, 50 mg/mL Asc-2-phosphate trisodium salt, 20 mM β-GP	2.5 ng/mL TGF-β3	3D (PEG hydrogels) in vitro

Continued

Table 2. Continued

References	Cell source	Chondrogenic medium composition	Soluble factors	Environmental factors
Cao et al. 2015	BM-MSCs (rat)	DMEM-HG, 100 nM Dex, 50 µg/mL Asc-2-phosphate, ITS+1 liquid media supplement (100×), 5% FBS, 2 mM L-glutamine Added: 0.5 mg/mL aphidicolin, 50 mM 18-α-glycyrrhetinic acid as needed	10 ng/mL TGF-β1	2D (RGD microdomains on PEG hydrogels) in vitro

DMEM, Dulbecco's modified eagle medium; Dex, dexamethasone; AMSC, adipose-derived mesenchymal stem cell; bFGF, basic fibroblast growth factor; β-GP, β glycerophosphate; αMEM, α minimal essential medium; FBS, fetal bovine serum; BM-MSC, bone marrow-derived mesenchymal stem cell; PCL, polycaprolactone; UC-MSC, umbilical cord mesenchymal stem cell; FGF-2, fibroblast growth factor 2; PEG, poly(ethylene glycol); RGD, arginine-glycine-aspartate; MGF, mechano growth factor.

ACKNOWLEDGMENTS

This work is supported by the National Institutes of Health (R01 AR048756, R01 AR068073, R21 AR067527) (A.G.M.) and a National Science Foundation Graduate Research Fellowship (E.J.L.).

REFERENCES

- Acharya C, Adesida A, Zajac P, Mumme M, Riesle J, Martin I, Barbero A. 2012. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol* **227**: 88–97.
- Agata H, Asahina I, Yamazaki Y, Uchida M, Shinohara Y, Honda MJ, Kagami H, Ueda M. 2007. Effective bone engineering with periosteum-derived cells. *J Dent Res* **86**: 79–83.
- Aguiari P, Leo S, Zavan B, Vindigni V, Rimessi A, Bianchi K, Franzin C, Cortivo R, Rossato M, Vettor R, et al. 2008. High glucose induces adipogenic differentiation of muscle-derived stem cells. *Proc Natl Acad Sci* **105**: 1226–1231.
- Alsalameh S, Amin R, Gemba T, Lotz M. 2004. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. *Arthritis Rheum* **50**: 1522–1532.
- Anderson JA, Little D, Toth AP, Moorman CT, Tucker BS, Ciccotti MG, Guilak F. 2014. Stem cell therapies for knee cartilage repair: The current status of preclinical and clinical studies. *Am J Sports Med* **42**: 2253–2261.
- Apel A, Groth A, Schlesinger S, Bruns H, Schemmer P, Buchler MW, Herr I. 2009. Suitability of human mesenchymal stem cells for gene therapy depends on the expansion medium. *Exp Cell Res* **315**: 498–507.
- Ashton BA, Allen TD, Howlett CR, Eaglesom CC, Hattori A, Owen M. 1980. Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. *Clin Orthop Relat Res*: 294–307.
- Babur BK, Futrega K, Lott WB, Klein TJ, Cooper-White J, Doran MR. 2015. High-throughput bone and cartilage micropellet manufacture, followed by assembly of micropellets into biphasic osteochondral tissue. *Cell Tissue Res* **361**: 755–768.
- Basmanav FB, Kose GT, Hasirci V. 2008. Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials* **29**: 4195–4204.
- Bianchi G, Banfi A, Mastrogiacomo M, Notaro R, Luzzatto L, Cancedda R, Quarto R. 2003. Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. *Exp Cell Res* **287**: 98–105.
- Birmingham E, Niebur GL, McHugh PE, Shaw G, Barry FP, McNamara LM. 2012. Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. *Eur Cells Mater* **23**: 13–27.
- Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. 2005. Isolation and multilineage differentiation of bovine bone marrow mesenchymal stem cells. *Cell Tissue Res* **319**: 243–253.
- Brandl E, Sommer F, Goepferich A. 2007. Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior. *Biomaterials* **28**: 134–146.
- Brennan MA, Renaud A, Gamblin AL, D'Arros C, Nedellec S, Trichet V, Layrolle P. 2015. 3D cell culture and osteogenic differentiation of human bone marrow stromal cells plated onto jet-sprayed or electrospun micro-fiber scaffolds. *Biomed Mater* **10**: 045019.
- Buckwalter JA, Glimcher MJ, Cooper RR, Recker R. 1995. Bone biology. II: Formation, form, modeling, remodeling, and regulation of cell function. *J Bone Joint Surg Am* **77**: 1276–1289.
- Bueno DF, Kerkis I, Costa AM, Martins MT, Kobayashi GS, Zucconi E, Fanganiello RD, Salles FT, Almeida AB, do Amaral CER. 2008. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in

J. Lam et al.

- cleft lip and/or palate patients. *Tissue Eng Part A* **15**: 427–435.
- Bunnell BA, Flaatt M, Gagliardi C, Patel B, Ripoll C. 2008. Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods* **45**: 115–120.
- Bunyaratavej P, Hullinger TG, Somerman MJ. 2000. Bone morphogenetic proteins secreted by breast cancer cells upregulate bone sialoprotein expression in preosteoblast cells. *Exp Cell Res* **260**: 324–333.
- Burr DB, Allen MR. 2013. *Basic and applied bone biology*. Academic, London.
- Byers BA, Mauck RL, Chiang IE, Tuan RS. 2008. Transient exposure to transforming growth factor β 3 under serum-free conditions enhances the biomechanical and biochemical maturation of tissue-engineered cartilage. *Tissue Eng Part A* **14**: 1821–1834.
- Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, Lattanzi W, Logroscino G. 2014. Bone substitutes in orthopaedic surgery: From basic science to clinical practice. *J Mater Sci Mater Med* **25**: 2445–2461.
- Cao B, Li Z, Peng R, Ding J. 2015. Effects of cell–cell contact and oxygen tension on chondrogenic differentiation of stem cells. *Biomaterials* **64**: 21–32.
- Caplan AI. 1991. Mesenchymal stem cells. *J Orthop Res* **9**: 641–650.
- Caron MM, Emans PJ, Coolen MM, Voss L, Surtel DA, Cremers A, van Rhijn LW, Welting TJ. 2012. Redifferentiation of dedifferentiated human articular chondrocytes: Comparison of 2D and 3D cultures. *Osteoarthritis Cartilage* **20**: 1170–1178.
- Castren E, Sillat T, Oja S, Noro A, Laitinen A, Kontinen YT, Lehenkari P, Hukkanen M, Korhonen M. 2015. Osteogenic differentiation of mesenchymal stromal cells in two-dimensional and three-dimensional cultures without animal serum. *Stem Cell Res Ther* **6**: 167.
- Cha BH, Jung MJ, Moon BK, Kim JS, Ma Y, Arai Y, Noh M, Shin JY, Kim BS, Lee SH. 2016. Administration of tauroursodeoxycholic acid enhances osteogenic differentiation of bone marrow-derived mesenchymal stem cells and bone regeneration. *Bone* **83**: 73–81.
- Chang HX, Yang L, Li Z, Chen G, Dai G. 2011. Age-related biological characterization of mesenchymal progenitor cells in human articular cartilage. *Orthopedics* **34**: e382–388.
- Chang TC, Hsu MF, Wu KK. 2015. High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy. *PLoS ONE* **10**: e0126537.
- Chim H, Miller E, Gliniak C, Alsberg E. 2012. Stromal-cell-derived factor (SDF) 1- α in combination with BMP-2 and TGF- β 1 induces site-directed cell homing and osteogenic and chondrogenic differentiation for tissue engineering without the requirement for cell seeding. *Cell Tissue Res* **350**: 89–94.
- Chimal-Monroy J, Diaz de Leon L. 1997. Differential effects of transforming growth factors β 1, β 2, β 3 and β 5 on chondrogenesis in mouse limb bud mesenchymal cells. *Int J Dev Biol* **41**: 91–102.
- Choong CSN, Huttmacher DW, Triffitt JT. 2006. Co-culture of bone marrow fibroblasts and endothelial cells on modified polycaprolactone substrates for enhanced potentials in bone tissue engineering. *Tissue Eng* **12**: 2521–2531.
- Cigan AD, Nims RJ, Albro MB, Esau JD, Dreyer MP, Vunjak-Novakovic G, Hung CT, Ateshian GA. 2013. Insulin, ascorbate, and glucose have a much greater influence than transferrin and selenous acid on the in vitro growth of engineered cartilage in chondrogenic media. *Tissue Eng Part A* **19**: 1941–1948.
- Colnot C. 2011. Cell sources for bone tissue engineering: Insights from basic science. *Tissue Eng Part B Rev* **17**: 449–457.
- Correia C, Bhumiratana S, Yan LP, Oliveira AL, Gimble JM, Rockwood D, Kaplan DL, Sousa RA, Reis RL, Vunjak-Novakovic G. 2012. Development of silk-based scaffolds for tissue engineering of bone from human adipose-derived stem cells. *Acta Biomater* **8**: 2483–2492.
- Cukierman E, Pankov R, Stevens DR, Yamada KM. 2001. Taking cell–matrix adhesions to the third dimension. *Science* **294**: 1708–1712.
- Dahlin RL, Ni M, Meretoja VV, Kasper FK, Mikos AG. 2014. TGF- β 3-induced chondrogenesis in co-cultures of chondrocytes and mesenchymal stem cells on biodegradable scaffolds. *Biomaterials* **35**: 123–132.
- D’Andrea LD, Iaccarino G, Fattorusso R, Sorriento D, Carannante C, Capasso D, Trimarco B, Pedone C. 2005. Targeting angiogenesis: Structural characterization and biological properties of a de novo engineered VEGF mimicking peptide. *Proc Natl Acad Sci* **102**: 14215–14220.
- Dawson E, Mapili G, Erickson K, Taqvi S, Roy K. 2008. Biomaterials for stem cell differentiation. *Adv Drug Deliv Rev* **60**: 215–228.
- De Bari C, Dell’Accio F, Tylzanowski P, Luyten FP. 2001. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* **44**: 1928–1942.
- de Mendonça Costa A, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, Cerruti H, Alonso N, Passos-Bueno MR. 2008. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. *J Craniofac Surg* **19**: 204–210.
- Dexheimer V, Frank S, Richter W. 2012. Proliferation as a requirement for in vitro chondrogenesis of human mesenchymal stem cells. *Stem Cells Dev* **21**: 2160–2169.
- Diao H, Wang J, Shen C, Xia S, Guo T, Dong L, Zhang C, Chen J, Zhao J, Zhang J. 2009a. Improved cartilage regeneration utilizing mesenchymal stem cells in tgf- β 1 gene-activated scaffolds. *Tissue Eng Part A* **15**: 2687–2698.
- Diao Y, Ma Q, Cui F, Zhong Y. 2009b. Human umbilical cord mesenchymal stem cells: Osteogenesis in vivo as seed cells for bone tissue engineering. *J Biomed Mater Res A* **91**: 123–131.
- Dimitriou R, Tsiridis E, Giannoudis PV. 2005. Current concepts of molecular aspects of bone healing. *Injury* **36**: 1392–1404.
- Discher DE, Janmey P, Wang YL. 2005. Tissue cells feel and respond to the stiffness of their substrate. *Science* **310**: 1139–1143.
- Discher DE, Mooney DJ, Zandstra PW. 2009. Growth factors, matrices, and forces combine and control stem cells. *Science* **324**: 1673–1677.
- Dudas JR, Marra KG, Cooper GM, Penascino VM, Mooney MP, Jiang S, Rubin JP, Losee JE. 2006. The osteogenic

- potential of adipose-derived stem cells for the repair of rabbit calvarial defects. *Ann Plast Surg* **56**: 543–548.
- Duttenhoefer F, Lara de Freitas R, Loibl M, Bittermann G, Geoff Richards R, Alini M, Verrier S. 2015. Endothelial progenitor cell fraction contained in bone marrow-derived mesenchymal stem cell populations impairs osteogenic differentiation. *Biomed Res Int* **2015**: 659542.
- Duval E, Baugé C, Andriamanalijaona R, Bénateau H, Lelercq S, Dutoit S, Poulain L, Galéra P, Boumédiène K. 2012. Molecular mechanism of hypoxia-induced chondrogenesis and its application in in vivo cartilage tissue engineering. *Biomaterials* **33**: 6042–6051.
- Engler AJ, Sen S, Sweeney HL, Discher DE. 2006. Matrix elasticity directs stem cell lineage specification. *Cell* **126**: 677–689.
- Faia-Torres AB, Guimond-Lischer S, Rottmar M, Charnley M, Goren T, Maniura-Weber K, Spencer ND, Reis RL, Textor M, Neves NM. 2014. Differential regulation of osteogenic differentiation of stem cells on surface roughness gradients. *Biomaterials* **35**: 9023–9032.
- Fan J, Varshney RR, Ren L, Cai D, Wang DA. 2009. Synovium-derived mesenchymal stem cells: A new cell source for musculoskeletal regeneration. *Tissue Eng Part B Rev* **15**: 75–86.
- Fickert S, Fiedler J, Brenner RE. 2004. Identification of subpopulations with characteristics of mesenchymal progenitor cells from human osteoarthritic cartilage using triple staining for cell surface markers. *Arthritis Res Ther* **6**: R422–R432.
- Friedenstein AJ. 1980. Stromal mechanisms of bone marrow: Cloning in vitro and retransplantation in vivo. *Hematol Blood Transfus* **25**: 19–29.
- Friedenstein AJ, Petrakova KV, Kurolova AI, Frolova GP. 1968. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* **6**: 230–247.
- Futrega K, Palmer JS, Kinney M, Lott WB, Ungrin MD, Zandstra PW, Doran MR. 2015. The microwell-mesh: A novel device and protocol for the high throughput manufacturing of cartilage microtissues. *Biomaterials* **62**: 1–12.
- Gershovich JG, Dahlin RL, Kasper FK, Mikos AG. 2013. Enhanced osteogenesis in cocultures with human mesenchymal stem cells and endothelial cells on polymeric microfiber scaffolds. *Tissue Eng Part A* **19**: 2565–2576.
- Gieni RS, Hendzel MJ. 2008. Mechanotransduction from the ECM to the genome: Are the pieces now in place? *J Cell Biochem* **104**: 1964–1987.
- Glass KA, Link JM, Brunger JM, Moutos FT, Gersbach CA, Guilak F. 2014. Tissue-engineered cartilage with inducible and tunable immunomodulatory properties. *Biomaterials* **35**: 5921–5931.
- Gopal K, Amirhamed HA, Kamarul T. 2014. Advances of human bone marrow-derived mesenchymal stem cells in the treatment of cartilage defects: A systematic review. *Exp Biol Med (Maywood)* **239**: 663–669.
- Gronthos S, Graves SE, Ohta S, Simmons PJ. 1994. The STRO-1⁺ fraction of adult human bone marrow contains the osteogenic precursors. *Blood* **84**: 4164–4173.
- Guillotin B, Bourget C, Remy-Zoligadri M, Bareille R, Fernandez P, Conrad V, Amédée-Vilamitjana J. 2004. Human primary endothelial cells stimulate human osteoprogenitor cell differentiation. *Cell Physiol Biochem* **14**: 325–332.
- Hagmann S, Moradi B, Frank S, Dreher T, Kammerer PW, Richter W, Gotterbarm T. 2013. Different culture media affect growth characteristics, surface marker distribution and chondrogenic differentiation of human bone marrow-derived mesenchymal stromal cells. *BMC Musculoskelet Disord* **14**: 223.
- Haimi S, Suuriniemi N, Haaparanta AM, Ellä V, Lindroos B, Huhtala H, Rätty S, Kuokkanen H, Sándor GK, Kellomäki M. 2008. Growth and osteogenic differentiation of adipose stem cells on PLA/bioactive glass and PLA/ β -TCP scaffolds. *Tissue Eng Part A* **15**: 1473–1480.
- Hayashi O, Katsube Y, Hirose M, Ohgushi H, Ito H. 2008. Comparison of osteogenic ability of rat mesenchymal stem cells from bone marrow, periosteum, and adipose tissue. *Calcif Tissue Int* **82**: 238–247.
- Heino TJ, Hentunen TA, Väänänen HK. 2004. Conditioned medium from osteocytes stimulates the proliferation of bone marrow mesenchymal stem cells and their differentiation into osteoblasts. *Exp Cell Res* **294**: 458–468.
- Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A, Geiger F, Richter W. 2007. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGF β receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol* **211**: 682–691.
- Hildebrandt C, Büth H, Thielecke H. 2009. Influence of cell culture media conditions on the osteogenic differentiation of cord blood-derived mesenchymal stem cells. *Ann Anat* **191**: 23–32.
- Hiraoka K, Grogan S, Olee T, Lotz M. 2006. Mesenchymal progenitor cells in adult human articular cartilage. *Biorheology* **43**: 447–454.
- Hoch AI, Mittal V, Mitra D, Vollmer N, Zikry CA, Leach JK. 2015. Cell-secreted matrices perpetuate the bone-forming phenotype of differentiated mesenchymal stem cells. *Biomaterials* **74**: 178–187.
- Holland TA, Tabata Y, Mikos AG. 2005. Dual growth factor delivery from degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. *J Control Release* **101**: 111–125.
- Honda Y, Ding X, Mussano F, Wiberg A, Ho C-m, Nishimura I. 2013. Guiding the osteogenic fate of mouse and human mesenchymal stem cells through feedback system control. *Sci Rep* **3**: 3420.
- Hubka KM, Dahlin RL, Meretoja VV, Kasper FK, Mikos AG. 2014. Enhancing chondrogenic phenotype for cartilage tissue engineering: Monoculture and coculture of articular chondrocytes and mesenchymal stem cells. *Tissue Eng Part B Rev* **20**: 641–654.
- Hunziker EB, Driesang IM, Morris EA. 2001. Chondrogenesis in cartilage repair is induced by members of the transforming growth factor- β superfamily. *Clin Orthop Relat Res*: S171–S181.
- Huttenlocker AK, Woodward HN, Hall BK. 2013. The biology of bone. In *Bone histology of fossil tetrapods* (ed. Padian K, Lamm ET), pp. 13–34. University of California Press, Berkeley, CA.
- Hwang NS, Im SG, Wu PB, Bichara DA, Zhao X, Randolph MA, Langer R, Anderson DG. 2011. Chondrogenic prim-

J. Lam et al.

- ing adipose-mesenchymal stem cells for cartilage tissue regeneration. *Pharm Res* **28**: 1395–1405.
- Im GI, Shin YW, Lee KB. 2005. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthr Cartil* **13**: 845–853.
- Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. 1997. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem* **64**: 295–312.
- Jang BJ, Byeon YE, Lim JH, Ryu HH, Kim WH, Koyama Y, Kikuchi M, Kang KS, Kweon OK. 2008. Implantation of canine umbilical cord blood-derived mesenchymal stem cells mixed with β -tricalcium phosphate enhances osteogenesis in bone defect model dogs. *J Vet Sci* **9**: 387–393.
- Jiang Y, Tuan RS. 2015. Origin and function of cartilage stem/progenitor cells in osteoarthritis. *Nat Rev Rheumatol* **11**: 206–212.
- Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. 1998. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* **238**: 265–272.
- Jones BA, Pei M. 2012. Synovium-derived stem cells: A tissue-specific stem cell for cartilage engineering and regeneration. *Tissue Eng Part B Rev* **18**: 301–311.
- Kadiyala S, Jaiswal N, Bruder SP. 1997. Culture-expanded, bone marrow-derived mesenchymal stem cells can regenerate a critical-sized segmental bone defect. *Tissue Eng* **3**: 173–185.
- Kanichai M, Ferguson D, Prendergast PJ, Campbell VA. 2008. Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: A role for AKT and hypoxia-inducible factor (HIF)-1 α . *J Cell Physiol* **216**: 708–715.
- Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. 2006. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* **24**: 1294–1301.
- Kestendjieva S, Kyurkchiev D, Tsvetkova G, Mehadjiev T, Dimitrov A, Nikolov A, Kyurkchiev S. 2008. Characterization of mesenchymal stem cells isolated from the human umbilical cord. *Cell Biol Int* **32**: 724–732.
- Khan IM, Bishop JC, Gilbert S, Archer CW. 2009. Clonal chondroprogenitors maintain telomerase activity and Sox9 expression during extended monolayer culture and retain chondrogenic potential. *Osteoarthr Cartil* **17**: 518–528.
- Kim HJ, Im GI. 2009. Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: Greater doses of growth factor are necessary. *J Orthop Res* **27**: 612–619.
- Kim KS, Lee JH, Ahn HH, Lee JY, Khang G, Lee B, Lee HB, Kim MS. 2008. The osteogenic differentiation of rat muscle-derived stem cells in vivo within in situ-forming chitosan scaffolds. *Biomaterials* **29**: 4420–4428.
- Kim S, Kang Y, Krueger CA, Sen M, Holcomb JB, Chen D, Wenke JC, Yang Y. 2012. Sequential delivery of BMP-2 and IGF-1 using a chitosan gel with gelatin microspheres enhances early osteoblastic differentiation. *Acta Biomater* **8**: 1768–1777.
- Kim K, Lam J, Lu S, Spicer PP, Lueckgen A, Tabata Y, Wong ME, Jansen JA, Mikos AG, Kasper FK. 2013a. Osteochondral tissue regeneration using a bilayered composite hydrogel with modulating dual growth factor release kinetics in a rabbit model. *J Control Release* **168**: 166–178.
- Kim SE, Yun YP, Lee JY, Shim JS, Park K, Huh JB. 2013b. Co-delivery of platelet-derived growth factor (PDGF-BB) and bone morphogenic protein (BMP-2) coated onto heparinized titanium for improving osteoblast function and osteointegration. *J Tissue Eng Regen Med* **9**: E219–E228.
- Knippenberg M, Helder MN, Doulabi BZ, Wuisman P, Klein-Nulend J. 2006. Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. *Biochem Biophys Res Commun* **342**: 902–908.
- Kofron MD, Laurencin CT. 2006. Bone tissue engineering by gene delivery. *Adv Drug Del Rev* **58**: 555–576.
- Koga H, Muneta T, Ju YJ, Nagase T, Nimura A, Mochizuki T, Ichinose S, von der Mark K, Sekiya I. 2007. Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. *Stem Cells* **25**: 689–696.
- Koga H, Shimaya M, Muneta T, Nimura A, Morito T, Hayashi M, Suzuki S, Ju YJ, Mochizuki T, Sekiya I. 2008. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther* **10**: R84.
- Koga H, Engebretsen L, Brinckmann JE, Muneta T, Sekiya I. 2009. Mesenchymal stem cell-based therapy for cartilage repair: A review. *Knee Surg Sports Traumatol Arthrosc* **17**: 1289–1297.
- Koh JT, Ge C, Zhao M, Wang Z, Krebsbach PH, Zhao Z, Franceschi RT. 2006. Use of a stringent dimerizer-regulated gene expression system for controlled BMP2 delivery. *Mol Ther* **14**: 684–691.
- Kreja L, Brenner RE, Tautzenberger A, Liedert A, Friemert B, Ehrnthaller C, Huber-Lang M, Ignatius A. 2010. Non-resorbing osteoclasts induce migration and osteogenic differentiation of mesenchymal stem cells. *J Cell Biochem* **109**: 347–355.
- Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G, Papaccio G. 2005. A new population of human adult dental pulp stem cells: A useful source of living autologous fibrous bone tissue (LAB). *J Bone Miner Res* **20**: 1394–1402.
- Lam J, Lu S, Meretoja VV, Tabata Y, Mikos AG, Kasper FK. 2014. Generation of osteochondral tissue constructs with chondrogenically and osteogenically predifferentiated mesenchymal stem cells encapsulated in bilayered hydrogels. *Acta Biomater* **10**: 1112–1123.
- Lam J, Lu S, Kasper FK, Mikos AG. 2015. Strategies for controlled delivery of biologics for cartilage repair. *Adv Drug Deliv Rev* **84**: 123–134.
- Langenbach F, Handschel J. 2013. Effects of dexamethasone, ascorbic acid and β -glycerophosphate on the osteogenic differentiation of stem cells in vitro. *Stem Cell Res Ther* **4**: 117.
- Lee EJ, Kasper FK, Mikos AG. 2014. Biomaterials for tissue engineering. *Ann Biomed Eng* **42**: 323–337.
- Leijten JC, Georgi N, Wu L, van Blitterswijk CA, Karperien M. 2013. Cell sources for articular cartilage repair strategies: Shifting from monocultures to cocultures. *Tissue Eng Part B Rev* **19**: 31–40.

- Leijten J, Georgi N, Moreira Teixeira L, van Blitterswijk CA, Post JN, Karperien M. 2014. Metabolic programming of mesenchymal stromal cells by oxygen tension directs chondrogenic cell fate. *Proc Natl Acad Sci* **111**: 13954–13959.
- Levorson EJ, Santoro M, Kasper FK, Mikos AG. 2014. Direct and indirect co-culture of chondrocytes and mesenchymal stem cells for the generation of polymer/extracellular matrix hybrid constructs. *Acta Biomater* **10**: 1824–1835.
- Li YM, Schilling T, Benisch P, Zeck S, Meissner-Weigl J, Schneider D, Limbert C, Seufert J, Kassem M, Schütze N, et al. 2007. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. *Biochem Biophys Res Commun* **363**: 209–215.
- Li CY, Wu XY, Tong JB, Yang XX, Zhao JL, Zheng QF, Zhao GB, Ma ZJ. 2015. Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. *Stem Cell Res Ther* **6**: 55.
- Liao HT, Chen CT. 2014. Osteogenic potential: Comparison between bone marrow and adipose-derived mesenchymal stem cells. *World J Stem Cells* **6**: 288–295.
- Lim SM, Oh SH, Lee HH, Yuk SH, Im GI, Lee JH. 2010. Dual growth factor-releasing nanoparticle/hydrogel system for cartilage tissue engineering. *J Mater Sci Mater Med* **21**: 2593–2600.
- Lin X, Takahashi K, Campion SL, Liu Y, Gustavsen GG, Pena LA, Zamora PO. 2006. Synthetic peptide F2A4-K-NS mimics fibroblast growth factor-2 in vitro and is angiogenic in vivo. *Int J Mol Med* **17**: 833–839.
- 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 β -TCP ceramics. *Biomaterials* **29**: 4792–4799.
- Luo Z, Jiang L, Xu Y, Li H, Xu W, Wu S, Wang Y, Tang Z, Yonggang L, Yang L. 2015. Mechano growth factor (MGF) and transforming growth factor (TGF)- β 3 functionalized silk scaffolds enhance articular hyaline cartilage regeneration in rabbit model. *Biomaterials* **52**: 463–475.
- Lutolf MP, Gilbert PM, Blau HM. 2009. Designing materials to direct stem-cell fate. *Nature* **462**: 433–441.
- Luu HH, Song WX, Luo X, Manning D, Luo J, Deng ZL, Sharff KA, Montag AG, Haydon RC, He TC. 2007. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orth Res* **25**: 665–677.
- Majumdar MK, Banks V, Peluso DP, Morris EA. 2000. Isolation, characterization, and chondrogenic potential of human bone marrow-derived multipotential stromal cells. *J Cell Physiol* **185**: 98–106.
- Maniopoulos C, Sodek J, Melcher AH. 1988. Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats. *Cell Tissue Res* **254**: 317–330.
- Massagué J. 1990. The transforming growth factor- β family. *Annu Rev Cell Biol* **6**: 597–641.
- Mazor M, Lespessailles E, Coursier R, Daniellou R, Best TM, Toumi H. 2014. Mesenchymal stem-cell potential in cartilage repair: An update. *J Cell Mol Med* **18**: 2340–2350.
- Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ. 2012. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. *Adv Drug Del Rev* **64**: 1257–1276.
- Mellor LE, Mohiti-Asli M, Williams J, Kannan A, Dent M, Guilak F, Lobo EG. 2015. Extracellular calcium modulates chondrogenic and osteogenic differentiation of human derived adipose stem cells: A novel approach for osteochondral tissue engineering using a single stem cell source. *Tissue Eng Part A*. **21**: 2323–2333.
- Meretoja VV, Dahlin RL, Kasper FK, Mikos AG. 2012. Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. *Biomaterials* **33**: 6362–6369.
- Meretoja VV, Dahlin RL, Wright S, Kasper FK, Mikos AG. 2013. The effect of hypoxia on the chondrogenic differentiation of co-cultured articular chondrocytes and mesenchymal stem cells in scaffolds. *Biomaterials* **34**: 4266–4273.
- Meretoja VV, Dahlin RL, Wright S, Kasper FK, Mikos AG. 2014. Articular chondrocyte redifferentiation in 3D co-cultures with mesenchymal stem cells. *Tissue Eng Part C Methods* **20**: 514–523.
- Moutsatsos IK, Turgeman G, Zhou S, Kurkalli BG, Pelled G, Tzur L, Kelley P, Stumm N, Mi S, Muller R. 2001. Exogenously regulated stem cell-mediated gene therapy for bone regeneration. *Mol Ther* **3**: 449–461.
- Nayak TR, Jian L, Phua LC, Ho HK, Ren Y, Pastorin G. 2010. Thin films of functionalized multiwalled carbon nanotubes as suitable scaffold materials for stem cells proliferation and bone formation. *ACS Nano* **4**: 7717–7725.
- Nayerossadat N, Maedeh T, Ali PA. 2012. Viral and nonviral delivery systems for gene delivery. *Adv Biomed Res* **1**: 27.
- Needham CJ, Shah SR, Dahlin RL, Kinard LA, Lam J, Watson BM, Lu S, Kasper FK, Mikos AG. 2014. Osteochondral tissue regeneration through polymeric delivery of DNA encoding for the SOX trio and RUNX2. *Acta Biomater* **10**: 4103–4112.
- Nicolaidou V, Wong MM, Redpath AN, Ersek A, Baban DF, Williams LM, Cope AP, Horwood NJ. 2012. Monocytes induce STAT3 activation in human mesenchymal stem cells to promote osteoblast formation. *PLoS ONE* **7**: e39871.
- Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, Pearce S, Kasten P. 2010a. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. *Biomaterials* **31**: 3572–3579.
- Niemeyer P, Szalay K, Luginbühl R, Südkamp NP, Kasten P. 2010b. Transplantation of human mesenchymal stem cells in a non-autogenous setting for bone regeneration in a rabbit critical-size defect model. *Acta Biomater* **6**: 900–908.
- Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B. 1999. Chondroprogenitor cells of synovial tissue. *Arthritis Rheum* **42**: 2631–2637.
- Nurminskaya M, Magee C, Faverman L, Linsenmayer TE. 2003. Chondrocyte-derived transglutaminase promotes maturation of preosteoblasts in periosteal bone. *Dev Biol* **263**: 139–152.
- Nuttelman CR, Tripodi MC, Anseth KS. 2004. In vitro osteogenic differentiation of human mesenchymal stem

J. Lam et al.

- cells photoencapsulated in PEG hydrogels. *J Biomed Mater Res A* **68**: 773–782.
- Occhetta P, Centola M, Tonnarelli B, Redaelli A, Martin I, Rasponi M. 2015. High-throughput microfluidic platform for 3D cultures of mesenchymal stem cells, towards engineering developmental processes. *Sci Rep* **18**: 10288.
- O'Sullivan J, D'Arcy S, Barry FP, Murphy JM, Coleman CM. 2011. Mesenchymal chondrogenitor cell origin and therapeutic potential. *Stem Cell Res Ther* **2**: 8.
- Packer L. 1997. *Vitamin C in health and disease*. CRC, Boca Raton, FL.
- Pape HC, Evans A, Kobbe P. 2010. Autologous bone graft: Properties and techniques. *J Orthop Trauma* **24**: S36–S40.
- Park Y, Sugimoto M, Watrin A, Chiquet M, Hunziker EB. 2005. BMP-2 induces the expression of chondrocyte-specific genes in bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. *Osteoarthritis Cartil* **13**: 527–536.
- Park JS, Chu JS, Tsou AD, Diop R, Tang Z, Wang A, Li S. 2011. The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF- β . *Biomaterials* **32**: 3921–3930.
- Parmar PA, Chow LW, St. Pierre J, Horejs C, Peng Y, Werkmeister J, Ramshaw J, Stevens MM. 2015. Collagen-mimetic peptide-modifiable hydrogels for articular cartilage regeneration. *Biomaterials* **54**: 213–225.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science* **284**: 143–147.
- Pneumatics SG, Triantafyllopoulos GK, Basdra EK, Papavassiliou AG. 2010. Segmental bone defects: From cellular and molecular pathways to the development of novel biological treatments. *J Cell Mol Med* **14**: 2561–2569.
- Puetzer JL, Petite JN, Lobo EG. 2010. Comparative review of growth factors for induction of three-dimensional in vitro chondrogenesis in human mesenchymal stem cells isolated from bone marrow and adipose tissue. *Tissue Eng Part B Rev* **16**: 435–444.
- Raiche AT, Puleo DA. 2004. In vitro effects of combined and sequential delivery of two bone growth factors. *Biomaterials* **25**: 677–685.
- Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. 2001. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* **98**: 2615–2625.
- Rey-Rico A, Venkatesan JK, Sohler J, Morni L, Cucchiari M, Madry H. 2014. Adapted chondrogenic differentiation of human mesenchymal stem cells via controlled release of TGF- β 1 from poly(ethylene oxide)-terephthalate/poly(butylene terephthalate) multiblock scaffolds. *J Biomed Mater Res A* **103**: 371–383.
- Rouwema J, Westerweel PE, de Boer J, Verhaar MC, van Blitterswijk CA. 2009. The use of endothelial progenitor cells for prevascularized bone tissue engineering. *Tissue Eng Part A* **15**: 2015–2027.
- Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. 2005. Comparison of human stem cells derived from various mesenchymal tissues: Superiority of synovium as a cell source. *Arthritis Rheum* **52**: 2521–2529.
- Samorezov JE, Alsberg E. 2015. Spatial regulation of controlled bioactive factor delivery for bone tissue engineering. *Adv Drug Del Rev* **84**: 45–67.
- Santo VE, Gomes ME, Mano JF, Reis RL. 2013. Controlled release strategies for bone, cartilage, and osteochondral engineering. Part I: Recapitulation of native tissue healing and variables for the design of delivery systems. *Tissue Eng Part B Rev* **19**: 308–326.
- Santo VE, Popa EG, Mano JF, Gomes ME, Reis RL. 2015. Natural assembly of platelet lysate-loaded nanocarriers into enriched 3D hydrogels for cartilage regeneration. *Acta Biomaterialia* **19**: 56–65.
- Seebach C, Henrich D, Kähling C, Wilhelm K, Tami AE, Alini M, Marzi I. 2010. Endothelial progenitor cells and mesenchymal stem cells seeded onto β -TCP granules enhance early vascularization and bone healing in a critical-sized bone defect in rats. *Tissue Eng Part A* **16**: 1961–1970.
- Seol YJ, Park YJ, Lee SC, Kim KH, Lee JY, Kim TI, Lee YM, Ku Y, Rhyu IC, Han SB. 2006. Enhanced osteogenic promotion around dental implants with synthetic binding motif mimicking bone morphogenetic protein (BMP)-2. *J Biomed Mater Res A* **77**: 599–607.
- Shafiee A, Seyedjafari E, Soleimani M, Ahmadbeigi N, Dinavand P, Ghaemi N. 2011. A comparison between osteogenic differentiation of human unrestricted somatic stem cells and mesenchymal stem cells from bone marrow and adipose tissue. *Biotechnol Lett* **33**: 1257–1264.
- Shen H, Ma Y, Luo Y, Liu X, Zhang Z, Dai J. 2015. Directed osteogenic differentiation of mesenchymal stem cell in three-dimensional biodegradable methylcellulose-based scaffolds. *Colloids Surf B Biointerfaces* **135**: 332–338.
- Shin M, Yoshimoto H, Vacanti JP. 2004. In vivo bone tissue engineering using mesenchymal stem cells on a novel electrospun nanofibrous scaffold. *Tissue Eng* **10**: 33–41.
- Shirasawa S, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. 2006. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: Optimal condition and comparison with bone marrow-derived cells. *J Cell Biochem* **97**: 84–97.
- Sitharaman B, Shi X, Walboomers XF, Liao H, Cuijpers V, Wilson LJ, Mikos AG, Jansen JA. 2008. In vivo biocompatibility of ultra-short single-walled carbon nanotube/biodegradable polymer nanocomposites for bone tissue engineering. *Bone* **43**: 362–370.
- Solchaga LA, Penick K, Porter JD, Goldberg VM, Caplan AI, Welter JE. 2005. FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. *J Cell Physiol* **203**: 398–409.
- Sotiropoulou PA, Perez SA, Salagianni M, Baxevanis CN, Papamichail M. 2006. Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. *Stem Cells* **24**: 462–471.
- Steinmetz NJ, Aisenbrey EA, Westbrook KK, Qi J, Bryant SJ. 2015. Mechanical loading regulates human MSC differentiation in a multi-layer hydrogel for osteochondral tissue engineering. *Acta Biomater* **21**: 142–153.
- Stockmann P, Park J, von Wilmsowsky C, Nkenke E, Felszeghy E, Dehner JF, Schmitt C, Tudor C, Schlegel KA. 2012. Guided bone regeneration in pig calvarial bone defects using autologous mesenchymal stem/progenitor cells—

- A comparison of different tissue sources. *J Craniomaxillofac Surg* **40**: 310–320.
- Sukegawa A, Iwasaki N, Kasahara Y, Onodera T, Igarashi T, Minami A. 2012. Repair of rabbit osteochondral defects by an acellular technique with an ultrapurified alginate gel containing stromal cell-derived factor-1. *Tissue Eng Part A* **18**: 934–945.
- Takamizawa S, Maehata Y, Imai K, Senoo H, Sato S, Hata R-I. 2004. Effects of ascorbic acid and ascorbic acid 2-phosphate, a long-acting vitamin C derivative, on the proliferation and differentiation of human osteoblast-like cells. *Cell Biol Int* **28**: 255–265.
- Thibault RA, Mikos AG, Kasper FK. 2013. Osteogenic differentiation of mesenchymal stem cells on demineralized and devitalized biodegradable polymer and extracellular matrix hybrid constructs. *J Biomed Mater Res A* **101**: 1225.
- Tibbitt MW, Anseth KS. 2009. Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol Bioeng* **103**: 655–663.
- Tierney EG, McSorley K, Hastings CL, Cryan S-A, O'Brien T, Murphy MJ, Barry FP, O'Brien FJ, Duffy GP. 2013. High levels of ephrinB2 over-expression increases the osteogenic differentiation of human mesenchymal stem cells and promotes enhanced cell mediated mineralisation in a polyethyleneimine-ephrinB2 gene-activated matrix. *J Control Release* **165**: 173–182.
- Tsutsumi S, Shimazu A, Miyazaki K, Pan H, Koike C, Yoshida E, Takagishi K, Kato Y. 2001. Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. *Biochem Biophys Res Commun* **288**: 413–419.
- Venugopal J, Rajeswari R, Shayanti M, Low S, Bongso A, R. Giri Dev V, Deepika G, Choon AT, Ramakrishna S. 2013. Electrospayed hydroxyapatite on polymer nanofibers to differentiate mesenchymal stem cells to osteogenesis. *J Biomater Sci Polym Ed* **24**: 170–184.
- Vishnubalaji R, Al-Nbaheen M, Kadalmani B, Aldahmash A, Ramesh T. 2012. Comparative investigation of the differentiation capability of bone-marrow- and adipose-derived mesenchymal stem cells by qualitative and quantitative analysis. *Cell Tissue Res* **347**: 419–427.
- Vo TN, Kasper FK, Mikos AG. 2012. Strategies for controlled delivery of growth factors and cells for bone regeneration. *Adv Drug Del Rev* **64**: 1292–1309.
- Wang Y, Volloch V, Pindrus MA, Blasioli DJ, Chen J, Kaplan DL. 2007. Murine osteoblasts regulate mesenchymal stem cells via WNT and cadherin pathways: Mechanism depends on cell–cell contact mode. *J Tissue Eng Regen Med* **1**: 39–50.
- Wappler J, Rath B, Läufer T, Heidenreich A, Montzka K. 2013. Eliminating the need of serum testing using low serum culture conditions for human bone marrow-derived mesenchymal stromal cell expansion. *Biomed Eng Online* **12**: 15.
- Watson BM, Vo TN, Tatara AM, Shah SR, Scott DW, Engel PS, Mikos AG. 2015. Biodegradable, phosphate-containing, dual-gelling macromers for cellular delivery in bone tissue engineering. *Biomaterials* **67**: 286–296.
- Wright VJ, Peng H, Usas A, Young B, Gearhart B, Cummins J, Huard J. 2002. BMP4-expressing muscle-derived stem cells differentiate into osteogenic lineage and improve bone healing in immunocompetent mice. *Mol Ther* **6**: 169–178.
- Wu L, Leijten JC, Georgi N, Post JN, van Blitterswijk CA, Karperien M. 2011. Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. *Tissue Eng Part A* **17**: 1425–1436.
- Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. 2012. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. *Tissue Eng Part A* **18**: 1542–1551.
- Yang X, Walboomers XF, van den Beucken JJJP, Bian Z, Fan M, Jansen JA. 2008. Hard tissue formation of STRO-1–selected rat dental pulp stem cells in vivo. *Tissue Eng Part A* **15**: 367–375.
- Yang HJ, Kim KJ, Kim MK, Lee SJ, Ryu YH, Seo BE, Oh DY, Ahn ST, Lee HY, Rhie JW. 2014. The stem cell potential and multipotency of human adipose tissue-derived stem cells vary by cell donor and are different from those of other types of stem cells. *Cells Tissues Organs* **199**: 373–383.
- Yao Y, He Y, Guan Q, Wu Q. 2014. A tetracycline expression system in combination with Sox9 for cartilage tissue engineering. *Biomaterials* **35**: 1898–1906.
- Ye CP, Heng BC, Liu H, Toh WS, Cao T. 2007. Culture media conditioned by heat-shocked osteoblasts enhances the osteogenesis of bone marrow-derived mesenchymal stromal cells. *Cell Biochem Funct* **25**: 267–276.
- Yoon E, Dhar S, Chun DE, Gharibjanian NA, Evans GRD. 2007. In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model. *Tissue Eng* **13**: 619–627.
- Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. 2007. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res* **327**: 449–462.
- Young S, Patel ZS, Kretlow JD, Murphy MB, Mountziaris PM, Baggett LS, Ueda H, Tabata Y, Jansen JA, Wong M. 2009. Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. *Tissue Eng Part A* **15**: 2347–2362.
- Zeng Q, Li X, Beck G, Balian G, Shen FH. 2007. Growth and differentiation factor-5 (GDF-5) stimulates osteogenic differentiation and increases vascular endothelial growth factor (VEGF) levels in fat-derived stromal cells in vitro. *Bone* **40**: 374–381.
- Zhang X, Godbey WT. 2006. Viral vectors for gene delivery in tissue engineering. *Adv Drug Del Rev* **58**: 515–534.
- Zhang ZY, Teoh SH, Chong MS, Schantz JT, Fisk NM, Choolani MA, Chan J. 2009. Superior osteogenic capacity for bone tissue engineering of fetal compared with perinatal and adult mesenchymal stem cells. *Stem Cells* **27**: 126–137.
- Zhang F, Leong W, Su K, Fang Y, Wang DA. 2013. A transduced living hyaline cartilage graft releasing transgenic stromal cell-derived factor-1 inducing endogenous stem cell homing in vivo. *Tissue Eng Part A* **19**: 1091–1099.
- Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. 2001. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* **7**: 211–228.

Cold Spring Harbor Perspectives in Medicine

Honing Cell and Tissue Culture Conditions for Bone and Cartilage Tissue Engineering

Johnny Lam, Esther J. Lee, Elisa C. Clark and Antonios G. Mikos

Cold Spring Harb Perspect Med 2017; doi: 10.1101/cshperspect.a025734 originally published online March 27, 2017

Subject Collection [Tissue Engineering and Regenerative Medicine](#)

The Heart and Great Vessels

Ekene Onwuka, Nakesha King, Eric Heuer, et al.

Three-Dimensional Bioprinting Strategies for Tissue Engineering

Yu Shrike Zhang, Rahmi Oklu, Mehmet Remzi Dokmeci, et al.

Honing Cell and Tissue Culture Conditions for Bone and Cartilage Tissue Engineering

Johnny Lam, Esther J. Lee, Elisa C. Clark, et al.

Tissue Engineering Functional Gastrointestinal Regions: The Importance of Stem and Progenitor Cells

Andrew Trecartin and Tracy Grikscheit

Historical Perspective and Future Direction of Blood Vessel Developments

Sashka Dimitrievska and Laura E. Niklason

Craniofacial Tissue Engineering

Weibo Zhang and Pamela Crotty Yelick

The Self-Assembling Process and Applications in Tissue Engineering

Jennifer K. Lee, Jarrett M. Link, Jerry C.Y. Hu, et al.

Biologic Scaffolds

Alessandra Costa, Juan Diego Naranjo, Ricardo Londono, et al.

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>