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APPLICATION OF BIOMATERIALS TO *IN VITRO* PLURIPOTENT STEM CELL DISEASE MODELING OF THE SKELETAL SYSTEM

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Abstract

Disease-specific pluripotent stem cells can be derived through genetic manipulation of embryonic stem cells or by reprogramming somatic cells (induced pluripotent stem cells). These cells are a valuable tool to study human diseases *in vitro* in order to dissect their pathomechanisms and develop novel therapeutics. Although pluripotent stem cell-derived models have successfully recapitulated the abnormalities of some skeletal diseases *in vitro*, this field is still at its early stages, and it could greatly benefit from the direct application of biomaterial research. Biomaterial-based systems may be utilized to enhance the differentiation processes of pluripotent stem cells in order to create more homogeneous and physiologically relevant *in vitro* disease models. Moreover, inducing the disease phenotype may be facilitated by the guidance of biomaterials. This review presents a comprehensive summary of existing biomaterial applications in human disease modeling and their potential on skeletal disease models. By utilizing disease-specific pluripotent stem cells, current biomaterial-based systems for *in vitro* models could be extrapolated to study skeletal diseases in a petri dish.

1 Introduction

Pluripotent stem cells (PSCs) have the ability to differentiate into any one of the three germ layers: endoderm, ectoderm, and mesoderm.[1-3] Therefore, *in vitro* models of different cell lineages can be generated from disease- and patient-specific PSCs. Modeling diseases *in vitro* offers a cost-effective and ethically more acceptable approach to testing therapeutics. The drug development process costs approximately 1.7 billion US dollars per drug,[4, 5] and a large number of drugs fail the clinical trial phase. Human PSC-based disease models may respond to therapies in a more physiologically relevant manner than traditional animal models, thereby helping to bridge the gap between *in vivo* preclinical testing and human clinical trials.

PSCs have been generated for a large number of diseases, of genetic and sporadic origin.[6-9] The application of PSCs to *in vitro* models has been particularly popular in cardiovascular and neurological research, but has lagged behind in the musculoskeletal field. Nevertheless, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been generated from a handful of monogenic (single gene mutation) and multifactorial skeletal diseases. These studies serve as a proof-of-concept for using human PSC models to understand skeletal disorders and test possible therapeutics.

In order to develop *in vitro* models, successful and efficient differentiation of PSCs into specific cell types is required. Most stem cell differentiation protocols are based on mesenchymal stem cell (MSC) differentiation, and they have typically fallen short for PSCs. Current PSC differentiation methods have been limited and inefficient, yielding heterogeneous compositions of differentiated cells and immature cells that inadequately resemble functional cells in the human body.[10] The assistance of biomaterials may play a pivotal part in improving PSC differentiation and engineering physiological relevant disease models. Biomaterials have tunable properties that can be utilized to enhance the differentiation process and improve current systems employed in *in vitro* disease models (i.e. 2D monolayer and 3D pellet/micromass culture systems). Moreover, biomaterials can also be employed to better recapitulate the disease phenotype, by inducing cellular responses from disease-specific microenvironments.

Here we focus on the role of PSCs and biomaterials in *in vitro* models for skeletal diseases. There is a distinct advantage in applying biomaterials research to PSC-derived *in vitro* disease models, and it could advance research on numerous genetic musculoskeletal diseases. The goal of this review is to present an overview of existing PSC-derived skeletal *in vitro* models and the potential of biomaterials to enhance disease models for skeletal lineages. The biomaterial-based culture systems discussed here may be extrapolated to improve current and develop new *in vitro* disease models in order to understand complex mechanisms of the pathology.

2 Pluripotent Stem Cells in Skeletal Disease Modeling

Disease- and patient-specific PSCs offer a powerful tool to dissect pathomechanisms during embryonic development and disease progression, and to develop novel therapeutic interventions via *in vitro* human models. Skeletal tissue-specific and adult stem cells are difficult to harvest, represent a source of patient morbidity, and possess limited proliferation ability. In contrast, researchers have the technology to derive PSCs from any tissue. Pluripotent cells can be expanded to produce large numbers of disease-specific stem cells, which in turn can be differentiated into numerous cell types in order to study different aspects of the same disease. PSC technology is particularly advantageous in orphan diseases or in diseases for which no relevant animal model is available. For many monogenic diseases, the animal models show no or only an approximate resemblance to the human disease.[11] For polygenic and complex diseases, we are only able to recapitulate certain aspects of the disease *in vivo*. In this case, human *in vitro* disease models may be able to provide valuable insight complementary to available animal models.

2.1 Embryonic stem cells

ESCs are obtained from the undifferentiated, inner cell mass of the blastocyst (Figure 1). Disease-specific ESCs can be derived by the genetic modification of existing ESC lines or by the generation of new ESCs from embryos carrying genetic diseases detectable via pre-implantation genetic diagnosis (Figure 1).[11] Somantic cell nuclear transfer using adult cell donors offers another route to patient-specific ESCs; however, technical, logistical and ethical difficulties have, to date, presented insuperable difficulties.[12]

More than 500 rare genetic skeletal disorders have been described, and challenges from studying these diseases comes from a lack of suitable animal models and unavailability of skeletal tissues for studies.[13] Although only known genetic diseases can be modeled through ESCs, these models can provide unprecedented understanding of disease progression and pathophysiology for these orphan diseases. Moreover, one benefit of the targeted manipulation of an ESC line is the availability, in principle, of a perfect control (the unmodified line) to examine mutation-specific cellular differences.[11, 14]

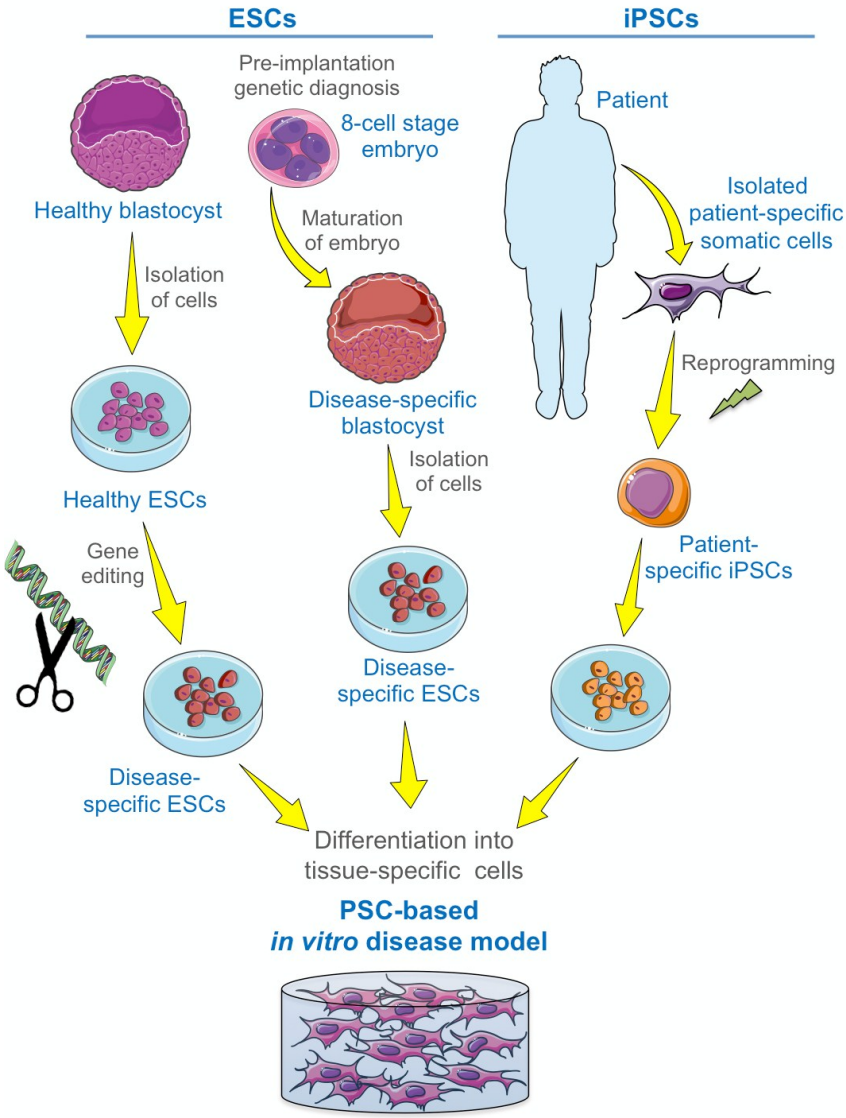

Figure 1. Overview of PSC disease modeling process. ESCs are isolated from blastocysts. Disease-specific ESCs can be obtained by screening for the disease-specific mutation via pre-implantation genetic diagnosis, or by inducing the mutation with gene editing techniques. iPSCs, on the other hand, are derived from the reprogramming of somatic stem cells. These disease- and patient-specific PSCs can be differentiated into tissue-specific cells to create *in vitro* disease models. This figure was produced using Servier Medical Art, available from http://www.servier.com/Powerpoint-image-bank.

2.2 Induced Pluripotent Stem Cells

The discovery of iPSCs revolutionized the field of stem cell research and widened the possibility of patient-specific disease modeling and personalized medicine. iPSCs are generated by reprogramming somatic cells taken from donors of all ages (Figure 1).[9, 15] Skin, synovial fluid, blood, hair, etc.—virtually any cellular tissue or fluid can be used as a cell source for iPSCs. Therefore, iPSCs can be derived from living patients suffering from any genetic disease, simple or complex. The patient's clinical history is an important benefit of iPSC disease modeling. Many genetic diseases display variable penetrance and severity of clinical symptoms from patient to patient (e.g. familial rheumatoid arthritis[16], Duchenne muscular distrophy[17]), and knowledge of the clinical history could inform experimental design and interpretation of findings.[11] For polygenic and complex diseases, human iPSC (hiPSC) models can elucidate the correlation between patterns of gene expression, pathological mechanisms, and disease phenotype.

iPSCs share essential characteristics with ESCs and even yielded newborn offsprings from tetraploid complementation with efficiencies comparable to ESCs.[18-20] However, key differences between ESCs and iPSCs are emerging that can influence their role in disease modeling. Reprogramming of iPSCs is not perfect. It has been shown that iPSCs contain epigenetic memory of their cell of origin at early passages; early iPSCs show distinct cellular and molecular characteristics based on their cell type of origin.[21-23] This property may be advantageous for studying the same cell type derived from iPSCs as its cell of origin. There is a functional significance of the donor cell gene expression, where iPSC differentiation back into the cell of origin brings an advantage over iPSC differentiation into an unrelated lineage.[21, 22, 24]

It is important to note, however, that not all the differentially methylated regions between iPSCs and ESCs belong to the cell-of-origin memory, indicating that iPSCs also accumulate novel aberrant epigenetic states.[24, 25] Residual DNA methylation patterns and resulting gene expression of the somatic cell of origin are lost in later passages of iPSCs,[21, 22, 26] when the cells are considered more stable.[21] Nevertheless, the nature of epigenetic misregulation and the underlying disease mutation should be carefully considered before initiating an iPSC-based approach to model the disease of interest.[11]

Current research in iPSC reprogramming systems may enhance the process to prevent aberrant epigenetic states. Engineered biomaterials can potentially aid in iPSC reprogramming through controlled spatiotemporal presentation and kinetics of reprogramming factor delivery.[27] Well-defined biomaterial substrates can also regulate the epigenetic state of iPSCs.[28] Biomaterials have a wide range of applicability in iPSC technology, including reprogramming, expansion, and differentiation.

2.3 In Vitro Skeletal Disease Models

Differentiation of PSCs into somatic cells has fallen short of requirements, resulting in low efficiency and heterogeneous populations.[10, 12] Despite these shortcomings, pure differentiated cell populations may not be necessary to permit important benefits accruing from the use of disease-specific PSCs.[12] PSC lines have been generated for a handful of skeletal diseases with simple Mendelian inheritance as well as complex etiology, shown in Table 1.

Table 1. Skeletal diseases for which PSCs have been generated and used to model the disease phenotype *in vitro*.

Marfan Disorder	FBN1 mutation	Blastocytes with mutation	ESCs micromass culture in chondrogenic medium	Impaired ability of osteogenic differentiation; chondrogenesis ability in the absence of exogenous TGF β.	[29]
		Dermal fibroblasts	iPSCs micromass culture in chondrogenic medium		
Metatropic dysplasia	TRPV mutation	Fibroblasts	iPSC micromass culture in chondrogenic medium	Impaired ability of chondrogenic differentiation	[30]
Achondroplasia; thalatophoc dysplasia type 1	FGFR3 (G380R); FGFR3 (R248C) mutations	Dermal fibroblasts	High cell-density iPSC-chondrocytes cultured in suspension, under chondrogenic medium	Impaired ability of chondrogenic differentiation	[31]
Neonatal-onset multisystem inflammatory disease	NLRP3 mutation	Unknown	2D micromass and 3D pellet cultures of iPSC-chondroprogenitor cells in chondrogenic medium	Enhanced chondrogenesis ; increased ECM production	[32]
Familial osteochondritis dissecans	Heterozygous ACAN mutation	Dermal fibroblasts	iPSC micromass culture in chondrogenic medium	Impaired chondrogenesis ; irregular ECM assembly and composition	[33]
Fibrodysplasia osteogenesis imperfecta	ACVR1 mutation	Dermal fibroblasts	iPSC-EB pellet culture in chondrogenic medium; iPSC monolayer culture in osteogenic medium	Enhanced chondrogenesis; increased mineralization	[34]

Marfan syndrome (MFS) is a heritable connective-tissue disorder caused by a mutation in the FBN1 gene, which encodes fibrillin-1, an extracellular protein.[29] Quarto *et al*[29] modeled MFS by deriving human ESCs (hESCs) from a blastocyst carrying the FBN1 mutation and by reprogramming hiPSCs from MFS patient dermal cells. Both PSC models faithfully recapitulated disease phenotypes in differentiated cells. Results showed that MFS-PS-derived cells manifest impaired osteogenic differentiation and undergo chondrogenesis in the absence of TGF-β, because of an enhanced TGF-β signaling.[29] MFS-PSC osteogenesis was rescued by inhibition of TGF-β signaling, while chondrogenesis is not perturbed and occurs in a TGF-β cell-autonomous fashion.[29]

Skeletal dysplasias (SDs) are caused by abnormal chondrogenesis during cartilage growth plate differentiation.[30] Saita and colleagues studied early stages of cartilage formation using iPSCs derived from a patient with a lethal form of metatropic dysplasia, caused by a dominant mutation in the calcium channel gene TRPV4.[30] Mutant iPSCs were able to recapitulate the disease phenotype, reflecting molecular evidence of aberrant chondrogenic development processes.[30] In another study, Yamashita *et al*[31] studied iPSC models from achondroplasia SD and thalatophoric dysplasia type 1, both of which are caused by gain-of-function mutations in the gene FGFR3. When FGFR3 protein accumulates, it affects downstream pathways to suppress the

differentiation and proliferation of chondrocytes.[35] Disease-specific iPSCs showed higher expression of FGFR3 protein, manifested abnormal cartilage formation, and displayed decreased proliferation and increased apoptosis after chondrogenic differentiation.[35] This iPSC disease model served as a platform to study treatment with statin, which resulted in the degradation of mutated FGFR3 protein and restored normal chondrogenic differentiation.[31]

Neonatal-onset multisystem inflammatory disease (NOMID) causes, among other clinical manifestations, tumor-like expansive lesions in epiphesial portions of long bones.[32] Clinical and pathological findings suggest that mutant NLRP3 induces epiphyseal overgrowth in NOMID patients via mechanisms unrelated to the NLRP3 inflammasome.[32] Yokoyama *et al*[32] recently showed, in an iPSC-derived NOMID model, that SOX9 is overexpressed via the cAMP/PKA/CREB signaling pathway in NOMID-iPSC-chondrocytes with the NLRP3 mutation, and this causes overproduction of the extracellular matrix (ECM) independently of the NLRP3 inflammasome. By using disease-specific iPSC-based *in vitro* model of chondrogenesis, researchers revealed a previously unidentified connection in NOMID.

A recent study from Xu *et al*[33] dissected the mechanisms underlying irregular chondrogenesis in familial osteochondritis dissecans (FOCD) using iPSC-derived chondrocytes. FOCD is characterized by the development of large cartilage lesions in multiple joints, short statue, and early onset of severe osteoarthritis. This disease is caused by a heterozygous mutation in the ACAN gene, which results in an amino acid replacement in the G3 aggrecan C-type lectin domain.[36] This study found that the mutation leads to the retention of the aggrecan core in the endoplasmic reticulum (ER) of chondrocytes and subsequently induces ER stress.[33] Through *in vitro* disease modeling, Xu *et al*[33] elucidated for the first time the cellular pathomechanism caused by the mutation. This abnormal processing of aggrecan results in the irregular assembly of the ECM in chondrocytes, leading to rapid joint destruction and development of osteoarthritis.[33]

Fibrodysplasia ossificans progressive (FOP) is a rare, debilitating genetic disease caused by hyperactive mutations in ACV1 gene.[35] Activating the ACVR1 gene leads to increased mineralization that causes abnormal endochondral bone formation in patients' soft tissues.[10] Consistent with the disease manifestation, FOP-iPSCs exhibited enhanced mineralization and chondrogenesis *in vitro*.[34] iPSCs have also recently been generated from rheumathoid arthritis[37] and osteoarthritis[37, 38] patients; however, follow-up studies of cellular pathomechanisms or therapeutic drug screening have not yet been reported.

PSC models are complimentary and powerful tools to gain further insights into human molecular pathogenesis. By understanding the underlying pathophysiology, researchers can apply effective strategies that evoke and/or enhance disease-relevant phenotypes in cell models and, hopefully, discover new drugs.[35] The application of biomaterials could tremendously benefit this field. For example, 3D biomaterial-based models may overcome current hurdles to model disorders at the tissue level. Biomaterials can also improve the differentiation process and even present the proper pathogenic stimuli to induce disease-relevant phenotypes.

3 Application of Biomaterials to PSC-derived *In Vitro* Disease Models

The application of biomaterial-based culture systems may enhance disease-specific tissue engineering; thereby, providing important new insights into the pathogenesis of the disease. The biomaterials field has established methodologies to precisely control a broad range of properties that influence cell behavior, including sequestration and release of bioactive molecules, degradation rate, cell-recognizable surface chemistries, surface topography, and mechanical stiffness. The overall success of tissue organization and development is highly dependent upon these properties, since they can ultimately dictate cell adherence, nutrient/waste transport, matrix synthesis, matrix organization, and cell differentiation.[39] Furthermore, 3D scaffolds create an environment that better simulates the *in vivo* milieu, compared to conventional cell culture systems.[40] PSCs have been shown to express significantly higher levels of ECM-related genes, as well as genes that regulate cell growth, proliferation and differentiation in 3D scaffolds compared to 2D tissue culture plates.[41]

Biomaterials may also assist *in vitro* disease models by stimulating the pathological microenvironment. Diseased cells may respond differently to mechanical stimuli, chemical signaling, or oxidative stress. Biomaterial-based systems may provide a platform to study different aspects of the cellular microenvironment, thereby further elucidating pathological mechanisms.

3.1 PSC-derived In Vitro Disease Models

Cellular functions are influenced not only by cell-autonomous programs but also by microenvironmental stimuli, which include neighboring cells, ECM, soluble factors, and physical forces.[42] Engineered biomaterials may provide a powerful tool for studying disease-relevant cellular function by closely mimicking the natural microenvironments of cells and tissues. For example, Zhang *et al*[43] recently demonstrated that a 3D hydrogel culture of iPSC-derived neurons can induce *in vivo*-like responses related to Alzheimer's disease, not recapitulated with conventional 2D culture. Aβ oligomer production has been implicated to be the direct cause of pathological symptoms of Alzheimer's disease.[43] In this model, the 3D self-assembling peptide hydrogel served as an interaction platform between iPSC-neurons and Aβ oligomer. This 3D environment showed clear effects on cytoskeleton remodeling, a critical event in the progress of Alzheimer's disease that was not observed in the conventional 2D culture.[43] 3D culture systems do not only model morphology and structure of cells and their connections more accurately, but are also fundamental for the study of human diseases related to abnormal ECM remodeling.

In another instance, a 3D *in vitro* cardiac tissue model was developed to understand and treat cardiac arrhythmias and related cardiovascular diseases. The model was created using iPSC-derived cardiomyocytes (CMs), from healthy and long QT syndrome type 3 (LQT3) iPSCs.[44] Two-photon initiated polymerization (TRIP) with a UV-curable organic-inorganic hybrid polymer was used to create the 3D filamentous scaffolds with precisely controlled structural alignment, spatial resolution, and mechanical properties.[27, 44] Tailoring these parameters modulated the contractility of residing CMs and, more importantly, recapitulated the abnormal contractility of long QT syndrome in the LQT3-iPSC-CMs-seeded scaffold, which was not seen in the control-iPSC-CMs.[27, 44] The LQT3 *in vitro* model was further tested by exposure to a panel of drugs. The LQT3-iPSC-CMs were found to be more susceptible to pharmacological

interference when grown in a 3D scaffold with lower fiber stiffness, compared to those cultured in 3D with stiffer fibers or on 2D surfaces.[27, 44]

Moreover, biomaterials can be used to induce the pathogenic phenotype by introducing mechanical or pathogenic stimuli such as relevant chemical agents or toxins in a spatiotemporal manner. As one example, Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration and weakness. The skeletal muscle phenotype of DMD is thought to be due to both the presence of dystrophin mutations and cumulative mechanical stretch injury from muscle use.[45] *In vitro* PSC mechanistic studies have successfully recapitulated the initial pathology of DMD, by showing abnormal electrical response of DMD skeletal myotubes.[17] However, the pathologic mechanotransduction of DMD skeletal muscle has not been studied to date and it may be fundamental to the disease progression and the development of a therapeutic intervention. In this case, biomaterial-based cultures may be used to apply mechanical stress to PSC-derived skeletal muscle to appropriately model this disease *in vitro*.[45]

The application of biomaterials to PSC-derived disease models has been most extensively used to study cardiovascular, neurological, and hepatic disorders; nevertheless, this approach has immense potential to impact the musculoskeletal field as well. The next section discusses the potential of current biomaterials being applied to ESCs and iPSCs to create *in vitro* cartilage and bone constructs. Although biomaterials have not been directly applied to disease modeling of skeletal diseases yet, the following are examples of biomaterial-based for PSC cartilage and bone engineering *in vitro* which may be used with disease-specific cells in order to elucidate the pathology and test pharmacological therapies.

3.2 Application of Biomaterial-Based PSC-derived In Vitro Systems to Skeletal Disease Modeling

Engineering skeletal tissue constructs (i.e. bone and cartilage) from disease-specific PSCs may allow scientists to better study the pathological manifestation and dissect signaling pathways related to the disease of interest. In this scenario, biomaterials may help create a more physiologically relevant tissue construct in order to better recapitulate and understand the disease. It is well known that 3D cell-cell and cell-matrix interactions regulate a variety of cell signaling pathways to enable tissue development,[40, 46] and those interactions may be crucial for the presentation of other aspects of pathological phenotypes, such as ECM deposition and remodeling. Toh *et al*[47] engineered cartilage from human ESC (hESC)-chondrogenitor cells encapsulated in a hyaluronic acid-based poly(ethelene glycol)-diacrylate (PEGDA) hydrogel. Constructs demonstrated characteristic time-dependent patterns of matrix synthesis, with an initial robust increase in GAG content before plateauing and a slower onset in type II collagen deposition.[47] This hydrogel system could be of great utility to dissect time-dependent mechanisms during ECM deposition in diseased PSC-derived chondrocytes.

The application of biomaterials cannot only enhance PSC-derived tissue engineering for disease modeling, but could also provide a scaffold for in-depth characterization of the cellular response to mechanotransduction and exogenous molecules. It is well established that mechanical loading is essential to development, growth, and maintenance of the skeletal system, and biomaterials facilitate the assessment of the effects of mechanical stimuli on 3D PSC

constructs. Terraciano *et al*[48] used an RGD-modified PEGDA hydrogel as a platform to examine the effect of mechanical stimuli on the chondrogenic differentiation of hESC-embryonic body-derived cells, subjecting the constructs to compressive tests. PEGDA gels have been previously characterized as viscoelastic material with a very minimal viscous response.[48] In articular cartilage, chondrocytes respond to mechanical compression and remodel their ECM ultimately changing the composition, structure and biomechanical properties. Applying models like this to diseased cells can provide us with tremendous understanding of their mechanobiology and possible disease progression.

In another example, a type II collagen scaffold was used to study the cell biology of murine ESC (mESC)-derived osteoprogenitor cells, particularly how they are influenced by mechanical stimulation during cell differentiation and maturation. Results indicated that mechanical pre-stimulation of this mESC-seeded scaffold yielded significant differences in the structure and organization of mineralization present in the collagen matrix.[49] Specifically, scaffolds loaded for 40 hours after 5 days of differentiation and then left to fully differentiate for 30 days produced a highly structured honeycombed-shaped mineralization in the matrix (Figure 2); an outcome that was previously shown to be indicative of late-osteoblast/early osteocyte activity[49]. This is a great example of how biomaterial-based system can create more physiologically relevant *in vitro* models to study dysosteogenesis or other bone-related diseases. Biomaterials may serve as a platform for cell-cell and cell-matrix interactions and for mechanical stimulus.

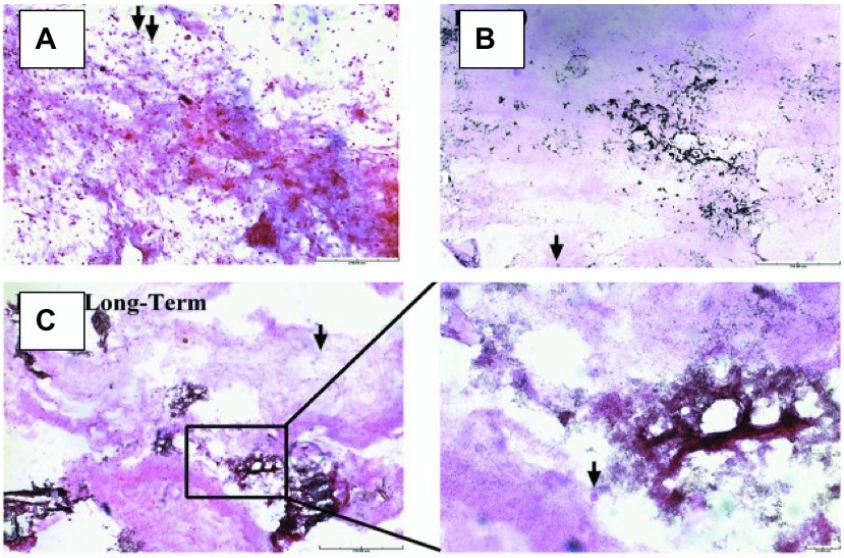

Figure 2. Von Kossa staining of type I collagen hydrogels. **(A)** Staining of unloaded hydrogels at 5 days resulted in no visual positive staining, while **(B)** unloaded hydrogels at 30 days showed positive staining for mineralization of the matrix. **(C)** 5-day loading followed by 30 days of differentiation without mechanical stimulus showed the greatest concentration of mineralization, where a distinct honeycomb structure was observed. Brown/black staining indicates positive staining for mineralization. Black arrows indicate counterstained cells. Pictures taken from Damaraju *et al*.[49]

Moreover, applying biomaterials to *in vitro* disease models may provide a solution for PSCs with impaired differentiation. For example, hiPSCs from gingival fibroblasts have been proved to have weak osteogenesis capability, seemingly because of their epigenetic memory.[21, 50]

Ji *et al*[51] enhanced the osteogenic activity of these cells by tailoring the properties of their 3D porous nHAp/chitosan-gelatin (CG) scaffold. The intimate adhesion between nHAp and the complex organic matrix of a CG scaffold improves the microhardness of the composite.[52] Therefore, morphology and crystallography of nHAp particles can influence the morphology and adsorption characteristics of the composite.[52] In fact, results showed that bone-specific gene expressions were different during the osteogenic induction process of rod-like and sphere-shaped nHAp in CG scaffolds.[51] Overall, osteogenic gene expression in the rod-like scaffold group increased less significantly compared with the sphere-shaped scaffold group, indicating that sphere-shaped nHAp can better increase the osteogenic differentiation of hiPSCs.[51] Sphere-shaped nHAp induced a denser ECM in gingival hiPSCs. In this study, biomaterials provided a solution to the limited osteogenic ability of hiPSCs caused by their epigenetic memory. Clinically discarded gingival tissues could represent an important source of hiPSCs to model human diseases, and the direct application of biomaterials enables their study in bone diseases.

4 Summary and Concluding Remarks

PSCs represent a unique opportunity to study complex molecular and cellular mechanisms in disease progression and pathophysiology in a petri dish. PSCs can be generated through genetic manipulation (ESCs) as well as from somatic cells from living patients with full medical records (iPSCs). *In vitro* disease modeling in the past has been limited due to the lack of cell sources, and so, PSC technology promises to boost this field as well as positively impact the pharmaceutical industry. Nevertheless, PSC technology in *in vitro* disease modeling is still in its developmental stages and has some hurdles to overcome before being able to engineer physiological relevant tissues for *in vitro* models.

In vitro models require efficient and robust differentiation methods that correctly replicate a wild type cell phenotype in order to then study the pathological cell phenotype of a disease. However, conventional culture systems often yield a heterogeneous population or functionally impaired resultant cells. Many existing methods for PSC differentiation are still complex, laborious, and cost-inefficient.[10] The application of biomaterials research may directly address these current issues in *in vitro* modeling and launch forward the concept of "disease in a petri dish". Known material-PSC interactions can be harnessed to enhance *in vitro* models by better mimicking natural ECM and promoting 3D cell-cell interactions. Furthermore, biomaterials may also serve as a platform to assess different aspects of the disease, or even induce the disease phenotype via delivery of pathogenic agents or stress induction.

The PSC-material systems discussed in this review widen our understanding of their use in disease modeling and pave the way to start utilizing these concepts to enhance *in vitro* PSC skeletal models. There is a need for superior differentiation of PSCs, tissue architecture and composition that more closely resembles the diseased tissue or organ being studied, and a platform to test how different properties of the microenvironment may affect the disease. The integration of PSCs with biomaterials may be essential to tackle these challenges.

The idea of modeling a disease in a petri dish could not only drastically lower costs of drug screening, but could also provide a platform to study orphan diseases for which animal models are not available or feasible. In that sense, PSC technology offers a unique, exciting

opportunity to broaden the horizons of pathomechanistic studies and the subsequent therapeutic interventions, and it may very well depend on biomaterials to reach that level.

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