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Bioengineering Human Cartilage–Bone Tissues for Modeling of Osteoarthritis

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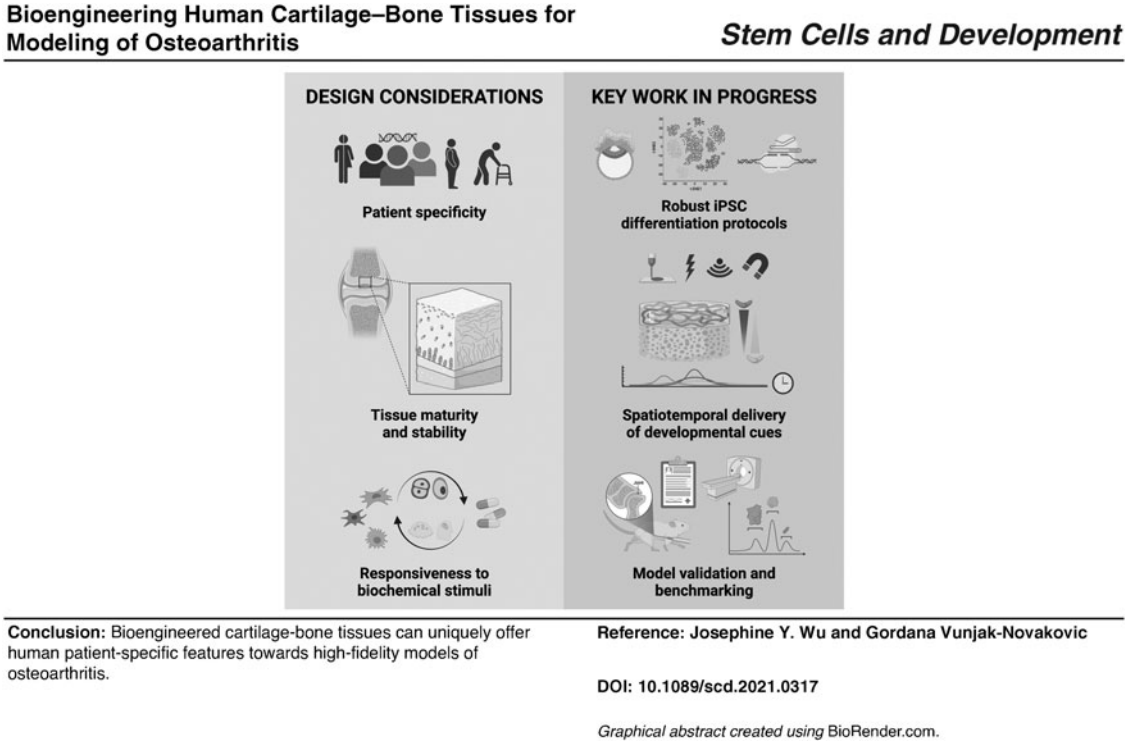
Osteoarthritis (OA) is the most common joint disease worldwide, yet we continue to lack an understanding of disease etiology and pathology and effective treatment options. Essential to tissue homeostasis, disease pathogenesis, and therapeutic responses are the stratified organization of cartilage and cross talk at the osteochondral junction. Animal models may capture some of these features, but to establish clinically consistent therapeutics, there remains a need for high-fidelity models of OA that meet all the above requirements in a human patient-specific manner. In vitro bioengineered cartilage–bone tissue models could be developed to recapitulate physiological interactions with human cells and disease-initiating factors. In this study, we highlight human induced pluripotent stem cells (hiPSCs) as the advantageous cell source for these models and review approaches



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for chondrogenic fate specification from hiPSCs. To achieve native-like stratified cartilage organization with cartilage–bone interactions, spatiotemporal cues mimicking development can be delivered to engineered tissues by patterning of the cells, scaffold, and environment. Once healthy and native-like cartilage–bone tissues are established, an OA-like state can be induced through cytokine challenge or injurious loading. Bioengineered cartilage–bone tissues fall short of recapitulating the full complexity of native tissues, but have demonstrated utility in elucidating some mechanisms of OA progression and enabled screening of candidate therapeutics in patient-specific models. With rapid progress in stem cells, tissue engineering, imaging, and high-throughput omics research in recent years, we propose that advanced human tissue models will soon offer valuable contributions to our understanding and treatment of OA.

Keywords: osteoarthritis, chondrogenesis, osteogenesis, tissue engineering, stem cells

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Osteoarthritis and Cartilage

Although osteoarthritis (OA) is the most common joint disease affecting over 500 million people worldwide, it remains a chronic degenerative condition with poorly understood etiology and pathology. OA prevalence increased by nearly 50% between 1990 and 2020 and is further increasing in our aging population [1]. Short of surgical interventions, OA management is generally focused on analgesia with paracetamol, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and intra-articular corticosteroids.

Repurposing disease-modifying antirheumatic drugs (DMARDs) for rheumatoid arthritis (RA) showed little success with OA, and none of the disease-modifying OA drugs (DMOADs) have successfully completed clinical trials [2]. The deficit of meaningful OA management strategies is due, at least in part, to the lack of high-fidelity models that would help understand disease progression and test novel treatments [3].

Healthy human articular cartilage has a specific stratification (superficial, transitional, deep, and calcified zones), with each layer having distinct cell and matrix properties (chondrocyte phenotype, collagen alignment, and proteoglycan-to-water ratio) that are essential to its function. Friction and wear properties at the articular surface are enhanced by the compositional heterogeneity of cartilage [4], while the calcified zone features small chondrocytes in the apatite-filled matrix, which limits transport from the underlying bone [5]. While cartilage is devoid of vascularization, innervation, or lymphatics, its interactions with the subchondral bone play a role in development, homeostasis, and disease [6].

In joint diseases, cartilage degeneration progresses in a layer-dependent manner. OA typically starts with small lesions in the superficial layers, and compensatory matrix production from the deeper layers, until the calcified layers wear away to expose bone. In addition, pathogenesis is mediated by disruptions in tissue cross talk at the osteochondral interface [7,8].

Mouse models are often used to study OA, yet fail to recapitulate key aspects of human cartilage anatomy such as size, thickness, and zonal organization [9]. Although rat models display thicker cartilage with zonal structure, naturally occurring OA is uncommon in rats [10]. In general, animals cannot fully mimic human biology or serve as patient-specific models of disease, which leads to clinically inconsistent therapeutics.

Cell Sources for Engineered Cartilage–Bone Models

With advances in stem cell and tissue engineering, cartilage–bone in vitro tissue models could serve as a viable alternative because of their biological fidelity and incorporation of human cells and disease-initiating factors. To build patient-specific models that can capture disease heterogeneity while minimizing clinical invasiveness, human induced pluripotent stem cells (hiPSCs) are an advantageous choice as they can be derived from small blood samples.

Primary articular chondrocytes, a mature primary cell source, can be harvested from arthroscopic biopsies of low weight-bearing joint regions for autologous chondrocyte implantation therapies (eg, Carticel, MACI) [11]. However, their use is limited by donor site morbidity, dedifferentiation after extended in vitro expansion, and potentially inferior function due to patient age or disease.

While bone marrow (BM)-derived mesenchymal stromal/stem cells (MSCs) have traditionally been used for their musculoskeletal differentiation potential, they have limited expansion capability. In contrast, hiPSCs can be expanded near indefinitely, allowing for sufficient cell numbers, multiple cell types generated from the same source, and genetic modification as needed.

As cells undergo specification into chondrogenic, osteogenic, and stromal lineages, their differentiation should not be done in isolation to recapitulate physiological developmental processes with intertissue cross talk and responsiveness to stimuli [11]. Further maturation of bioengineered cartilage–bone tissues will require spatiotemporal specification of growth factors and other cues. Finally, reliable methods to induce and benchmark the OA disease state from stable and mature engineered tissue models are required.

Chondrogenic Fate Specification

Starting from hiPSCs and other pluripotent stem cells, approaches for differentiation into the relevant musculoskeletal lineages can be classified broadly into single-step, MSC-based, and developmentally guided protocols. In choosing an approach for generating chondrocytes from hiPSCs, the fundamental issue of how simple is complex enough will determine how much development needs to be recapitulated and how much cell heterogeneity can be

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tolerated in a tissue model. Additional related considerations include protocol robustness, reliance on serum or growth factors, throughput, and cost.

Single-step protocols, also called nonspecific differentiation, aim to go directly from pluripotent to differentiated cell types without intermediate lineage specification, starting from monolayers, embryoid bodies, micromasses, or coculture systems with pluripotent stem cells. While some of these single-step protocols show promise in addition to the ease of use, they result in heterogeneous and inefficiently differentiated cell populations that require downstream sorting and isolation. These methods remain far less adopted than protocols with an intermediate fate specification step.

In musculoskeletal contexts, MSCs from BM or adipose tissue were initially used as the predominant cell source and thus many widely used differentiation protocols rely on MSCs as the starting point. To reconcile with the use of hiPSCs, protocols and commercially validated kits for differentiation into MSC lineages are now available.

These MSCs meet the three validation criteria set forth by the International Society for Cellular Therapy: (1) adherence to uncoated tissue culture plastic with elongated cell morphology; (2) high expression of mesenchymal surface markers, CD73, CD90, and CD105, and low expression of lineage markers, CD19, CD34, CD45, CD79α, and HLA-DR; and (3) the ability to tridifferentiate into adipogenic, chondrogenic, and osteogenic lineages [12].

Recent studies have elucidated key differences between BM- and hiPSC-derived MSCs (hiMSCs) using more extensive functional, phenotypic, and genetic characterization. The hiMSCs have gene expression profiles similar to vascular progenitor cells, which can also tridifferentiate along adipogenic, chondrogenic, and osteogenic pathways, but under different conditions than BM-MSCs [13]. When designing inductive conditions with hiMSCs in mind, these findings should not preclude the use of hiPSCs as the starting point for engineered tissue models.

Others have taken inspiration from development to design differentiation protocols more closely recapitulating in vivo processes. Of note, craniofacial skeletal tissues originate from the neural crest, while the remainder of the skeleton originates from the mesoderm, with paraxial and lateral plate mesoderm giving rise to axial and appendicular skeletal tissues, respectively. Developmentally guided methods target multiple pathways in a coordinated and specific manner, resulting in distinct cell types from particular tissue regions [14]. Even so, recent single-cell transcriptomic analysis of chondrogenesis following developmentally guided differentiation from hiPSCs still displays some off-target differentiation [15].

With the rise of hiPSC-derived chondrogenic progenitors, many groups have started to benchmark these cells against primary or BM-MSC-derived cells [13,16]. Reporters for key chondrogenic markers such as collagen II have been used to assess and purify differentiated cells [17], while advances in single-cell sequencing technologies resulted in more thorough evaluation of differentiation strategies [15].

Some groups have also begun to leverage these insights to genetically modify cell lines to modulate expression of favorable or unfavorable factors, for example, by constitutively activating bone morphogenetic protein-2 (BMP-2) for increased chondrogenic capacity [18]. With the rise of CRISPR-Cas9 technologies, cells can be edited with high efficiency to study OA disease pathways. In human chondrocytes, knockout of matrix metallopeptidase (*MMP*)-13 expression enhanced accumulation of collagen II [19].

In chondrogenic micromass cultures of murine iPSCs, targeted deletion of the gene encoding interleukin (IL)-1 receptor type I conferred some immunity against IL-1-mediated tissue degradation [20]. In this study, we have outlined three general approaches for differentiating hiPSCs in order of increasing complexity—single-step, MSC-based, and developmentally guided protocols—keeping in mind their use in bioengineered human cartilage–bone models.

Other reviews cover the use of pluripotent stem cells in skeletal tissue engineering more broadly, including for clinical therapeutic use [21]. Beyond optimizing cell fate specification through temporal coordination of key signaling pathways in isolated cell cultures, we will next consider spatiotemporal regulation of relevant cues and tissue cross talk in three-dimensional (3D) settings while continuing to draw inspiration from development.

Spatiotemporal Cues in Forming Cartilage–Bone Tissues

It is a well-established paradigm that cells exhibit more native-like behavior when cultured under physical and biochemical conditions mimicking the in vivo microenvironment [22]. Chondrocytes in particular are known to dedifferentiate over time in monolayer cultures on stiff substrates and express more collagen I and III and to redifferentiate when suspended in soft 3D hydrogels and recover their chondrogenic phenotype in terms of collagen II and proteoglycan expression [23].

First-generation tissue engineering was able to capture some of these differences, largely using homogeneous isotropic constructs fabricated in bulk. However, spatiotemporal gradients of transforming growth factor beta (TGF-β), a widely used factor for driving chondrogenesis and promoting tissue growth, are critical to the development of stratified native-like cartilage [24].

Importantly, engineered cartilage tissues that fail to recapitulate the appropriate tissue anisotropy become unstable and undergo endochondral ossification once subjected to in vivo implantation [25,26]. Similarly, BMP-2 can promote chondrogenic differentiation, osteogenic differentiation, or endochondral ossification in the same system, but requires careful regulation [27].

Our group and others have shown the utility of including bone substrate for promoting chondrogenesis, suggesting that cartilage–bone cross talk is important in not only disease pathogenesis but also homeostasis of healthy tissues [28–30]. In next-generation tissue engineering, we seek the means for introducing spatiotemporal patterning in vitro to more closely mimic developmental processes and ultimately recapitulate the stratified organization of human cartilage with cartilage–bone interactions.

There have been numerous efforts to introduce some degree of native-like tissue organization, targeting one or multiple fundamental pillars of tissue engineering—cells, scaffold, and environment. Early on, some groups tried to pattern subpopulations of chondrocytes isolated from zonal slices of bovine articular cartilage and reencapsulated in

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hydrogels [31,32]. Others have since included porous, hollow fiber materials for enhanced nutrient transport and MSCs in coculture as support cells [33].

Even so, cell-based patterning methods in their current state suffer from limited cell numbers and lack of patient specificity. Without the ability to robustly generate specific subpopulations of chondrocytes through hiPSC differentiation protocols, we turn to more recent efforts in osteochondral organoids.

Organoids have garnered much success as in vitro 3D tissue models with basic self-organization to study aspects of development, function, and disease for the kidney, lung, gut, brain, and retina, on top of a rapidly growing list [34]. Although chondrogenic micromasses have been used for some time, only recently have true osteochondral organoids—recapitulating organ-specific hallmarks in terms of multicellularity, function, and architecture—been formed in vitro from murine iPSCs by mirroring endochondral ossification bone development [35]. These organoids offer a scaffold- and bioreactor-free platform for studying cross talk at the cartilage–bone interface with potential for patient and disease specificity, trading some features of model complexity for ease of use and throughput.

Scaffolds are necessary for building more complex engineered tissues and can be used to provide both physical and biochemical cues. Multilayered scaffolds have been fabricated to mimic depth-dependent heterogeneity in cartilage, for example, by tuning the percentage of agarose in each layer to adjust stiffness [36]. Electrospun scaffolds with trizonal fiber organization demonstrated more native-like compressive properties than randomly aligned scaffolds [37].

Freeze-dried extracellular matrix (ECM) derived from porcine articular cartilage and growth plate tissues and used in bilayered cartilage–bone constructs could spatially direct the differentiation of seeded BM-MSCs [38]. With advances in 3D bioprinting, non-ECM-derived scaffolds can also be functionalized with biochemical cues to promote chondrogenesis or osteogenesis at precise locations. Cartilage- and bone-promoting peptides presented in different combinations and arrangements within one continuous construct worked synergistically to guide tissue formation in the absence of differentiation factors [39].

Growth factors can also be incorporated in specific scaffold locations with varying release profiles; this has been demonstrated with angiogenic vascular endothelial growth factor and osteogenic BMP-2 gradients to regulate bone healing [40]. Cartilage–bone interactions are also commonly introduced to the model through the scaffold. Our laboratory has demonstrated the technical feasibility and biological utility of interfacing decellularized bone matrix with engineered cartilage toward forming better stratified tissues [28,29].

Others have layered chondrogenic micromasses at varying stages of the endochondral ossification process to create cartilage- and bone-like regions within a single construct in a modular approach, but critically relied on in vivo implantation to eventually establish a cohesive tissue [41]. Overall, scaffolds present a highly useful opportunity for spatially introducing both physical and biochemical cues, but with a general lack of control over temporal dynamics, especially once cells are added and remodel their surroundings.

Targeting the environment external to the engineered tissue to deliver spatiotemporal cues has garnered more attention recently, with engineering advancements making these approaches technically feasible. Work from our laboratory showed that recapitulation of physiological spatiotemporal TGF-β gradients in an in vitro Transwell system resulted in enhanced zonal organization of engineered human cartilage with more phenotypic stability, presenting a promising strategy for stratified cartilage tissue engineering [42].

Dual-chamber, microfluidic organ-on-a-chip bioreactors have also been used to establish tissue-specific microenvironments with distinct chondrogenic and osteogenic medium streams. Encapsulation of human BM-MSCs or hiMSCs in gelatin with culture over 4 weeks resulted in an osteochondral tissue-on-a-chip with the formation of a nascent, functional osteochondral junction [30,43].

Others have demonstrated methods to establish the osteochondral interface through BMP-2 gradients, creating distinct cartilage and bone regions with an interface resembling the native tidemark using buoyancy-driven gradients [44] or magnetic field alignment of BMP-2-conjugated superparamagnetic nanoparticles [45]. Magnetic alteration of a hydrogel scaffold, in lieu of tagging growth factors or cells of interest, can also be used to the same effect [46].

In the context of bone regeneration and using optogenetically modified LIM homeobox 8 (*LHX8*) and *BMP-2* genes, light-activated expression could selectively drive the proliferative or osteogenic differentiation potential of rat BM-MSCs [47]. The use of external force fields for patterning in tissue engineering has been extensively described [48]. These newer strategies trend toward overcoming the limitations of traditional environmental patterning methods, which rely on maintenance of a chemical gradient and have an inherent lack of fast and specific spatiotemporal control, making them difficult to translate to complex settings such as multitissue platforms or loading bioreactors.

Combining several of these methodologies, we recently created autologous cartilage–bone constructs with native-like features at a clinical scale, recapitulating zonally organized cartilage with low friction coefficients as well as mature subchondral bone matrix after 6 months of orthotopic in vivo implantation [49]. Comparing the histological and functional properties at 5 weeks of in vitro culture versus 6 months postimplantation, these cartilage–bone grafts served as templates for remodeling and regeneration, rather than immediate replicates and replacements of the native tissue.

In studies done by others and ourselves, in vitro models may capture critical aspects of the osteochondral unit in a patient-specific manner, but continue to fall short of the full complexity of tissue architecture and cross talk offered by in vivo models.

We considered the perennial question of how simple is complex enough in the context of chondrogenic fate specification from pluripotent stem cells at the cellular, tissue, and systemic scales. In general, in vitro models should emulate functional physiological responses of their in vivo counterparts based on the biological question of interest. OA is a heterogeneous and multifactorial disease typically affecting older adults, with disrupted cartilage–bone tissue architecture and cross talk facilitating disease progression.

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Therefore, a robust in vitro model for studying OA should feature patient specificity, cellular maturity, stratified cartilage organization, and osteochondral interactions. Beyond these design requirements for the osteochondral tissue unit, there are systemic contributors to OA such as circulating factors (proinflammatory cytokines, adipokines, and hormones), which may also offer potential therapeutic targets [50–52]. An in vitro model should be responsive to such stimuli, which will likely require integration into more complex microphysiological systems.

In the last decade, there has been rapidly growing interest in organ-on-a-chip research and, in turn, substantial progress in advancing the individual in vitro tissue models that comprise these microphysiological systems [53]. Although the existing in vitro model approach may be incomplete, bioengineered cartilage–bone tissues are already demonstrating utility in modeling some aspects of OA.

Model Validation and Benchmarking

Nearly as challenging as engineering native-like human cartilage–bone tissues is generation of in vitro models of OA, particularly because the underlying causes and pathways are poorly understood. The methods for inducing OA-like catabolic changes in vitro broadly fall into the categories of cytokine stimulation and physical loading. Cytokines such as IL-1β and tumor necrosis factor alpha have been implicated, although their exact mechanisms in OA are not known beyond their presence in the joint following synovial inflammation [50,54].

Although loading parameters can be precisely tuned, their effects largely depend on the scaffold material. As OA is a slowly progressive disease, supraphysiological levels of cytokines or loading are used for in vitro models, typically to replicate the degradative hallmarks of end-stage disease [55].

Some of the aforementioned tissue platforms have been studied under OA-like conditions. Using the osteochondral tissue-on-a-chip models, the OA state in a human BM-MSC-based model was induced through a 7-day IL-1β challenge to the cartilage component alone or the bone component alone and analyzed for tissue-specific markers and MMP expression [43]. Interestingly, IL-1β treatment of the bone component created a catabolic response in the cartilage component, which was stronger than after direct treatment of the cartilage component, demonstrating effective cartilage–bone cross talk in the model and suggesting a more active role of osteoblasts in OA degeneration.

In the follow-up study using the hiMSC-based model, the authors recapitulated these findings and additionally tested drug responses [30]. Celecoxib, a commonly prescribed NSAID for OA, showed no adverse effect on healthy tissues and downregulated catabolic and proinflammatory cytokines in pathologic tissues. OA tissues that only had a drug-treated cartilage component still showed some rescued phenotype in the bone component. Overall, these studies offer a strong case for the utility of engineered cartilage–bone tissues for elucidating some mechanisms behind OA progression and for screening candidate therapeutics. To the best of our knowledge, loading-induced OA has only been studied using explants, but not engineered cartilage–bone tissues.

Key players have been identified in OA pathology, but findings are largely observational rather than mechanistic, leaving us without consensus on the best model of OA. It is unclear whether changes in cartilage or bone serve as the primary trigger in OA and which effects of synovial inflammation on the osteochondral unit are conferred through interactions with synovial fibroblasts versus their secretory cargo alone [56,57].

Typically, models can be benchmarked to tissue histology or imaging, changes in biomarkers, or known drug responses, but these present challenges in OA. Human clinical samples, already scarce, tend to come from patients with advanced disease, making it difficult to identify early disease mechanisms that can be therapeutically targeted. Earlier stage disease samples may be available from animals, with some similarities between naturally occurring OA in domestic animals and humans [58].

Similarly, imaging tends to capture late-stage disease well after the onset of symptoms. With a heterogeneous OA disease population, molecular changes and drug responses can be highly variable. For clinically relevant benchmarking of a model, there remains an ongoing need for advances in diagnostic imaging and biomarker discovery, particularly to capture early-stage OA disease features.

Outlook

Judging by the pace at which critical fields—stem cell biology, tissue engineering, imaging, and high-throughput omics—have progressed in the last decade, we anticipate that the development of more advanced human tissue models will soon capture more of the complexity of OA and other joint diseases, ultimately leading to improvements in therapeutic discovery and clinical management.

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