Trends in Biotechnology

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Review

Mimicking the Articular Joint with *In Vitro* Models

Susanna Piluso,[1,2,3] Yang Li,[1,3] Florencia Abinzano,[1,3] Riccardo Levato,[1,3] Liliana Moreira Teixeira,[2,3,4] Marcel Karperien,[2] Jeroen Leijten,[2] René van Weeren,[3,4] and Jos Malda,[1,3,4,*]

Treating joint diseases remains a significant clinical challenge. Conventional *in vitro* cultures and animal models have been helpful, but suffer from limited predictive power for the human response. Advanced models are therefore required to mimic the complex biological interactions within the human joint. However, the intricate structure of the joint microenvironment and the complex nature of joint diseases have challenged the development of *in vitro* models that can faithfully mimic the *in vivo* physiological and pathological environments. In this review, we discuss the current *in vitro* models of the joint and the progress achieved in the development of novel and potentially more predictive models, and highlight the application of new technologies to accurately emulate the articular joint.

Highlights

While different *in vitro* and *in vivo* models of joint disorders have been developed, there are no effective tools for the evaluation of new therapies for joint diseases, such as osteoarthritis (OA).

Recent advances in (bio)fabrication technologies enable the generation of *in vitro* models than can further recapitulate articular physiology with the potential of replacing animal models.

The validation of these advanced *in vitro* models is crucial to exploit their translational potential.

The Synovial Joint: A Complex Organ

The proper functioning of the joint depends on the maintenance of joint homeostasis, a dynamic equilibrium between anabolic and catabolic processes within all the components of the joint [1,2]. The joint is a complex multitissue organ encompassing the articular cartilage, the subchondral bone, the synovial membrane, and, in some joints, additional intra-articular structures, such as ligaments and menisci (Box 1). The synovium is essential for joint homeostasis; in fact, synovial macrophages are responsible for the maintenance of a fine balance between proinflammatory and anti-inflammatory **cytokines** (see Glossary) in the synovial fluid [3]. Synovial inflammation is now recognized to play a key role in the progression of joint diseases, with the release of inflammatory cytokines being mediated by the crosstalk between synovium and cartilage [4]. Further, alterations in the composition and structure of the subchondral bone can affect the behavior of the overlying cartilage, suggesting the existence of a physical and molecular crosstalk between the two tissues [5,6]. The intricate interaction between these different tissue structures and cell types makes it quite challenging to recapitulate both healthy and pathological [e.g., in the case of **osteoarthritis (OA)** or **rheumatoid arthritis (RA)**] joint physiology in a model. Clearly, conventional 2D *in vitro* static cultures cannot accurately recreate this level of complexity.

Therefore, to unravel the intricate mechanisms involved in joint homeostasis and disease, a wide range of *in vivo* models have been used [7,8]. Examples include models in small animals (i.e., in the mouse, rat, rabbit, or guinea pig) that are often used for initial drug screening, as these are generally cheaper and easier to handle than the large animal models. In contrast, large animals (i.e., in the dog, goat, sheep, pig, or horse) show more similarities to humans in terms of joint anatomy and cartilage morphology, but are more expensive and require specialized facilities [9,10]. Nevertheless, animal models allow for the study of diseases in the naturally

[1]Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
[2]Department of Developmental BioEngineering, Technical Medical Centre, University of Twente, Enschede, The Netherlands
[3]Regenerative Medicine Utrecht, Utrecht University, Utrecht, The Netherlands
[4]Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

*Correspondence: j.malda@umcutrecht.nl (J. Malda).

Box 1. Tissues of the Joint

Articular cartilage is a highly specialized connective tissue that covers the ends of our long bones. It is an avascular, alymphatic, and aneural tissue that consists of a dense ECM with a low density of cells (about 1–2%) [5]. Subchondral bone is the layer of bone beneath the cartilage. Together with the cartilage it forms a biocomposite, known as the osteochondral unit, which is specialized to transfer load during weight-bearing and joint motion. The synovium constitutes the envelope of the articular joints and, by acting as an interface with the systemic blood circulation, ensures nutrient supply to the articular cartilage via the synovial fluid. Moreover, it supplements the synovial fluid with the key molecules necessary for joint function (i.e., lubricin, hyaluronan, and immunomodulatory cytokines) [5]. The synovial membrane consists of two cell types, synovial fibroblasts, which constitute up to 75% of all cells in a healthy synovium, and macrophages. The macrophages can be classified into classically activated (M1) and alternatively activated (M2). The latter subtype is involved in the production of immunoregulatory factors [e.g., IL-10 and chemokine ligand (CCL)-18], whereas M1 macrophages produce proinflammatory mediators such as TNF-α, IL-1β, and IL-6, which play a key role in synovial inflammation [88]. The number of macrophages increases drastically during inflammation and, together with synovial fibroblasts, secrete proinflammatory cytokines and ECM-degrading enzymes. The production of cytokines attract inflammatory cells (T cells, macrophages, monocytes) into the synovium, resulting in the formation of a mass of inflamed tissue or pannus [3].

Additional components of a healthy knee joint are the menisci (i.e., wedge-shaped tissues that perform complex functions in load-bearing, load transmission, stabilization of the joint, shock absorption during movements, nutrition of articular cartilage, and lubrication [89]). Lubricin, a superficial zone protein, which has a key role in the maintenance of joint integrity, is highly expressed in healthy knee menisci. The expression of lubricin is downregulated in the knee menisci and synovial fluid of OA patients, leading to increased friction in the joint and cartilage degeneration [80]. Further, meniscus cells increased their production of matrix-degrading enzymes, cytokines, and chemokines in response to stimulation with proinflammatory factors (IL-1β, IL-6, or fibronectin fragments). This suggests that the role of the meniscus in OA goes beyond the mechanical aspect and might be due to biological interaction [90].

Tendons and ligaments are soft connective tissues composed of closely packed, highly aligned collagen fiber bundles that join bone to muscle and bone to bone, respectively. These structures transfer tensile loads to guide motion and stabilize the diarthrodial joint [91]. Tendon or ligament failure may result in joint destabilization and hence lead to damage by altering the biomechanical balance between neighboring tissues (e.g., meniscus and articular cartilage) in the joint [92]. Further, after anterior cruciate ligament injuries, the gene expression of key degradative enzymes (MMPs) was upregulated in the ligaments and the synovium, suggesting a close interaction between these tissues in response to injuries and a key role in cartilage degradation [93].

The knee joint also contains Hoffa's fat pad or infrapatellar fat pad (IFP), a soft tissue interposed between the joint capsule and the synovium. The IFP is a highly innervated tissue and, therefore, a common source of knee pain. Although its physiological function in the knee remains still elusive, early studies suggest that inflammation of this adipose tissue might play a role in the development and progression of OA [94].

occurring environment of the whole joint. However, due to species-specific differences [10,11], many therapeutic treatments fail when translating animal studies to human clinical trials [12]. Furthermore, ethical concerns and the societal ambition to reduce animal experimentation have driven the development of advanced *in vitro* models that can more accurately represent stages of human disease.

Recent advances in engineering and biology have resulted in the development of functional microscale units of human organs that are able to recapitulate human diseases [13,14]. A unique feature of these systems is their ability to recreate the complex tissue microenvironments and facilitate communication between different tissues, accurately mimicking the *in vivo* situation [15]. *In vivo*, cells reside in a highly sophisticated 3D microenvironment that provides biochemical and biomechanical cues guiding their behavior, including migration, proliferation, and differentiation [16]. Alterations of a specific **extracellular matrix (ECM)** component can already greatly impact the biochemical–biomechanical balance, disrupting tissue homeostasis and function [17,18].

Glossary

3D bioprinting: the automated process of patterning and assembling living and non-living materials with a spatially controlled organization, to produce bioengineered structures for application in regenerative medicine.
Chondrocytes: cartilage mature resident cells. They produce and maintain the extracellular matrix that keeps cartilage healthy and functional.
Cytokines and growth factors: signaling molecules released by cells, with the objective of affecting the behavior of other cells, including growth, proliferation, migration, inflammatory state, and differentiation.
Explant: extracted pieces of native tissues or organs that can be cultured in the laboratory.
Extracellular matrix (ECM): dense network of macromolecules, such as proteins and polysaccharides, that provides structural and biochemical support to the surrounding cells.
Hydrogel: hydrophilic polymer networks, which absorb and retain a large amount of water.
Mesenchymal stromal cells (MSCs): multipotent adult cells that can differentiate into bone, cartilage, adipose, and muscle tissue. They can be obtained from bone marrow, adipose tissue, and other sources.
Microfluidics: a multidisciplinary field between chemistry, physics, engineering, and micro/nanotechnology which deals with the precise manipulation of fluids at submillimeter scale.
Organ-on-a-chip: a multichannel microfluidic device with integrated microscale cellular co-cultures that offers physiological biochemical and/or biophysical stimulation to more realistically recapitulate the native microenvironment.
Osteoarthritis (OA): degenerative joint disease associated with cartilage destruction, inflammation of the synovial membrane, and subchondral bone remodeling.
Rheumatoid arthritis (RA): a systemic inflammatory disease that involves synovial cell proliferation and structural damage to cartilage, bone, and ligaments.

Key Figure

Schematic of the Synovial Joint

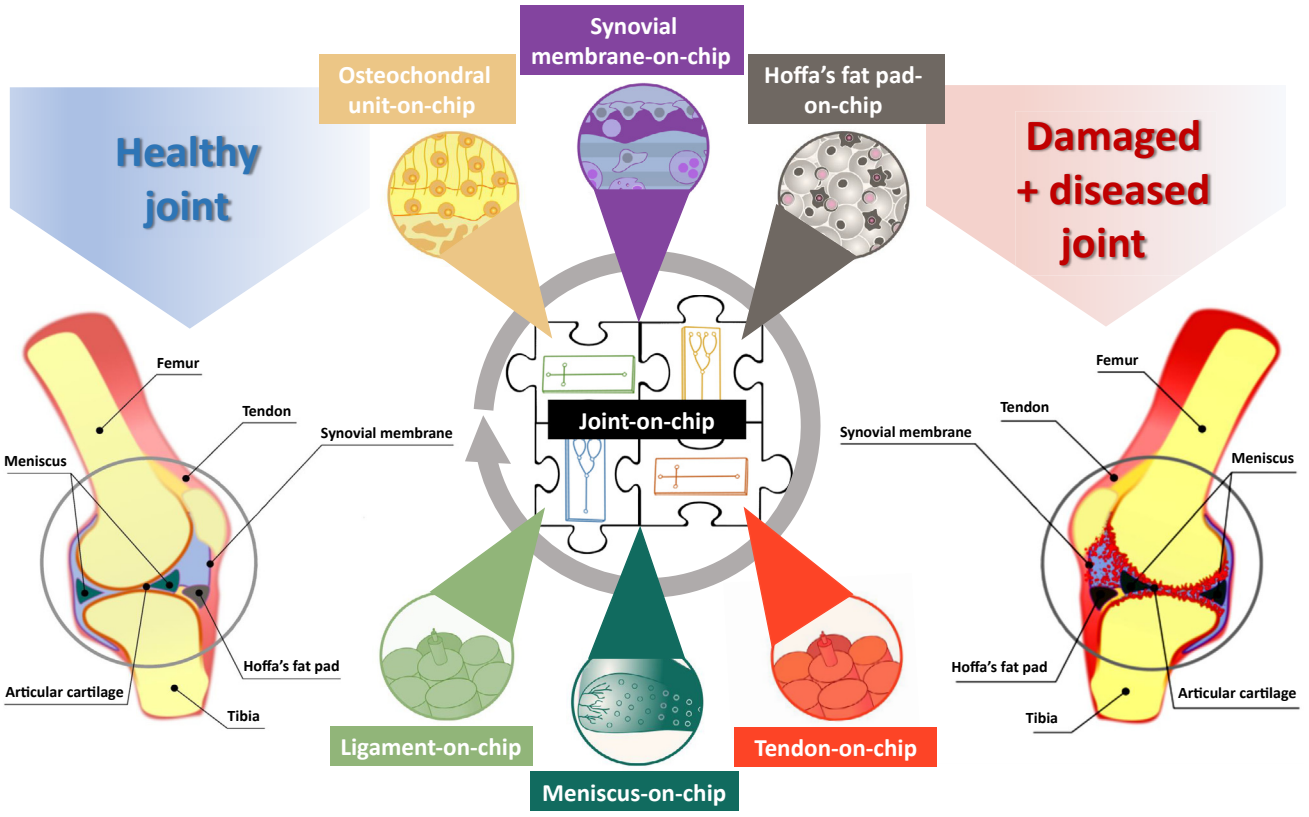

Figure 1. The synovial joint consists of several tissues that work closely together to realize and maintain joint function and homeostasis. These tissues comprise articular cartilage, subchondral bone, synovium, menisci, tendon, ligament and fat pad, which all are characterized by a specific structure and which are exposed to different biochemical and biomechanical stimulations. Impairment of function of one tissue leads to altered behavior of the other tissues, which can lead to joint degeneration. The ideal *in vitro* model must recapitulate this complex environment; the most suitable approach to realize such a model is the development of several joint tissues-on-chip, that can be connected with each other in a modular fashion.

Ideally, *in vitro* models of the joint must recapitulate the intricate microenvironment of the synovial cavity and capture the interactions between the various joint elements (Figure 1, Key Figure). The tissues that the joint is composed of are extremely sensitive to their mechanical environment, and loading plays a key role in the maintenance of joint homeostasis [19]. While moderate mechanical loading contributes to the maintenance of tissue integrity, reduced loading or overloading can trigger pathological changes in joint tissues [20]. Therefore, *in vitro* models of the joint should ideally allow for the inclusion of mechanical stimulation and reproduce the interactions between the different tissues.

Here, we review the existing *in vitro* models and we propose new avenues for the future development of more sophisticated models of the articulating joint.

The State of the Art: *In Vitro* Models of Tissues in the Joint

2D Monolayer Culture Models

To date, limited *in vitro* models exist to study the interactions between the different tissues of the joint. Within the majority of current *in vitro* models, the simulation of interactions is often limited to the introduction of biochemical cues or to co-culture of cells or tissues [21]. For example, interactions with the synovium are often simulated by supplementing the culture media with cytokines [22], or by addition of synovial fluid [23]. Alternatively, fibroblast-like synoviocytes can be included in the model [24]. Recently, an *in vitro* model using synovial cells was developed to study the response of the synovium to cartilage wear particles [25]. The model consisted of a dense cell sheet of fibroblast-like synoviocytes in culture with cartilage particles. The co-culture resulted in an increased production of cytokines and thickening of the cell sheet through increased collagen content. Interestingly, these results correlate with data from previous animal studies showing that cartilage debris can induce synovitis [25].

Choosing a cell source that is representative can, however, be a challenge, with primary **chondrocytes** and chondrogenically differentiated **mesenchymal stromal cells (MSCs)** being the most commonly chosen cells for this simplified view of the joint [21]. Additionally, cells cultured on 2D surfaces are prone to change their phenotype compared with their native *in vivo* milieu. To understand the pathways leading to de-differentiation, changes in gene expression were investigated in monolayer cultures of human chondrocytes [26].

Notwithstanding their relative simplicity, these models do provide valuable insights that can enhance our understanding of the events involved in joint diseases. However, these simple models provide information only on isolated events within a specific tissue and allow for changing only one factor at a time (cytokines, **growth factors**, osmotic pressure, etc.).

Biomaterial-Based 3D Culture Models

The most simplistic 3D culture model is based on aggregation of cells into spheres [27]. Previous studies have, indeed, shown that chondrospheres outperform single cells in terms of cartilage matrix production [28], allowing the study of cell-to-cell and cell-to-matrix interactions. This provides a powerful yet simple tool to study cartilage formation and to preliminarily screen drugs for OA [29]. Combining microaggregates with **hydrogels** further exploits the natural environmental cues to promote growth factor-free chondrogenic differentiation [30]. These constructs can also be subjected to mechanical loads to investigate the cell response to these types of stimuli [31,32]. Mechanical loads can induce changes in the pericellular and territorial matrix of chondrocytes [33]. Further, cellular response to mechanical stimuli depends on the material within which cells are encapsulated [34]. For example, hydrogels provide 3D matrices with properties similar to natural ECMs, such as high water content, porosity, and biocompatibility [35]. To capture the biological features of ECM, hydrogels can be modified to provide bioactive cues, such as the arginine-glycine-aspartic acid (RGD) adhesive motif or the peptide binding motif of N-cadherin that mimics cell–cell interactions, which might enhance chondrogenesis of MSCs [36,37]. Further, the dynamic remodeling of ECM through cell-responsive enzymatic degradation can be replicated by crosslinking hydrogels with difunctional matrix metalloproteinase (MMP)-degradable peptides [38]. The inclusion of protease-degradable crosslinks in hyaluronic acid hydrogels significantly affected the morphology of encapsulated MSCs, which displayed an elongated shape. Meanwhile, cells remained rounded in hydrogels that inhibited cellular remodeling [38].

Additionally, hydrogel mechanical properties can be reinforced and tailored to match the stiffness of the target tissue, for example, by combining the hydrogel with polymeric fibers

[39]. The fibers could also mimic tissue topography, which is known to influence cell behavior. For example, composites of electrospun fibers with bioglass-derived foams or biphasic scaffolds have been used to repair osteochondral defects [40]. Recently, an engineered scaffold consisting of hydrogels and electrospun fibers was developed to simulate the mechanical and topographical characteristics of native tendon tissue [41]. Additionally, hydrogels can be fabricated to mimic structural features of tissue interfaces, such as the tendon/ligament-to bone interface, by using biphasic scaffolds that mimic the alignment of collagen molecules [42]. Further, the combination of **microfluidic** mixing technologies with hydrogel functionalization approaches enable the preparation of hydrogels with a controlled spatial gradient of cells and biomolecule signals [43].

Table 1. Representative Examples of In Vitro Models of Joint Tissues

Tissue	Model	Findings	Refs
Fibroblast-like synoviocytes	2D monolayer culture	Treatment of fibroblast-like synoviocytes resulted in cell death and production of proinflammatory mediators (IL–1β, IL-6, and TNF-α)	[24]
	2D monolayer culture	Culturing fibroblast-like synoviocytes with cartilage wear particles resulted in an increase in proliferation and ECM content, similar to the thickening of synovial lining observed in OA patients	[25]
Periosteal stem cells	Biomaterial-based 3D culture	Encapsulation of macroaggregated periosteal stem cells into biomaterials resulted in improved *in vivo* cartilage tissue formation, compared with single cell-laden hydrogels	[30]
Cartilage	Tissue explants	Collagenase was used on cartilage explants to imitate early OA; mechanical loading affected the degraded cartilage more than healthy explants	[44]
		Ageing affected TGF and bone morphogenetic protein (BMP) signaling pathways, which might contribute to the development of OA	[82]
		Combining insulin-like growth factor-1 (IGF-1) and dexamethasone (Dex) prevented matrix loss in an inflammatory environment	[45]
Bone	Tissue explants	Trabecular bone explants were submitted to induced shear stress to analyze the effects on cilia expression of bone marrow cells	[83]
Meniscus	Tissue explants	Cytokines inhibited meniscal repair of explants *in vitro*	[84]
		Inducing overexpression of TGF-β via recombinant adeno-associated virus (rAAV)-mediated gene transfer stimulated *in vitro* healing of meniscus explants	[85]
Tendon	Tissue explants	Cyclic loading induced expression of inflammatory markers on tendon fascicles	[86]
Synovium	Tissue explants	Synovial explants from rheumatoid arthritis patients were used to evaluate the effects of biologic disease modifying antirheumatic drug treatment *in vitro*; these results were correlated to clinical performance of the drugs	[87]
Cartilage and synovium	Co-culture	Adding osteoarthritis synovium to cartilage explants inhibited glycosaminoglycan production, and this effect was counteracted by the addition of triamcinolone	[55]
	Osteochondral plug	An osteochondral plug model with independent compartments for cartilage and bone was used to evaluate the effect of cell distribution within a hydrogel for cartilage repair applications	[51–53]
Osteochondral microtissue	Multichamber bioreactor	The effects of IL-1β were evaluated on an osteochondral microtissue model, where MSCs seeded within a hydrogel were maintained using separate chondral and osseous compartments in which the cells differentiated into cartilage and bone-like tissues	[50]
Osteochondral unit and synovial lining	Multichamber bioreactor	The synovial lining was reproduced by incorporating MSCs seeded within a polyethylene glycol hydrogel on the osteochondral interface, consisting of a collagen hydrogel	[74]

Tissue Culture Models

Tissue **explants**, however, keep the cells in their natural microenvironment (Table 1). Cartilage explants have provided valuable information on the whole tissue response to several stimuli, recreating both physiological and pathological environments in diverse joint tissues. For example, to emulate the damage observed in early stages of OA, cartilage explants were first treated with collagenase and then subjected to repetitive mechanical stress. This model provided novel information on the mechanisms associated with cartilage degeneration (e.g., mechanical loading) [44]. In another study to elucidate the mechanism of action of dexamethasone, a potential therapeutic drug, cartilage explants were treated with inflammatory cytokines and subjected to mechanical injury to simulate early stages of OA [45]. A major drawback is that only a limited number of explants can be obtained from each donor and there is a high intradonor variability between samples, depending on the location of extraction from the joint [46]. This variation is even greater between donors, presenting a challenge for reproducibility. Explants commonly feature cell death on the edges where the tissue was cut [21]. Long-term studies are also complicated, as the properties of cartilage and bone explants change over time in terms of mechanical properties and ECM composition [47]. Moreover, although it is relatively easy to obtain explants from animal sources, availability of healthy human donor tissue is limited.

Bioreactors

Bioreactors are devices able to culture cells under a controlled environment (e.g., temperature, pH, nutrient supply, mechanical stimuli). Different types of bioreactors have been developed based on tissue and application, such as spinner flasks, rotating wall vessels, and perfusion bioreactors [48,49]. In a recent work, a bioreactor consisting of two separate compartments has been used to recreate an engineered osteochondral unit. The two microenvironments could be individually controlled, thus allowing control of conditions in the bone and cartilage part, following exposure to proinflammatory cytokines [50]. Further, the design of this bioreactor was further advanced to enable continuous optical monitoring during culture [48].

Preserving the integrity of an osteochondral unit during *in vitro* culture has been shown to extend the life and quality of explants [51]. Previous work on osteochondral explants demonstrated that bioreactor platforms (six-well plate format) providing distinct media to the cartilage and bone regions of the implant allow for long-term culture of the osteochondral plugs, while maintaining cartilage tissue content, structure, and mechanical properties [52]. This platform was also used to investigate the effect of the spatial chondrocyte distribution in cartilage repair mechanisms [53]. Despite the fact that this osteochondral plug-based model provided new insight into the interaction between tissues, it is still limited to the interplay between bone and cartilage and has not been yet adapted to represent inflammatory joint conditions.

Co-Culture of Tissue Explants

The above-described *in vitro* models focus on individual tissues of the joint or the osteochondral unit, without including interactions with other elements of the joint. This aspect was highlighted in a recent study describing co-cultures of bovine cartilage explants with explants of fibrous joint capsule and synovium [54]. Specifically, the co-incubation of mechanically injured cartilage with fibrous joint capsule and synovium resulted in increased proteolytic degradation of aggrecan by both MMPs and aggrecanase. This enhanced proteolytic activity was not observed when cartilage was cultured alone [54]. Similarly, the co-incubation of explants of human articular cartilage with human synovium resulted in the production of MMPs and inflammatory cytokines that were not detected in the cartilage explant monoculture [55]. The cytokines and degradative enzymes detected in the synovium–cartilage co-cultures were similar to those found in the synovial fluid of patients with OA, suggesting that co-cultures have a higher predictive power

compared with monocultures. This further suggests that *in vitro* models of the joint must be multitissue systems in which the synovium plays a crucial role.

The Challenge: More Advanced *In Vitro* Technologies for Mimicking Articular Function

Although static culture of tissue explants and co-cultures can recapitulate some *in vivo* functionalities of the joint tissues, these models still lack mechanical stimuli, such as tension, compression, and shear stress that cells experience *in vivo*.

Advances in biofabrication and **microfluidics** technologies provide the opportunity to recreate dynamic flow conditions and mechanical stimulation (Figure 2) that may aid the development of more predictive models of the human synovial joint. To this end, a variety of 3D culture techniques, including 3D human organoids, human **organ-on-a-chip** [56], and biofabricated tissue-like structures [57], have been explored to model physiological and pathological human conditions.

An overview of the current *in vitro* models used to culture articular joint tissues is depicted in Figure 3, highlighting advantages and limitations of each model type, thereby enabling a

Joint tissue	Main cells	Type of mechanical load
Synovial membrane	Fibroblast-like synovial cells Macrophage-like synovial cells T and B cells	Tension Fluid induced shear strain
Osteochondral unit	Chondrocytes Osteoblasts, osteocytes, and osteoclasts Microvascular and nerve cells	Shear stress Tension Compression
Ligament tendon	Ligamentoblast/ ligamentocytes Tenoblasts/tenocytes Fibrochondrocytes Vascular cells	Tension Fluid induced shear
Hoffa's fat pad	Adipocytes Vascular and immune cells Pericytes and mesenchymal progenitor cells	Tension Fluid induced shear stress
Meniscus	Fibroblast-like cells Chondrocyte-like cells Nerve and vascular cells	Shear, tension, and compression

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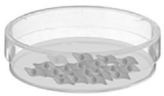
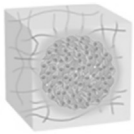
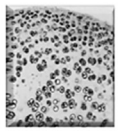
Figure 2. Mechanical Stimulation of the Synovial Joint. Illustrative table with representative examples of the joint tissues with their major cellular components and type of mechanical load.

(A)

Current *in vitro* models	Advantages	Limitations
2D monolayer 	• Reproducibility and compatibility with high-throughput screening • Simple and easy to work • Models isolated events	• Reductionistic • Challenge to select a representative cell source • Cells prone to change their phenotype
3D biomaterial based 	• Allow the study of cell-to-cell and cell-to-matrix interactions • Can provide natural environmental cues	• Reductionistic • Lacks mechanical actuation • No tissue–tissue interface • No gradients of chemicals and oxygen
Tissue explants 	• The cells are cultured in their 3D native microenvironment • Enables whole tissue response to several stimuli	• High intra- and interdonor variation; reproducibility challenge • Limited number of explants from each donor • Static
Bioreactors 	• Extended culture time and quality of explants/tissues • Semidynamic 3D culturing	• No tissue–tissue interface • No gradients of chemicals and oxygen
***Ex vivo* multitissue** 	• Higher predictive power compared with monocultures • Includes interactions with other cells/tissues	• Static • Limited 3D environment • No direct tissue–tissue interface

(B)

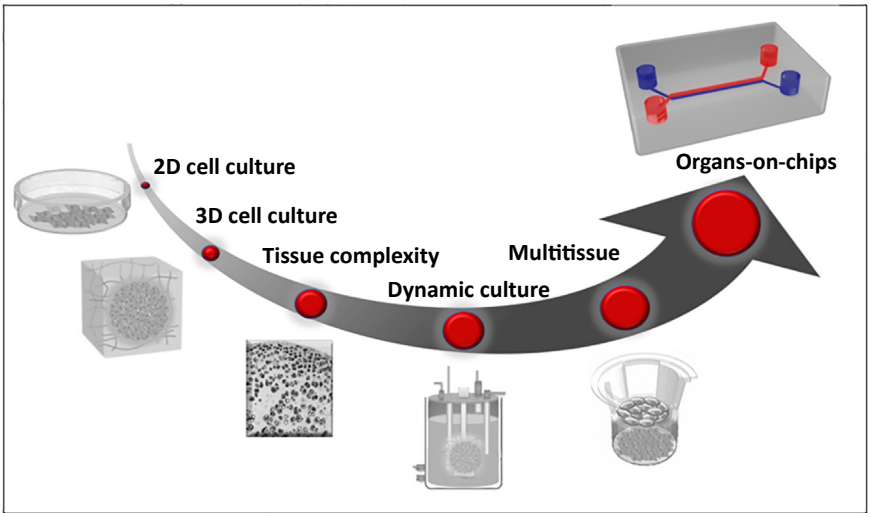


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Figure 3. Current and Envisioned Models of the Articular Joint Tissues. (A) Illustrative table with most relevant *in vitro* cell/tissue culture models of the joint, highlighting the advantages and drawbacks of each model. (B) Schematic representation of the evolution of current *in vitro* models towards advanced organs-on-chip models.

comparison between models (A). The evolution throughout the model systems is illustrated in Figure 3B, featuring increasing complexity steps towards organ-on-chip platforms. Organs-on-chip are microfluidic devices that can simulate physiological functions of tissues and organs [14]. Numerous organs-on-chip models have been developed so far, however, there are still issues to be addressed. First, we should choose a matrix (e.g., a hydrogel) with a suitable composition and topography to mimic the ECM of the different tissues. For example, cartilage exhibits a highly organized structure which is different from the bone or synovium tissue. Further, the stiffness of the matrix is also an important factor, since the mechanical loads experienced by the cells might be affected by the stiffness of the matrix (i.e., shielding) [58]. The need to find suitable flow rates for each of the different tissue models will also be challenging, especially when connecting the different tissues. Another important issue is related to the source of cells. The majority of the microfluidic devices have been operated using cell lines, even though human-derived primary cells would be a better mimic of the human physiology. The main limitations for using human primary cells are the limited donor availability, potential variability, and their costs [59].

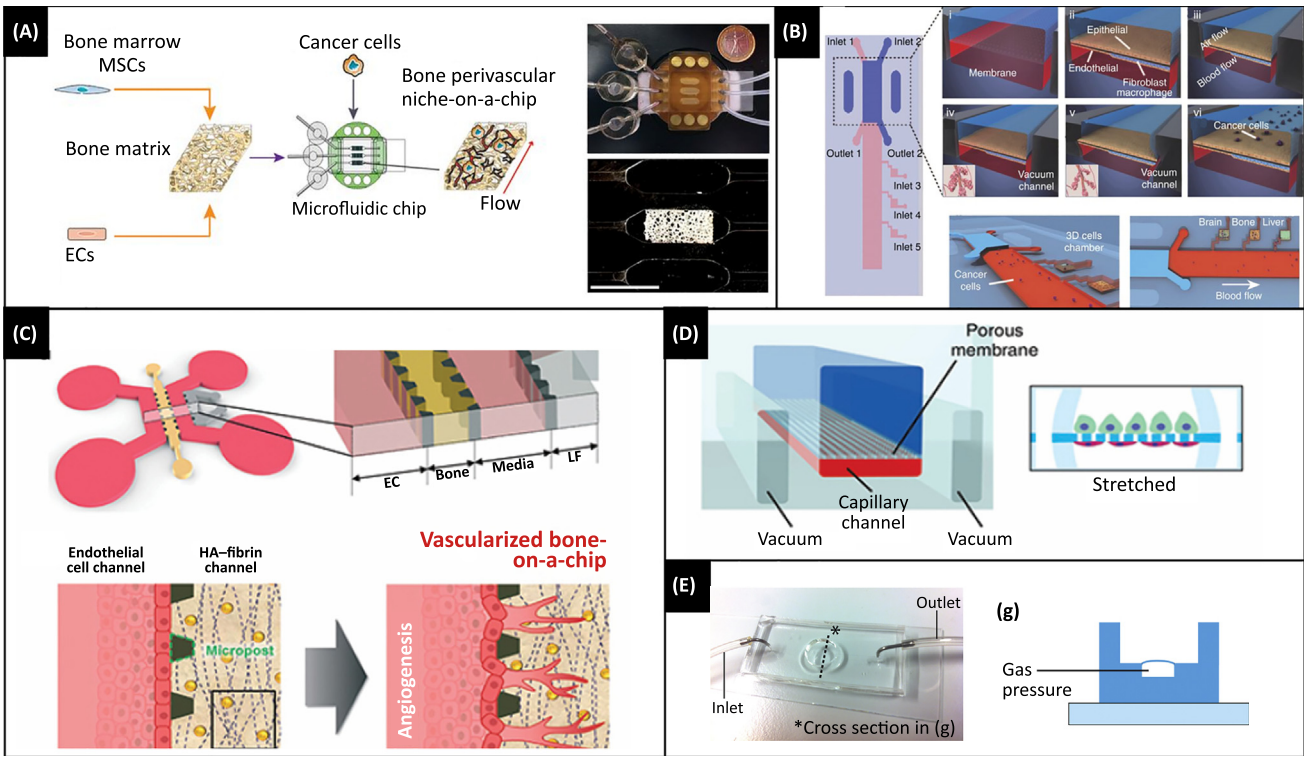
Based on the fundamental understanding of functional and pathological organs, a combination of technological approaches (e.g., soft lithography, microfluidics, **3D bioprinting**) is adopted to simulate *in vitro* the native 3D organization by incorporating biomaterial scaffolds, continuous vascular-like perfusion, mechanical stimulation, and chemical cues within the same platform. 3D bioprinting represents a promising technology for replicating tissue–tissue interfaces due to its inherent capability to deposit heterogeneous bioinks (living cells and/or biomaterials) in a defined spatiotemporal manner relevant to biological architectures. Biofabricated 3D *in vitro* models offer a great opportunity to investigate the physiological and pathological processes of multitissues, as well as to perform drug screening and toxicological studies. Up to now, numerous *in vitro* models of tissues and organs, ranging from bone to microvasculature, have been developed with a variety of shapes, length scales, resolutions, and mechanical properties of biomaterials and mostly using human-derived cells [56,60]. Recently, the performance of an osteochondral bioreactor was significantly improved by using 3D printing. Specifically, the new model enabled the fluid transport through the central chamber to be maximized and allowed for optical access within the 3D construct, while maintaining dimensions compatible with a 96-well plate [48]. Additionally, a recently described 3D printer bioreactor allows for the printing of 3D constructs directly inside the bioreactor, reducing both contamination risk and the risk of damage to the construct [61]. 3D-printing technology has gained increased attention in the fabrication of microfluidic systems. These devices are commonly fabricated using polydimethylsiloxane (PDMS), which is easy to mold, biocompatible, transparent, and inexpensive. Additionally, PDMS molding has very high resolution. However, the fabrication of the devices involves stacking and bonding different layers together, which increases the final cost and limits the 3D complexity that can be achieved [62]. Although, 3D-printing is an automated and assembly-free technology, with low-cost set-ups, limited only by the resolution of the printing process. Printing technologies such as stereolithography (SLA) or melt electrospinning writing (MEW) could significantly improve the resolution of perfusable channels. For example, a custom-built projection SLA has been recently used to prepare complex 3D structures with patterning features of <5 μm resolution [63], while MEW enables the fabrication of fibers of 4–7 μm [64].

Organ-on-Chip Technology

Microfluidic systems can be connected with each other to form multicompartment microfluidic devices. An important challenge when connecting different microfluidic devices involves the requirement for a common media, or blood substitute, suitable for each tissue of the interacting

system [65]. This multicompartment organization is ideal for the design of *in vitro* models of the joint, a multitissue organ where cells are exposed to different types of mechanical stimuli. For example, within the synovial joint, cells experience a high mechanical stress, both through load-bearing and by shear forces created by the motion of the synovial fluid during exercise [66]. The magnitude, direction, and type of stress applied was found to affect the type and severity of inflammation of the joints [67]. However, the biochemical effects of shear force on the synovial membrane have not been studied systematically in an *in vitro* platform, and a multicompartment microfluidic chamber would be a promising solution.

Depending on the orientation of the compartmentalized channels, mainly two types of configurations have been developed (Figure 4C,D) (i.e., upper–lower chambers separated by a semiporous, stretchable membrane and lateral chambers obtained by an array of microposts [68–70] or a removable template [69]). In addition, dynamic compressive bioreactors have been



Trends in Biotechnology

Figure 4. Recreating Microphysiologically Articular Joint-Relevant Environments with Organ-on-Chips. (A) Generation of the bone perivascular niche for studies of breast cancer colonization. Human bone marrow-derived mesenchymal stem cells (MSCs) and endothelial cells (ECs) were cultured in monolayers and in 3D decellularized bone matrix. Image reproduced with permission, from [78]. (B) Multiple 'organs-on-a-chip' platform to model metastasis from lung to brain, bone, and liver downstream organs. This modular strategy can be used to model the tissues from the articular joint. Image reproduced with permission, from [79]. (C) Scheme of a microfluidic vascularized bone tissue model for mimicking real bone angiogenesis. The bone-mimicking channel consists of a mixture of fibrin with hydroxyapatite. Image reproduced with permission, from [69]. (D) Schematic drawing and photograph of the microfluidic kidney glomerulus chip, with the urinary and capillary compartments of the glomerulus separated by a polydimethylsiloxane membrane. Cyclic strain is applied on the side channel to mimic the tissue stretch. It is envisioned that a similar design can be used for joint articular tissues, which involve similar mechanical tension, such as the synovial membrane or the tendons. Image reproduced with permission, from [80]. (E) Picture of a complete actuator chip implemented in mechano-stimuli responsive studies of neuronal cell networks on chip. The images represent a schematic drawing of its mechanism of actuation using gas flow. It is envisioned that a similar design can be used for joint articular tissues involving similar compressive actuation as the cartilage. Image reproduced with permission, from [81]. Abbreviation: HA, hydroxyapatite.

developed to screen the effect of biomaterials and of mechanical stimulation on cellular behaviors (Figure 4E) [71].

Recapitulating the Microphysiological Environment of the Synovial Joint

The current developments described above open up new ways to recapitulate whole organs or combinations of multiple organs in a body-on-a-chip fashion. However, the integration of different organs still faces some challenges, including maintaining the functionality of the different organs and the study of intertissue responses to drug administration. The development of these multicompartment systems, that can interact with each other in a physiologically relevant manner, would be crucial for the development of models of the joint, for example a joint-on-a-chip.

Organ-on-chip technology provides a platform to recreate physiologically relevant environments. Although this novel technology has been used to develop biomimetic systems of several organs (lung, kidney, liver), bone is the only tissue of the joint that has been developed on chip thus far. To mimic the *in vivo* microenvironment of bone, a microfluidic approach was used to develop a vascularized bone tissue model. Fibrin was used as a model of the ECM and combined with various concentrations of hydroxyapatite nanocrystals to mimic the bone structure. The presence of hydroxyapatite resulted in enhanced angiogenic properties, inducing improved sprout length, sprout speed, and lumen diameter [72]. Other studies focused mainly on bone marrow-on-chip. For example, a bone marrow-on-a-chip system was developed by first engineering new bone *in vivo* and then perfusing it in a microfluidic device able to reconstitute the hematopoietic niche physiology [73]. To test the functionality and organ-level response, the engineered bone marrow was exposed to varying doses of γ-radiation, and the results closely mimicked the effects observed in the bone marrow of live mice [73].

To reconstitute the microenvironment of the articular joint, an *in vitro* 3D microsystem model of the osteochondral unit was developed by fitting a multichamber bioreactor into a microfluidic base [50]. To mimic the chondrogenic and osteogenic tissues, human bone marrow stem cells (hBMSCs) were seeded within a hyaluronic acid-based hydrogel and a gelatin/hydroxyapatite construct, respectively. The two compartments were supplied by two different medium streams to reconstitute the chondral and osseous microenvironments and promote tissue-specific differentiation of hBMSCs. In a next step, the osteochondral unit was exposed to interleukin-1β (IL-1β) to evaluate the response to proinflammatory cytokines and the communication between the two compartments. The IL-1β treatment of the osseous compartment resulted in a strong catabolic response in the chondral layer, which was significantly higher than the response observed after local exposure to IL-1β, suggesting an active biochemical communication between the two layers. To better mimic the joint environment, the microtissue model was improved by incorporating an osteochondral interface, consisting of an MSC-laden collagen hydrogel, and a synovial lining produced with MSCs seeded on a polyethylene glycol hydrogel [74]. Although this microtissue represents a valuable model of the osteochondral unit, some key elements such as other joint components (menisci/tendons) and mechanical stimulation (e.g., compression, shear stress) are still missing.

Besides articular cartilage, subchondral bone, menisci, tendons, and fat pad, a key component of the joint organ is the synovium. Indeed, synovial inflammation is intimately associated with joint degeneration in diseases such as OA and RA [3]. Recent studies indicate that OA tissue and synovial fluid contain high levels of cytokines, and that chondrocytes and synovial cells in OA overproduce several inflammatory mediators, including IL-1β, tumor necrosis factor-α (TNF-α), and nitric oxide, which are characteristic of inflammatory arthritis. Further, the lipid

metabolism might also contribute to the pathogenesis of OA through inflammatory mediators, for instance, adipokines produced by adipose tissue such as present in Hoffa's fat pad [75]. It becomes clear that the synovium together with the osteochondral unit, menisci, tendons, and fat pad are essential components for the development of an advanced *in vitro* model of the joint. Further, synovial inflammation also represents an effective target for the development of novel therapeutic strategies. However, a major limitation so far is the inability to specifically target synovial cells, without affecting the whole-organism physiology [3]. This limitation could be overcome by fabricating individual joint components using microfluidic technology on chip to reconstitute the physiological context of each tissue, including mechanical and biochemical stimulation, and combine these individual tissues on chip in a multimodular device: the joint-on-chip. This approach provides the opportunity to understand the molecular pathways involved in the physiology and etiology of each tissue individually and may, as an example, aid in the discovery of synovium-targeting therapies. Furthermore, by designing interconnectable organ-on-chip models of cartilage, bone, synovium, tendons, and fat pad and arranging them as *in vivo*, it is also possible to study crosstalk between the various tissues. An interconnected model of the joint could be valuable for understanding joint homeostasis and to develop novel therapies to prevent and slow the progression of joint diseases.

Outstanding Questions

Can the complex microenvironment of the synovial joint be faithfully recapitulated using the organ-on-chip technology?

Is it possible to study onset of joint diseases and the sequence of pathological events recapitulating the complexity of human disease using a multicompartment joint-on-a-chip?

Will such a humanized joint model be able to predict the efficacy and toxicity of novel treatments for arthritis?

Can a multicompartment joint-on-a-chip enable the discovery of early biomarkers for osteoarthritis?

Validation of *In Vitro* Models of the Joint

These advanced models, due to their great level of physiological mimicry, have the potential to replace or be integrated with animal studies for preclinical testing. To achieve this, one important challenge still needs to be addressed to translate the 'on-chip' results to the clinic; that is, the validation of the model.

Here, a key step will be to demonstrate the predictive power of the multitissue model of the joint for human disease. Therefore, there is a strong need to develop validation methods that can assess the reproducibility, reliability, and the translational potential of those models [60]. A possible strategy could be the testing of a range of drugs with known effect against RA (in the case of the joint models) and compare the results with data obtained from *in vitro* tissue co-culture models, animal studies, and efficacy studies in patients. For instance, celecoxib, a nonsteroidal anti-inflammatory drug used for the treatment of OA and RA, has been used to evaluate the accuracy of a model based on the co-culture of a tissue-engineered cartilage construct with synovial fibroblasts and macrophages. The system could replicate only some of the *in vivo* responses of the drug [76]. An important limitation, however, is that this model only mimics cartilage–synovium interactions, while numerous studies demonstrated a close interaction between cartilage–subchondral bone and vasculature [49]. This further highlights the necessity to develop multitissue models that can better mimic the *in vivo* behavior. These multitissue devices could be advantageous for drug screening, enabling the testing of varying concentrations of a drug or combinations of drugs, simultaneously and in a cost-effective manner. Most importantly, these devices can be operated with human cells and thus are capable of further mimicking the human physiology and metabolism [77].

Concluding Remarks and Future Perspectives

Understanding the molecular mechanisms involved in joint homeostasis and disease is fundamental for the development of novel therapeutic treatments. A major challenge so far has been the lack of models that can faithfully mimic joint physiology in health and disease. The limited predictive power of existing animal models has driven the need to develop advanced *in vitro* models that can more accurately represent the *in vivo*-like environment. Recent advances in engineering and biology have enabled the development of *in vitro* models that recapitulate

complex 3D organ-level structures with integrated mechanical, biochemical, and physical stimulation (see Outstanding Questions). This technology has the potential to emulate the various components of the joint organ and the interplay between the different elements of the joint. In addition to a 'humanized' joint model, the development of joint models for animals is also important for the application in veterinary medicine. Specifically, the use of human and animal models under a similar experimental setting could bring to light species-specific differences. Ultimately, the development of an advanced *in vitro* model of the joint will assist in reducing or replacing the use of animal models in biomedical research, personalized medicine, and pharmaceutical studies.

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