Coculture Strategies in Bone Tissue Engineering: The Impact of Culture Conditions on Pluripotent Stem Cell Populations

Sathyanarayana Janardhanan, M.S.,¹ Martha O. Wang, B.S.,² and John P. Fisher, Ph.D.^{1,2}

The use of pluripotent stem cell populations for bone tissue regeneration provides many opportunities and challenges within the bone tissue engineering field. For example, coculture strategies have been utilized to mimic embryological development of bone tissue, and particularly the critical intercellular signaling pathways. While research in bone biology over the last 20 years has expanded our understanding of these intercellular signaling pathways, we still do not fully understand the impact of the system's physical characteristics (orientation, geometry, and morphology). This review of coculture literature delineates the various forms of coculture systems and their respective outcomes when applied to bone tissue engineering. To understand fully the key differences between the different coculture methods, we must appreciate the underlying paradigms of physiological interactions. Recent advances have enabled us to extrapolate these techniques to larger dimensions and higher geometric resolutions. Finally, the contributions of bioreactors, micropatterned biomaterials, and biomaterial interaction platforms are evaluated to give a sense of the sophistication established by a combination of these concepts with coculture systems.

Introduction

TRADITIONAL MOLECULAR BIOLOGY methods have been applied to study interactions between cell populations of different phenotypes involved in bone tissue development. The surge in the application of pluripotent mesenchymal stem cell (MSC) populations has renewed interest in understanding cell interactions in different schemes of tissue development. Early studies placed related cell types in direct contact to evoke the exchange of signaling molecules by the formation of gap junctions. With the development of tools within *in vitro* biology, these populations were then cultured in proximity, but at a finite distance, which eliminates the chance of cell-to-cell contact, encouraging communication by means of soluble protein expression into the surrounding media in an indirect coculture setting.¹ The introduction of biomaterials in the area of bone tissue development introduced a third component to these *in vitro* experiments. Biomaterials enabled the use of larger populations and provided an accurate reproduction of microenvironments for the coculture of MSCs and related differentiated cells. Most preliminary biomaterial-based coculture experiments were focused on demonstrating the targeted differentiation that was achieved as a result of premeditated juxtaposition of known cell types and previously established capacity of such materials to support the differentiation.

In more recent studies, these developments are used to decipher intercellular signaling and the key factors involved at much higher resolutions using techniques, including gene expression quantification (microarrays, microRNA assays), biomaterial design (stereolithography, microfluidics), and antibody research.^{2–5} The various forms of intercellular signaling that can occur in any given coculture system have been previously described.⁶ Cells are known to interact by cell-to-cell contact or soluble cytokine secretion.⁷ The modalities of intercellular communication impact the regulatory control of these processes and the significance of each interaction is often exploited while developing tissue engineering strategies.⁸

Research in the field of bone tissue engineering provides us information identifying specific factors, pathways, and mechanisms involved in formation of bone precursor cells, mineralized matrix, and the physiological characteristics of these cells in a tissue-engineered environment.^{9–14} Of particular focus in this review is the body of tissue engineering that deals with applying MSCs to differentiate and mature into osteoblasts and eventually deposit mineralized matrix. Molecular signals play an important role in this differentiation process. Additionally, it is widely acknowledged that the intercellular signaling during endochondral and intramembranous ossification are significant to comprehending processes such as wound healing or host tissue

¹Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, Maryland.

²Fischell Department of Bioengineering, University of Maryland, College Park, Maryland.

incorporation of biomaterials.^{15–18} This is especially true with cell-seeded implants that can interact with the surrounding cells in the host tissue. To understand the implications of such signaling pathways, *in vitro* strategies are often employed to identify and establish relationships between different cell types and how closely their interactions resemble known theories of bone tissue formation. Coculture techniques provide this opportunity to place cells in suitable proximity to induce differentiation or growth in a fashion that closely correlates to the biological phenomena we aim to understand.^{1,5}

The microenvironment around pluripotent stem cells exerts a considerable influence on the differentiation of the population. The two main contributing factors that define a microenvironment are the physical form in which the individual cell populations are presented and the properties of the individual morphogens present in the system. A biomaterial may be used to augment the nature of cellular interaction permitted by the host environment and subsequently influencing the physiological characteristics such as proliferation, differentiation, and protein expression.^{19,20} Secreted proteins play a major role in the intercellular communication. Proteins involved in such signaling can be intracellular, matrix bound, or secreted into the adjoining media volume. The spatial distribution of these cells and their related matrices influence the accessibility and local concentration of these proteins. In addition to the choice of biomaterial and cell-secreted protein content, the relationship between the coculture method, and the signaling modality provides an additional degree of freedom that can be used to understand and apply these interactions. For instance, one of the earliest coculture experiments established that MSCs and chondrocytes cocultured on the same pellet can deposit a cartilaginous matrix.⁸ The indirect coculture of similar cells has been shown to yield different results in a number of other studies, raising the need for a consensus on the role of factors in the engineering of mineralized bone tissue and their occurrence as a function of the method used.^{21–23} Through the course of this review, we attempt to present a synthesis of outcomes of coculture strategies in bone tissue

engineering and how recent advancements can be applied to further our understanding of molecular interactions, and, finally, to succeed in the ultimate goal of engineering viable bone tissue (Fig. 1).

Common Platforms in CoCulture Tissue Engineering

Two enabling platforms have contributed to studying relationships in coculture systems: micromass culture and indirect coculture.

Micromass culture

Micromass culture involves high-density *in vitro* culture of cell populations²⁴ (Fig. 2A). The interest in this technique of cell culture grew out of studies such as those attempting to differentiate embryonic stem cells into chondrogenic nodes.²⁵ The high-density cell populations concentrated into a small volume enable the creation of three-dimensional (3D) constructs that consist strictly of cells and cell-based matrix products, thus eliminating the need for a biomaterial to host these cells.^{26,27} This scaffold-free culture can be especially important in certain types of studies that aim at understanding only the signal transduction cascades involved and avoid any influences exerted by the presence of a biomaterial. In these studies, close tracking of a gene, a protein, and matrix expression is necessary to understand the underlying mechanisms that control processes like differentiation and growth.^{28,29} The micromass system provides a platform to track short-term expression trends that can yield large sample populations for gene and protein studies.

The micromass platform is used extensively for growth factor delivery.³⁰ With regard to bone tissue engineering, this can be especially important to understand the effects of exogenously delivered factors that constitute one of the central tenets of the field. The high-density cell populations enable a greater efficiency in delivering of these factors and the biomaterial-free construct ensures less loss during transport as compared to traditional delivery methods. One can argue that this mode of delivery brings about a kinetic disadvantage to the cells at the core of the micromass. However, with

FIG. 1. Scale of lengths involved in coculture interactions can be very important in designing strategies for MSC differentiation. Modes of interaction between cell populations influence the growth and differentiation of MSCs by determining the local microenvironment. Cells from a mesenchymal lineage respond to influences exerted by morphological conditions as well as a range of factors that can be intracellular, matrix bound, or soluble. The availability and abundance of these factors is highly dependent on the spatiotemporal relation of the intercellular communication, thus making the study of these scales of length important to tissue engineering applications. MSC, mesenchymal stem cell.

FIG. 2. (A) Micromass coculture: high volumes of cells may be pelleted together in a microcentrifuge tube. This micromass coculture allows for the study of signaling pathways without any interference from biomaterials present in a scaffold. Pelleted cells may then be placed into a well for further culturing or may be kept in the microcentrifuge tube. (B) Indirect coculture: cell populations may be cocultured without physical contact through use of a transwell system. This indirect coculturing allows for any soluble factors to be shared through media, while individual populations may be cultured using different techniques. For example, in this figure, chondrocytes are cultured in alginate beads above the monolayer culture of MSCs. (C) *Ex-vivo* coculture: explanted tissues may be cocultured with cells as an *ex-vivo* study. This allows for the maintenance of the microenvironment of the explant while sharing any signaling molecules through the media. For example, culturing native tissue explants with target MSCs enable the solubilization of such factors that can otherwise be hard to synthesize *in vitro* or introduce exogenously. (D) Conditioned media: conditioned media studies may be used to evaluate the impact of cell secreted factors on differentiating cells. Conditioned media studies are often used to evaluate cell cross talk within cell signaling pathways.

suitable optimization, a scaffold-free delivery system can be advantageous in the delivery of small molecules in tissue engineering systems.

Another application of micromass cultures is in transfection studies. The reasons why transfecting micromass cultures can be more effective as compared to biomaterial or monolayer cultures are similar to the reasons mentioned with regard to growth factor delivery.^{31,32} The tightly packed volume of cells enables effective delivery of vehicles in a small region of space, which can otherwise be compromised by absorptive factors such as materials or media in other culture systems. The core strength of micromass cultures is the enhanced cell densities and confined volumes that create a sharp advantage while preparing cells for tissue culture applications. The capacity of this platform to be used in cocultures flows immediately from these advantages in enhancing intercellular interactions. In some cocultures, MSC-chondrocyte, MSC-epithelial cell or mesenchymal-hematopoietic stem cell culture, a number of added growth factors are used to influence differentiation and growth in addition to the secreted factors. The combination of traditional coculture techniques with the unique advantages offered by high-density micromass cultures creates a powerful platform for high-throughput preparation of cells for tissue culture. Although using micromass techniques partially or entirely for *in vivo* applications has yet to be demonstrated, the platform provides opportunity for studying interactions and directing differentiation.^{15,17,33,34}

Indirect cocultures

The indirect coculture system is often utilized to study paracrine interactions between distinct cell populations. It differs from the micromass system in that it allows for various physical geometries within the culture in which individual cell population can be hosted before, during, and after the coculture. Cells to be used in coculture can be cultured in various forms, including monolayer, 3D scaffolds, or even in micromass culture. The physically distinct locations of the cell populations help track individual phenotype changes and gene expression characteristics.^{21–23,27,35} The shared media volume also aids in accounting for factors that may be constitutively expressed by one or more of the cell populations, as well as for any temporal changes in the secretory protein expression profile that is typical of many primary cell populations involved in tissue regeneration.

In the context of bone tissue engineering, this form of coculture has been exploited for the study of differentiation processes of MSC. For instance, the endochondral ossification process involves a spatiotemporal gene expression gradation in chondrocytes, mesenchymal cells, and eventually osteoblasts. This gene expression progressively causes it to change the matrix composition and to secrete factors that trigger pathways causing switches in the phenotype and characteristics. The temporal changes during this process can be studied effectively through an indirect coculture system. Paracrine signals between the distinct populations are

exchanged via soluble media at all times, while the individual cell populations can still be kept isolated. The second advantage of this system is that the cell populations can be in different physical forms. Recent research has cast light on the phenotype changes especially in chondrocytes with changes in morphology. While attempting to understand processes such as endochondral ossification, it is important to be able to carry out studies without introducing an added effect of morphological changes owing to culture of cells *in vitro* (Table 1). Some specific examples of indirect coculture techniques are discussed below.

***In vitro* cocultures.** The early *in vitro* cocultures of chondrocytes and osteoblasts aimed to understand the effects of molecules believed to play a role in the process of bone development.¹ Most of these early studies focused on the factors' impact on growth conditions (e.g., proliferation and secretion of other known factors). These studies established that proteins secreted by osteoblasts stimulated the proliferation of chondrocytes.¹ Additionally, they concluded that the ratio of the active to latent forms of tumor growth factor- β 1 had a role to play in the phenomena.¹ Later studies investigating cell differentiation identified a number of factors that could play a role in the communication between chondrocytes and osteoblasts in during differentiation. Cell communication through paracrine signaling has demonstrated that it plays a role in the selective osteogenic differentiation of MSC when exposed to soluble factors secreted by nonhypertrophic chondrocytes cultured in a transwell membrane system^{22,23} (Fig. 2B). These results are different from the work of other studies, which demonstrated a higher expression of cartilaginous matrix or a chondrogenic differentiation.⁸ Other studies have shown with a transwell system, the role of cartilage explant secreted factors on the chondrogenic differentiation of MSCs.³⁶ The modes of paracrine signaling in each of these studies bear a unique significance for future studies. A similar osteogenic inductive effect was discovered on bone marrow stromal cells by primary articular cartilage chondrocytes in 3D scaffolds.³⁶

As noted earlier, chondrocytes cultured in 3D scaffolds retain their phenotype, so changes are a result of a biological response and not biased by dedifferentiation.^{37,38} However, it is yet to be established whether a micromass culture has any dedifferentiating impact on primary cell populations used in bone tissue engineering. Some studies note a 3D morphology is critical to the retention of the chondrogenic phenotype.³⁸ For example, embryonic bodies derived from embryonic stem cells differentiate better in the chondrogenic medium when cultured in a 3D scaffold as compared to a monolayer culture when subjected to an identical induction.²⁵ Other studies come to a similar conclusion in their review of endothelial cell–osteoblast interactions. They allude to the necessity of a suitable 3D environment to enhance cell survival and cell–cell interactions.^{39,40} As chondrocyte involvement precedes osteoblast involvement in the endochondral ossification process, maintaining the phenotype of these cells is essential in the application of cocultured constructs in bone tissue engineering.⁴¹

While chemical supplements can effect MSC differentiation, paracrine signaling from differentiated cell types can also be an effective technique to induce differentiation. This method of paracrine signaling to induce differentiation may be used in an implantable coculture system. The extent and nature of differentiation using chemical stimulants and/or exogenous factors is significantly different from differentiation via continuous coculture methods without exogenous factors.⁴² While supplements improve the *in vitro* differentiation of implantable cultures, further work is required to ascertain the optimal stage of *in vitro* osteogenic differentiation needed for appropriate bone formation and host tissue integration. Recent studies have investigated the roles played by a host of factors such as Indian Hedgehog, parathyroid hormone-related peptide, matrix metalloproteinase 9, matrix metalloproteinase 13, vascular endothelial growth factor, and certain members of the collagen family, including Col1A1, Col1A2, and ColX and other matrix protein such as fibromodulin, aggrecan, and versican in the differentiation of MSCs (Table 2).^{2,16,17,43}

TABLE 1. COCULTURE STRATEGIES AND ENSUING RESULTS

	Direct contact		Soluble media contact		
	Micromass	Pellet culture	<10 ⁻⁶ m	Transwell <10 ⁻³ m Soluble media	Scaffolds >10 ⁻² m
Type of coculture					
Length scale of intercellular signaling	<10 ⁻⁶ m	<10 ⁻³ m			
Modes of contact between cells	Gap junctions	Extracellular processes			
Bioreactor	No	No	No	No	Yes
Differentiated state of MSC					
MSC-chondrocyte	Chondrogenic			Osteogenic	Osteogenic
MSC-OA chondrocyte					Osteogenic
ECM proteins secreted					
Collagen types	II, X	II		II, X	
ECM-linked proteins		Aggrecan, GAG		OC	Aggrecan, OC
Non-ECM proteins secreted					
Growth factors				VEGF	
Cytokines	ALP			MMP13, ALP	PTHrP, ALP
Intracellular molecules		Sox9		Sox9	

A comparison between scales of length involved in intercellular interactions in different coculture systems in bone and cartilage tissue engineering.

MSC, mesenchymal stem cells; OA, osteoarthritic; ECM, extracellular matrix; GAG, glycosaminoglycan; OC, osteocalcin; VEGF, vascular endothelial growth factor; ALP, alkaline phosphatase; MMP13, matrix metalloproteinase 13; PTHrP, parathyroid hormone-related peptide.

TABLE 2. CYTOKINES INVOLVED IN COCULTURE SYSTEMS

Length scale	Reference	Type of coculture	Population	Reported protein expression							Other factors suggested
				MMP family	Col2	Col10	ALP	OC	Sox9	VEGF	
<10 ⁻⁶ m	71	Micromass	MSC-chondrocyte			X	X				
	26	Micromass	MSC-chondrocyte		X						
	27	Micromass	MSC-chondrocyte		X						
	72	Pellet	MSC-chondrocyte		X				X		Aggrecan
<10 ⁻³ m	8	Micromass	MSC-chondrocyte		X						
	73	Indirect	MSC-chondrocyte		X				X	X	
	36	Indirect monolayer	MSC-chondrocyte						X		
	22	Indirect monolayer	MSC-chondrocyte			X	X	X			
	1	Indirect monolayer	OB-chondrocyte								TGFB-1
	42	Indirect monolayer	OB-chondrocyte		X		X				Aggrecan
<10 ⁻² m	3	Indirect monolayer	OB-chondrocyte								OSF-1
	74	Indirect-scaffold	OB-chondrocyte								OSF-1, PTHrP
	21	Indirect-scaffold	MSC-chondrocyte	X	X		X	X			
	35	Indirect-monolayer	MSC-chondrocyte		X				X		Aggrecan
>10 ⁻² m	48	Bioreactor	MSC-epithelial		X						

Cytokines play an important role in a tissue engineering strategy and the nature of molecules expressed by coculture systems can influence the application of a certain method. OB, osteoblast; TGFB-1, transforming growth factor-B1; OSF-1, osteoblast specific factor-1.

The mechanisms of secretion of molecular signals believed to impact MSC differentiation vary largely. Some growth factors are secreted constitutively and their expression rates are not influenced by the ongoing differentiation state of the cells in the coculture. Other growth factors show a significant shift in expression in response to the activity during the coculture. These covariant factors demonstrate the potential of chemical cross talk that causes differentiation and subsequent production of important matrix-related factors.²¹ It is evident from the literature that paracrine signaling between progenitor cells and differentiated cell types provides an excellent starting point for developing therapeutic applications in injury and defect repair.^{4,44}

Ex vivo cocultures. Tissue explants are often used as a source of morphogenetic factors to influence the differentiation of cells. An intact tissue sample or a decellularized mass of a section of a tissue can yield a large quantity of signaling factors that can be applied to *in vitro* studies. This intact tissue sample may be placed in coculture with cells for an *ex-vivo* coculture (Fig. 2C). The role played by the microenvironment on the physiological characteristics and molecular behavior has been evaluated previously.^{35,36,45} Although well reproduced in a number of *in vitro* experiments, there is still need for the evaluation of the molecules that play an important role as molecular switches to elicit or aid differentiation cascades. Particularly, the roles of the extracellular matrix (ECM) components and the proteins that bind to the ECM, during differentiation and regulation of bone precursor cells, are yet to be characterized. Since the regulation of these matrix-related proteins is carried out in an autocrine fashion, the development of signaling cascades affecting their biosynthesis can happen over long periods of time. Using primary cells in short-term coculture experiments often does not provide enough temporal flexibility to enable the synthesis of these factors. Therefore, the lack of these factors often leads to an insufficient reproduction of the *in vivo* microenvironment conditions. Differentiated primary cells placed in coculture with precursor cells aid in expressing and recreating a number of these factors. *Ex vivo* experiments that culture native tissue explants with target MSCs enable the solubilization of such factors that can otherwise be hard to synthesize *in vitro* or introduce exogenously. Another important aspect of *ex vivo* cocultures is that cellular components introduced into the coculture system by the explants can play a role in the differentiation of MSCs.³¹ While it is not well documented if contact-interactions between cell processes originating from explanted tissues could regulate differentiation or proliferation of MSCs, one could hypothesize that under conditions that occur *in vivo*, these processes could act in regulating differentiation. These cell processes along with gap junctions and other paracrine interactions during the cell signaling cascades constitute the process of endochondral ossification. Of special interest in this scheme of signaling is the demonstrated chemotactic effect exerted by the explants on monolayer MSCs that are grown in coculture. This phenomenon can be exploited to distribute MSCs across the target areas of growth or within a macroporous scaffold.

Ex vivo systems pose one drawback in their application to coculture experiments. Since the explants are acquired primarily from the harvested tissue, the biological factors, such

as distribution of cells or cellular components in the donor site, can add a degree of uncertainty to the experimental design. While the overall signaling effects can be attained in the right media, the ability to optimize the level of induction can be limited due to the fixed densities of cells and factors in the explants. Nonetheless, such systems can be excellent platforms to understand the bridge between *in vitro* tissue development and *in vivo* implant integration.

***In vivo* cocultures.** The concept of *in vivo* cocultures applies to techniques used to implant heterogenous cell mixtures, usually after encapsulation in a scaffold or spheroids. Implanting a cell mixture ectopically can utilize the micro-environment of the implantation site and also the regulatory effect levied by the paracrine signaling effecting by the cocultured cell populations. This concept has been utilized in embryonic stem cell research to commit the differentiation to a predetermined lineage.²⁶ In other experiments, *in vitro* pretreatments of progenitor cells were followed by encapsulation and ectopic implantation *in vivo* to elicit differentiation. Such combination of techniques opens doors to a number of possible strategies that can be employed to repair defects. Materials that can be used as prevascularized scaffolds, cancellous scaffolds, or layered constructs form ideal candidates for *in vivo* bone tissue engineering applications. Previous research has demonstrated that ectopically implanted chondrocytes could provide soluble signals required for the differentiation of coimplanted and cocultured bone marrow stromal cells.⁴⁶ Furthermore, the corresponding *in vitro* coculture demonstrates the involvement of soluble factors rather than cell-cell contact as playing a role in the induction. The biological significance of such close appositions of cell populations should be completely understood to predict accurately their capacity to calcify and subsequently form bone. The stability of the individual phenotypes can be easily ascertained and optimized *in vitro* to balance influencing parameters.

Conditioned media culture. A number of differentiation pathways in the osteogenic signal expression cascade are triggered by proteins that are expressed by cells from the mesenchymal lineage without any pretreatment.²¹ Media used to incubate such cells can accrue a sufficient concentration of these proteins to elicit differentiation of MSCs when added exogenously. This method of preconditioning of media has been used to differentiate MSCs and is known as a preconditioned, or a conditioned media culture (Fig. 2D). The potential of this technique has identified the existence of cross talk between cocultured cell types. Preconditioned media may also elucidate the influence played by such intercellular signaling through modulating the signal expression. The period of incubation and volume of conditioned media required to differentiate a population of MSCs can be controlled. The related extents of differentiation from the modified preconditioned media allows for the correlation of secreted factor delivery with its consequence. This can be especially important while scaling up such operations to bioreactors, where this method of transferring soluble factors from one culture to another can be translated into a continuous process to ensure constant supply of signaling molecules as opposed to the pulsed delivery in a static conditioned media coculture.⁴⁷

Coculture bioreactors

Recent interest in bioreactors has enabled researchers to scale up traditional *in vitro* culture systems. Coculture bioreactor systems have been attempted in the past as extensions of *in vitro* cultures with mixed success. Since bioreactors typically allow for compartmentalization of the cells, they can provide a platform for multiple population cocultures that are otherwise difficult to establish *in vitro*. The additional degree of freedom introduced in bioreactor cultures is achieved through a range of material geometries and sizes to host the cells, most of which constitute the limitations of traditional *in vitro* systems.^{48,49} Many standard hydrogels are not directly compatible with a bioreactor due to their mechanical instability in a flow system. However, there is vast scope for optimization of parameters such as the mechanical strength, pore size, and introduction of multiple layers of reinforcement to provide the required properties, flow rates, and reactor geometry to circumvent the inability to use many standard hydrogels. Bioreactors represent an ideal manner to engineer bone tissue due to their ability to form biocompatible tissue that possesses not only the mechanical characteristics, but also the biochemical identity of bone tissues. If these two abilities are present, then it ensures complete integration when brought to a clinical setting. The limiting challenges that *in vitro* cultures face can be met by bioreactor cultures ability to create the ideal microenvironment through a large volume of culture space.

Beyond the obvious purpose of scale up, bioreactors have been employed for multiple purposes. The integrity of biomaterials in a bioreactor, in terms of their capacity to support growth and differentiation, can be viewed as a good measure of their performance and efficacy when used *in vivo*.⁵⁰ A number of bioreactor studies have explored different biomaterial parameters that affect flow such as scaffold architecture and adhesion characteristics. This can be especially important in bone tissue development due to the strong influence of oxygen levels on the expression and regulation of osteogenic markers such as osterix and osteonectin.⁵¹ As mentioned earlier, indirect coculture systems provide efficient means of studying growth factor involvement and delivery. With the introduction of convection, the biomaterials and cells used in these systems can be effectively made into various forms and orientations to yield a number of meaningful combinations that mimic *in vivo* tissue development. Studies have shown that convection improves distribution, growth of MSC in bioreactors, and added shear stress promotes greater differentiation.^{52–57} Other studies have successfully demonstrated that two or more distinct populations can be cocultured in a flow system to yield an implantable tissue engineering construct with well-defined and controllable properties. The ability to delineate and quantify limiting factors and culture outcomes in scaffold-based bioreactor cultures has enabled these reactors to play a role in developing bone tissues and endothelial, hematopoietic, cartilaginous, and other such peripheral cellular systems.^{58,59} MSCs in scaffold culture in bioreactors were demonstrated to play a role in effectively expanding and enriching a CD34+ hematopoietic stem cell population.^{60,61} MSC-endothelial cell coculture is a widely studied field to develop bone tissue engineering strategies for creating simultaneous calcification and vasculature of scaffolds. As mentioned earlier, the

capacity to compartmentalize different populations while using a common flow medium enables a whole range of bioreactor configurations to be used.^{62–64}

Challenges and Recent Advances

Tools that enable enhanced systematic evaluation of influencing parameters, such as high-resolution imaging, micro patterning, protein assembly, high-throughput gene expression methods, and advanced bioreactor techniques, are now applied to study coculture systems. While scaling up a coculture system, the influencing factors pose interesting challenges, like sustaining a uniform microenvironment while balancing the flow rate with the appropriate residence times for all soluble factors involved in the communication. With increases in the cell population size, it is important to understand the short scales of interaction between key signaling molecules. Increasing the cell population size raises nonuniformity issues due to flow and resolution. Large bioreactors compromise the detail of interaction, thus not realizing the full potential of intercellular signaling (Fig. 1). Thus, in large-scale cocultures, there is a simultaneous need for enhanced visibility of cell populations to one another and increased volume of cells. The central understanding of tissue engineering stresses the cooperative effects of cells, biomaterials, and signaling molecules. To support the characteristics of the cells and signaling factors while retaining the specificity of the interaction, biomaterials have now been enhanced by micropatterning to provide predictable spatial orientations between cells over large surface areas or volumes (two-dimensional micropatterning or 3D-controlled architecture). These trends are gaining prominence in recent years with the development of study into microarchitectures and patterned surfaces. Creating well-defined selectively adhesive regions over large surfaces addresses the issues with nonuniformity of interaction due to varying microenvironments. Additionally, the availability of biomaterials with well-defined microarchitecture resolves the issues with nonuniformity due to flow.

The potential to superimpose known techniques of biomaterial synthesis to produce hybrid bilayers could provide the solution to the issue of vascularization in bone tissue engineering. Most bone development strategies aim to achieve effective comaturation of osteoblastic and endothelial subpopulations to form an osseous body with components of a continuous vascular matrix. The development of microarchitected biomaterials with the employment of influences exerted by matrix-based collagens and other adhesive materials offers new tools to emulate early bone development *in vitro*. Newer bioreactors can provide predictable convective flow over a volume space regardless of the geometric orientation of the enclosed material, which allows for the maintenance of physiological conditions. A combination of these techniques can effectively address most physical challenges associated with a high-matrix-yield, low-variability cocultures.

MicroPatterning in Coculture Tissue Engineering

Developments in biomaterial lithography techniques have enabled the culture of multiple populations of cells in well-defined microarchitectures at high-geometric resolutions. Recent work by Takahashi *et al.* where they have adopted

capillary force lithography to yield a wide range of coculture patterns to understand aspects of hepatocyte-fibroblast cocultures, such as existence of gap junctions, more sophisticatedly.⁶⁵ This demonstrates how parameters such as spatial distribution and orientation of cells, which are generally random during culturing, can now be well defined to carry out high-resolution optical analyses. A similar platform was developed much earlier to study hepatocyte and non-parenchymal cell interactions.⁶⁶ This platform along with laser-mediated cell population recovery techniques pose excellent avenues in bone tissue engineering especially when studying transient phenotype changes.

With the growing knowledge of bioreactors and biomaterial–cell interactions, the need to visualize cells and more importantly the cross talk between distinct populations is gaining increasing relevance. Micropatterning of biomaterials to accommodate cells in very specific spatial configurations provides coculture tissue engineering a large scope to study interactions and quantify local phenomena such as matrix production, creation of gap junctions, and transport of signaling molecules that can be crucial while designing systems of increasing complexity.^{59,67–69} In bone tissue engineering, there is increased attention being given to controlled microarchitecture of scaffolds and its possible influence on differentiation.⁷⁰ The ability of cells to adhere better and form interconnected colonies in controlled architectures influences the growth, differentiation, and the ultimate suitability of these biomaterials. At a macroscopic level, this is encouraging for tissue engineering due to the higher predictability of flow parameters in controlled architecture scaffolds, which results in better scale up and compartmentalization. To use these micropatterned biomaterials for better imaging cell–cell interactions, the seeding densities often have to be much smaller and sometimes the cells are grown under conditions under which are less conducive for growth and adhesion.⁸ Some interesting work in high-resolution imaging of cocultured populations demonstrate the scope of this platform such as localizing enzymes and proteins secreted as part of a molecular cross talk or visualizing the existence of gap junctions and distribution of secreted matrix.

Conclusion

Coculture strategies provide excellent platforms for understanding biological interactions between cell types known to play a concerted role in key developmental processes that lead to tissue repair and regeneration. Although the concepts of coculture are relatively new to tissue engineering, they have been applied in a number of other fields such as cancer research, tumor biology, and a developmental biology. In bone tissue engineering, there is a need to gain understanding and control over both mechanical and biological properties of biomaterials to achieve successful clinical outcomes. One important challenge in utilizing cocultures is ensuring a uniform microenvironment across the entire span of the biomaterial. Ensuring a uniform microenvironment is difficult when cells are not directly in contact with one another. When this occurs, the peripheral cells are exposed to a different microenvironment as compared to cells at the core of the material. Suitable treatment methods can avoid this uncertainty by ensuring an active convective flow around the

material. Alternatively, this situation may be studied by applying such differential microenvironments to the experiments' advantage. There are a few questions that need to be addressed in the context of bone tissue engineering that may be addressed using a coculture strategy. Characterization of the effectiveness of secreted growth factors as a function of the paracrine communication length for different families of factors can be useful with the arrival of many new coculture strategies. Although this paracrine communication can vary significantly between cell types and conditions, a good grasp of the upper and lower bounds of the length of interaction can aid in streamlining the design of future platforms. The progress made over the years has developed a sound understanding of the influence of coculture on the growth and differentiation of pluripotent cells. The real challenge in utilizing cocultures to improve clinical tissue preparations is in optimizing physical characteristics of the system. Some of these physical characteristics are diffusive length, spatial distribution of factors, time scale of diffusion, and the biological significance of secreted factors. A good starting point would be to model an *in vivo* developmental process as a coculture system and derive these parameters to emulate similar results in a laboratory setting. The framework to produce a high-quality, low-variability matrix product is possible with the systematic assessment of these process parameters and their association to the developmental process along with the introduction of improved resolution and visibility of neighboring populations. Such frameworks will provide the tools to produce reliable products from known biological associations, thus strengthening product development pipelines that make tissue engineering products commercially available. Additionally, the frameworks will be able to provide the required confidence to standards and regulatory bodies through quantifiable parameters that influence their ultimate success. The path to developing frameworks based on physical and physiological parameters can be carried out by targeting bone development research that assesses the biochemistry of secreted factors, gradation of signaling pathways during development, period of maturation of the matrix, and its relation to spatially sensitive organization of enabling cell populations. Advanced biomaterials, bioreactors, and enhanced knowledge of the pathways will provide the context for further development of cocultures with the aim of engineering calcified matrix within the target microenvironment.

Acknowledgment

This research was supported by the National Institutes of Health (R01 AR061460).

Disclosure Statement

No competing financial interests exist.

References

- Lacombe-Gleize, S., Gregoire, M., Demignot, S., Hecquet, C., and Adolphe, M. Implication of TGF beta 1 in co-culture of chondrocytes-osteoblasts. *In Vitro Cell Dev Biol Anim* **31**, 649, 1995.
- Barry, F., Boynton, R.E., Liu, B., and Murphy, J.M. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res* **268**, 189, 2001.
- Sanchez, C., Deberg, M.A., Piccardi, N., Msika, P., Reginster, J.Y., and Henrotin, Y.E. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage* **13**, 988, 2005.
- Grassel, S., and Ahmed, N. Influence of cellular microenvironment and paracrine signals on chondrogenic differentiation. *Front Biosci* **12**, 4946, 2007.
- Spalazzi, J.P., Dionisio, K.L., Jiang, J., and Lu, H.H. Osteoblast and chondrocyte interactions during coculture on scaffolds. *IEEE Eng Med Biol Mag* **22**, 27, 2003.
- Hendriks, J., Riesle, J., and van Blitterswijk, C.A. Co-culture in cartilage tissue engineering. *J Tissue Eng Regen Med* **1**, 170, 2007.
- Yoon, D.M., and Fisher, J.P. Chondrocyte signaling and artificial matrices for articular cartilage engineering. *Tissue Eng* **585**, 67, 2007.
- Tsuchiya, K., Chen, G.P., Ushida, T., Matsuno, T., and Tateishi, T. The effect of coculture of chondrocytes with mesenchymal stem cells on their cartilaginous phenotype *in vitro*. *Mater Sci Eng C-Bio S* **24**, 391, 2004.
- Hoemann, C.D., El-Gabalawy, H., and McKee, M.D. *In vitro* osteogenesis assays: influence of the primary cell source on alkaline phosphatase activity and mineralization. *Pathol Biol (Paris)* **57**, 318, 2009.
- Goldring, M.B., Tsuchimochi, K., and Ijiri, K. The control of chondrogenesis. *J Cell Biochem* **97**, 33, 2006.
- Takagi, M., Kitabayashi, T., Koizumi, S., Hirose, H., Kondo, S., Fujiwara, M., *et al.* Correlation between cell morphology and aggrecan gene expression level during differentiation from mesenchymal stem cells to chondrocytes. *Biotechnol Lett* **30**, 1189, 2008.
- Boskey, A.L., Stiner, D., Binderman, I., and Doty, S.B. Type I collagen influences cartilage calcification: an immunoblocking study in differentiating chick limb-bud mesenchymal cell cultures. *J Cell Biochem* **79**, 89, 2000.
- Heng, B.C., Cao, T., and Lee, E.H. Directing stem cell differentiation into the chondrogenic lineage *in vitro*. *Stem Cells* **22**, 1152, 2004.
- Yoon, D.M., Hawkins, E.C., Francke-Carroll, S., and Fisher, J.P. Effect of construct properties on encapsulated chondrocyte expression of insulin-like growth factor-1. *Biomaterials* **28**, 299, 2007.
- Ortega, N., Behonick, D.J., and Werb, Z. Matrix remodeling during endochondral ossification. *Trends Cell Biol* **14**, 86, 2004.
- James, C.G., Stanton, L.A., Agoston, H., Ulici, V., Underhill, T.M., and Beier, F. Genome-wide analyses of gene expression during mouse endochondral ossification. *PLoS One* **5**, e8693, 2010.
- Colnot, C. Cellular and molecular interactions regulating skeletogenesis. *J Cell Biochem* **95**, 688, 2005.
- Kozhevnikova, M.N., Mikaelian, A.S., and Starostin, V.I. Molecular and genetic regulation of osteogenic differentiation of mesenchymal stromal cells. *Izv Akad Nauk Ser Biol* **261**, 2008.
- Kim, K., Dean, D., Lu, A., Mikos, A.G., and Fisher, J.P. Early osteogenic signal expression of rat bone marrow stromal cells is influenced by both hydroxyapatite nanoparticle content and initial cell seeding density in biodegradable nanocomposite scaffolds. *Acta Biomaterialia* **7**, 1249, 2010.
- Coates, E.E., and Fisher, J.P. Phenotypic variations in chondrocyte subpopulations and their response to *in vitro* culture and external stimuli. *Ann Biomed Eng* **38**, 3371, 2010.

21. Thompson, A.D., Betz, M.W., Yoon, D.M., and Fisher, J.P. Osteogenic differentiation of bone marrow stromal cells induced by coculture with chondrocytes encapsulated in three-dimensional matrices. *Tissue Eng Part A* **15**, 1181, 2009.
22. Gerstenfeld, L.C., Barnes, G.L., Shea, C.M., and Einhorn, T.A. Osteogenic differentiation is selectively promoted by morphogenetic signals from chondrocytes and synergized by a nutrient rich growth environment. *Connect Tissue Res* **44 Suppl 1**, 85, 2003.
23. Gerstenfeld, L.C., Cruceta, J., Shea, C.M., Sampath, K., Barnes, G.L., and Einhorn, T.A. Chondrocytes provide morphogenic signals that selectively induce osteogenic differentiation of mesenchymal stem cells. *J Bone Miner Res* **17**, 221, 2002.
24. Handschel, J.G., Depprich, R.A., Kubler, N.R., Wiesmann, H.P., Ommerborn, M., and Meyer, U. Prospects of micro-mass culture technology in tissue engineering. *Head Face Med* **3**, 4, 2007.
25. Chen, U., and Kosco, M. Differentiation of mouse embryonic stem cells *in vitro*: III. Morphological evaluation of tissues developed after implantation of differentiated mouse embryoid bodies. *Dev Dyn* **197**, 217, 1993.
26. Hwang, N.S., Varghese, S., Puleo, C., Zhang, Z., and Elisseeff, J. Morphogenetic signals from chondrocytes promote chondrogenic and osteogenic differentiation of mesenchymal stem cells. *J Cell Physiol* **212**, 281, 2007.
27. Jiang, J., Nicoll, S.B., and Lu, H.H. Co-culture of osteoblasts and chondrocytes modulates cellular differentiation *in vitro*. *Biochem Biophys Res Commun* **338**, 762, 2005.
28. Stanton, L.A., Sabari, S., Sampaio, A.V., Underhill, T.M., and Beier, F. p38 MAP kinase signalling is required for hypertrophic chondrocyte differentiation. *Biochem J* **378**, 53, 2004.
29. Woods, A., and Beier, F. RhoA/ROCK signaling regulates chondrogenesis in a context-dependent manner. *J Biol Chem* **281**, 13134, 2006.
30. Ghosh, P., Wu, J., Shimmion, S., Zannettino, A.C., Gronthos, S., and Itescu, S. Pentosan polysulfate promotes proliferation and chondrogenic differentiation of adult human bone marrow-derived mesenchymal precursor cells. *Arthritis Res Ther* **12**, R28, 2010.
31. Kumar, D., and Lassar, A.B. The transcriptional activity of Sox9 in chondrocytes is regulated by RhoA signaling and actin polymerization. *Mol Cell Biol* **29**, 4262, 2009.
32. Steinert, A., Weber, M., Dimmler, A., Julius, C., Schutze, N., Noth, U., *et al.* Chondrogenic differentiation of mesenchymal progenitor cells encapsulated in ultrahigh-viscosity alginate. *J Orthop Res* **21**, 1090, 2003.
33. Colnot, C.I., and Helms, J.A. A molecular analysis of matrix remodeling and angiogenesis during long bone development. *Mech Dev* **100**, 245, 2001.
34. Franceschi, R.T., Ge, C., Xiao, G., Roca, H., and Jiang, D. Transcriptional regulation of osteoblasts. *Ann N Y Acad Sci* **1116**, 196, 2007.
35. Wei, A., Chung, S.A., Tao, H., Brisby, H., Lin, Z., Shen, B., *et al.* Differentiation of rodent bone marrow mesenchymal stem cells into intervertebral disc-like cells following coculture with rat disc tissue. *Tissue Eng Part A* **15**, 2581, 2009.
36. Grassel, S., Rickert, M., Opolka, A., Bosserhoff, A., Angele, P., Grifka, J., *et al.* Coculture between periosteal explants and articular chondrocytes induces expression of TGF-beta1 and collagen I. *Rheumatology (Oxford)* **49**, 218, 2010.
37. Zaucke, F., Dinser, R., Maurer, P., and Paulsson, M. Cartilage oligomeric matrix protein (COMP) and collagen IX are sensitive markers for the differentiation state of articular primary chondrocytes. *Biochem J* **358**, 17, 2001.
38. Rai, M.F., Rachakonda, P.S., Manning, K., Palissa, C., Sittlinger, M., Ringe, J., *et al.* Molecular and phenotypic modulations of primary and immortalized canine chondrocytes in different culture systems. *Res Vet Sci* **87**, 399, 2009.
39. Hwang, N.S., Kim, M.S., Sampattavanich, S., Baek, J.H., Zhang, Z., and Elisseeff, J. Effects of three-dimensional culture and growth factors on the chondrogenic differentiation of murine embryonic stem cells. *Stem Cells* **24**, 284, 2006.
40. Grellier, M., Bordenave, L., and Amedee, J. Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering. *Trends Biotechnol* **27**, 562, 2009.
41. Cancedda, R., Castagnola, P., Cancedda, F.D., Dozin, B., and Quarto, R. Developmental control of chondrogenesis and osteogenesis. *Int J Dev Biol* **44**, 707, 2000.
42. Nakaoka, R., Hsiong, S.X., and Mooney, D.J. Regulation of chondrocyte differentiation level via co-culture with osteoblasts. *Tissue Eng* **12**, 2425, 2006.
43. Ilmer, M., Karow, M., Geissler, C., Jochum, M., and Neth, P. Human osteoblast-derived factors induce early osteogenic markers in human mesenchymal stem cells. *Tissue Eng Part A* **15**, 2397, 2009.
44. Phillips, J.E., Guldborg, R.E., and Garcia, A.J. Dermal fibroblasts genetically modified to express Runx2/Cbfa1 as a mineralizing cell source for bone tissue engineering. *Tissue Eng* **13**, 2029, 2007.
45. Granchi, D., Ochoa, G., Leonardi, E., Devescovi, V., Baglio, S.R., Osaba, L., *et al.* Gene expression patterns related to osteogenic differentiation of bone marrow-derived mesenchymal stem cells during *ex vivo* expansion. *Tissue Eng Part C Methods* **16**, 511, 2010.
46. Liu, X., Sun, H., Yan, D., Zhang, L., Lv, X., Liu, T., *et al.* *In vivo* ectopic chondrogenesis of BMSCs directed by mature chondrocytes. *Biomaterials* **31**, 9406, 2010.
47. Lichtenberg, A., Dumlu, G., Walles, T., Marinka, M., Ringes-Lichtenberg, S., Ruhparwar, A., *et al.* A multifunctional bioreactor for three-dimensional cell (co)-culture. *Biomaterials* **26**, 555, 2005.
48. Asnaghi, M.A., Jungebluth, P., Raimondi, M.T., Dickinson, S.C., Rees, L.E., Go, T., *et al.* A double-chamber rotating bioreactor for the development of tissue-engineered hollow organs: from concept to clinical trial. *Biomaterials* **30**, 5260, 2009.
49. Botchwey, E.A., Pollack, S.R., Levine, E.M., and Laurencin, C.T. Bone tissue engineering in a rotating bioreactor using a microcarrier matrix system. *J Biomed Mater Res* **55**, 242, 2001.
50. Williams, C., and Wick, T.M. Endothelial cell-smooth muscle cell co-culture in a perfusion bioreactor system. *Ann Biomed Eng* **33**, 920, 2005.
51. Inanc, B., Elcin, A.E., and Elcin, Y.M. Osteogenic induction of human periodontal ligament fibroblasts under two- and three-dimensional culture conditions. *Tissue Eng* **12**, 257, 2006.
52. Duffy, G.P., Ahsan, T., O'Brien, T., Barry, F., and Nerem, R.M. Bone marrow-derived mesenchymal stem cells promote angiogenic processes in a time- and dose-dependent manner *in vitro*. *Tissue Eng Part A* **15**, 2459, 2009.
53. Goldstein, A.S., Juarez, T.M., Helmke, C.D., Gustin, M.C., and Mikos, A.G. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. *Biomaterials* **22**, 1279, 2001.

54. Gomes, M.E., Sikavitsas, V.I., Behraves, E., Reis, R.L., and Mikos, A.G. Effect of flow perfusion on the osteogenic differentiation of bone marrow stromal cells cultured on starch-based three-dimensional scaffolds. *J Biomed Mater Res A* **67**, 87, 2003.
55. Holtorf, H.L., Jansen, J.A., and Mikos, A.G. Flow perfusion culture induces the osteoblastic differentiation of marrow stroma cell-scaffold constructs in the absence of dexamethasone. *J Biomed Mater Res A* **72**, 326, 2005.
56. Jin, Q.M., Zhao, M., Webb, S.A., Berry, J.E., Somerman, M.J., and Giannobile, W.V. Cementum engineering with three-dimensional polymer scaffolds. *J Biomed Mater Res A* **67**, 54, 2003.
57. Stiehler, M., Bunger, C., Baatrup, A., Lind, M., Kassem, M., and Mygind, T. Effect of dynamic 3-D culture on proliferation, distribution, and osteogenic differentiation of human mesenchymal stem cells. *J Biomed Mater Res A* **89**, 96, 2009.
58. Yu, X., Botchwey, E.A., Levine, E.M., Pollack, S.R., and Laurencin, C.T. Bioreactor-based bone tissue engineering: the influence of dynamic flow on osteoblast phenotypic expression and matrix mineralization. *Proc Natl Acad Sci U S A* **101**, 11203, 2004.
59. Zeng, Y., Lee, T.S., Yu, P., and Low, H.T. Numerical simulation of mass transport in a microchannel bioreactor with cell micropatterning. *J Biomech Eng* **130**, 031018, 2008.
60. Chen, X., Xu, H., Wan, C., McCaigue, M., and Li, G. Bioreactor expansion of human adult bone marrow-derived mesenchymal stem cells. *Stem Cells* **24**, 2052, 2006.
61. Jing, D., Fonseca, A.V., Alakel, N., Fierro, F.A., Muller, K., Bornhauser, M., *et al.* Hematopoietic stem cells in co-culture with mesenchymal stromal cells—modeling the niche compartments *in vitro*. *Haematologica* **95**, 542, 2010.
62. Bidwell, J.P., Alvarez, M., Feister, H., Onyia, J., and Hock, J. Nuclear matrix proteins and osteoblast gene expression. *J Bone Miner Res* **13**, 155, 1998.
63. Rubin, J., Rubin, C., and Jacobs, C.R. Molecular pathways mediating mechanical signaling in bone. *Gene* **367**, 1, 2006.
64. Sikavitsas, V.I., Temenoff, J.S., and Mikos, A.G. Biomaterials and bone mechanotransduction. *Biomaterials* **22**, 2581, 2001.
65. Takahashi, S., Yamazoe, H., Sassa, F., Suzuki, H., and Fukuda, J. Preparation of coculture system with three extracellular matrices using capillary force lithography and layer-by-layer deposition. *J Biosci Bioeng* **108**, 544, 2009.
66. Bhatia, S.N., Balis, U.J., Yarmush, M.L., and Toner, M. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *FASEB J* **13**, 1883, 1999.
67. Lee, J.Y., Shah, S.S., Yan, J., Howland, M.C., Parikh, A.N., Pan, T., *et al.* Integrating sensing hydrogel microstructures into micropatterned hepatocellular cocultures. *Langmuir* **25**, 3880, 2009.
68. Moon, J.J., Hahn, M.S., Kim, I., Nsiah, B.A., and West, J.L. Micropatterning of poly(ethylene glycol) diacrylate hydrogels with biomolecules to regulate and guide endothelial morphogenesis. *Tissue Eng Part A* **15**, 579, 2009.
69. Trkov, S., Eng, G., Di Liddo, R., Parnigotto, P.P., and Vunjak-Novakovic, G. Micropatterned three-dimensional hydrogel system to study human endothelial-mesenchymal stem cell interactions. *J Tissue Eng Regen Med* **4**, 205, 2010.
70. Kim, K., Yeatts, A., Dean, D., and Fisher, J.P. Stereolithographic bone scaffold design parameters: osteogenic differentiation and signal expression. *Tissue Eng Part B Rev* **16**, 523, 2010.
71. Giovannini, S., Diaz-Romero, J., Aigner, T., Heini, P., Mainil-Varlet, P., and Nesic, D. Micromass co-culture of human articular chondrocytes and human bone marrow mesenchymal stem cells to investigate stable neocartilage tissue formation *in vitro*. *Eur Cell Mater* **20**, 245, 2010.
72. Lettry, V., Hosoya, K., Takagi, S., and Okumura, M. Co-culture of equine mesenchymal stem cells and mature equine articular chondrocytes results in improved chondrogenic differentiation of the stem cells. *Jpn J Vet Res* **58**, 5, 2010.
73. Ahmed, N., Dreier, R., Göpferich, A., Grifka, J., and Grässel, S. Soluble signalling factors derived from differentiated cartilage tissue affect chondrogenic differentiation of rat adult marrow stromal cells. *Cell Physiol Biochem* **20**, 665, 2007.
74. Sanchez, C., Deberg, M.A., Piccardi, N., Msika, P., Reginster, J.Y., and Henrotin, Y.E. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage* **13**, 988, 2005.

Address correspondence to:

John P. Fisher, Ph.D.

Fischell Department of Bioengineering

University of Maryland

3238 Jeong H. Kim Engineering Building (# 225)

College Park, MD 20742

E-mail: jpfisher@umd.edu

Received: December 2, 2011

Accepted: March 14, 2012

Online Publication Date: July 6, 2012