

Progress of Microfluidic Hydrogel-based Scaffolds and Organ-on-Chips for the Cartilage Tissue Engineering

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Abstract

Cartilage degeneration is among the fundamental reasons behind disability and pain across the globe. Numerous approaches have been employed to treat cartilage diseases. Nevertheless, none have shown acceptable outcomes in the long run. In this regard, the convergence of tissue engineering and microfabrication principles can allow developing more advanced microfluidic technologies, thus offering attractive alternatives to current treatments and traditional constructs that have been used in tissue engineering applications. Herein, the current developments involving microfluidic hydrogel-based scaffolds, promising structures for cartilage regeneration, ranging from hydrogels with microfluidic channels to hydrogels prepared by the microfluidic devices, that enable therapeutic delivery of cells, drugs, and growth factors, as well as cartilage-related organ-on-chips are reviewed. Thereafter, cartilage anatomy and types of damage, and present treatment options are briefly overviewed. The various types of hydrogels are introduced, and the advantages of microfluidic hydrogel-based scaffolds and hydrogel-based microfluidic platforms over traditional hydrogels are thoroughly discussed. Furthermore, available technologies for fabricating microfluidic hydrogel-based scaffolds and microfluidic chips are presented. The preclinical and clinical applications of microfluidic hydrogel-based scaffolds in cartilage regeneration and the development of cartilage-

related microfluidic chips over time are further explained. The current developments, recent key challenges, and attractive prospects that should be considered so as to develop transitional microfluidic systems in cartilage repair are also highlighted and discussed in depth.

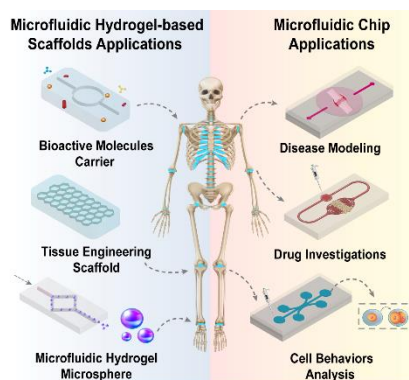


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1. Introduction

Articular cartilage (AC) structure consists of four distinct zones that can be damaged due to various joint pathologies: Trauma, osteochondritis dissecans, chondromalacia patellae, and childhood osteomyelitis.^[1,2] Cartilage injury is highly problematic because of an insufficient capacity for self-repair and dependency of the healing process on different factors, including depth, size, age, and location of the defect.^[3,4] Notably, cartilage defects can be observed in chondral and osteochondral (OC) layers depending upon the lesion's depth. Therefore, treating cartilage defects in the early stages is of great importance so as to prevent the appearing and progression of diseases such as osteoarthritis (OA) or rheumatoid arthritis (RA).

There are various treatment options for managing AC damage. Despite all the advantages of pharmacological treatment and laser emission for reducing pain and enhancing joint functions, the biomechanical properties of AC fail to meet the appropriate standard stage of regeneration.^[5,6]

Consequently, tissue engineering (TE) sheds light on restoring AC with better biomechanical function.^[7-9] The natural environment of chondrocyte cells requires specific mechanical strength (800-900 kPa) along with high water content (60-85 %).^[10] Accordingly, hydrogels, networks of cross-linked hydrophilic polymers possessing high water amounts, super absorption capacity, and extracellular matrix (ECM)-mimicking ability, are among ideal candidates for cartilage regeneration.^[11,12] Interestingly, a study revealed the superior capacity of mesenchymal stem cells (MSCs)-loaded hydrogels for chondrogenic differentiation, especially when dynamic compressive force was exerted on the hydrogel to supply mechanical requirements.^[13]

Although hydrogels offer multiple benefits in TE applications, some limitations have been reported, such as inefficient diffusion of solute^[14] and constraints upon sub-micron scale sized applications, causing difficulty in hydrogels' growth and development.^[15-20] Microfluidic systems replicate the critical perspective of the human body, which is essential for the better understanding of specific cell responses, fluidic and mechanical features of cell micro-environment, as well as tissue-hydrogel interactions. With advances in microfluidic hydrogel-based scaffolds, the mass transfer in these hydrogels has enhanced due to the incorporation of micro-channels, injection through the capillary needles has increased, the superior control over the distribution of chemical substances through micro-channels has become possible, and modeling of the native tissue as a three-dimensional (3D) structure to estimate a particular tissue's functional performance has been facilitated.^[21,22] In addition, the shear-thinning behavior of microfluidic hydrogel-based scaffolds enables injection for these hydrogels since solid-like performance is achieved after injection without the need to employ toxic chemical modifiers. Furthermore, as they possess a high surface-to-volume ratio, hydrogel-based scaffolds can have better cell interactions as compared to traditional ones.^[13] Among various technologies to fabricate microfluidic hydrogel-based scaffolds, a highly-organized construct can be obtained by means of utilizing a unique approach, the bioprinting. In this context, a bioink, a cells/growth factors (GFs)-laden hydrogel, precisely mimics the cartilage ECM and enables the fabrication of multi-layered and multi-material stratified structures with desirable chemical, mechanical, and biological gradients.^[18]

The investigation of the interactions of different stimuli with body tissues and the effects of a disease on the target tissue and other tissues of the host body involve a great deal of complexity. In order to study such grand challenges, the organ-on-chip (OoC) field has emerged.^[23,24] OoC devices, multi-cellular systems recapitulating physiological processes, integrate cellular micro-environment's key factors, including unique stimuli, biomolecules gradient, multiple types of cells, and barrier functions. Thus, OoC can either bridge the transitional gap between the patho/physiological processes in humans and *in vitro* platforms as well as animal models.^[25–27] Generally, this technology deepens our understanding of the biology itself, by means of allowing us to understand on how cells interact and regenerate during various injuries. The impacts of different therapeutic and environmental factors upon the behavior of cells in the regeneration process can also be identified. Furthermore, it has shown great promise for accelerating the evaluation procedures of drugs or under-developed products and bringing down their costs. With progress in this field and development of multi-OoC as well as body-on-a-chip, it will become possible to analyze the systemic effects of drugs on various tissues. Bearing these possibilities in mind, several OoCs have been developed throughout the years, and cartilage-related OoCs being one of them, have been investigated by scholars in different aspects, such as how to design an efficient platform, modeling diseases (*e.g.*, OA or RA), screening of various drugs, and applying diverse stimuli.^[28,29] While this field continues to grow, a timeline of cartilage-related OoCs depicting the most promising obtained results is specifically required. This is of critical importance for allowing scientists envisioning what novel experiments have been conducted in this scope so far, and how they can contribute to this fascinating branch of science, in the near future.

Despite the notable advances in cartilage defects' regeneration, literature focusing on preclinical and clinical achievements of microfluidic hydrogel-based scaffolds in the cartilage TE as well as cartilage-related OoCs is still scarce. This review represents a detailed overview of the AC structure and its damages, with a concentration on the treatment strategies. Interestingly, hydrogel-based engineered structures have been addressed as a promising approach to tackle the regeneration of damaged cartilage. While introducing various types of hydrogels employed in cartilage repair, the principal focus of the current review is on the current developments involving microfluidic hydrogel-

based scaffolds. The advantages of these hydrogel-based scaffolds over traditional ones are discussed in detail herein. The fundamental concept of microfluidic hydrogel-based scaffolds and their fabrication technologies, as well as microfluidic chips' manufacturing approaches, are also explained. Afterward, the preclinical and clinical achievements of microfluidic hydrogel-based scaffolds in cartilage regeneration as well as the development of cartilage-related OoCs from 2010 until 2022 are comprehensively reviewed. Ultimately, the state-of-the-art developments, current challenges and unmet needs, in addition to exciting prospects of microfluidic hydrogel-based scaffolds and OoCs for transitional cartilage research, are specifically mentioned.

2. Articular cartilage

Understanding the AC function and its structure is critical for perceiving cartilage diseases and damages as well as developing advanced treatment methods. AC is an alymphatic, aneural, and avascular form of the connective tissue with a thickness that can vary between 2 and 4 mm depending upon the location. It is made of hyaline cartilage comprising low-density (1-5 %) chondrocytes embedded within an ECM consisting of collagen, hyaluronic acid (HA), elastin, proteoglycans, and proteins.^[30–32] The AC is sorted into four main distinctive regions on the basis of diverse morphology, chemistry, and function, as well as the density of collagen (**Figure 1.A**).^[33]

Superficial zone: This region is the outermost and the thinnest layer of the AC, and directly contacts the articular joints' synovial fluid constituting 10 to 20 % of the entire thickness of the AC.^[34] This zone possesses the highest collagen and water as well as low proteoglycans contents, which leads to the integrity of this layer.^[35,36] The fibers of collagen within this region are effectively organized parallel with the joint's surface. These are firmly structured to withstand the shear forces that is caused by friction generated via the motion between AC surfaces.^[36,37]

Middle zone: The middle zone, located directly below the superficial region, constitutes 40 to 60 % of the AC volume. The middle layer is responsible for controlling compressive forces, being identified by the obliquely thicker collagen fibers, less cellular density, round-shaped chondrocytes, and increased proteoglycans content.^[30,35,36]

Deep zone: This zone forms around 30 % of the AC thickness, and has the highest glycoproteins content as well as the higher diameter of the collagen fibrils. Collagen fibers are arranged perpendicularly in the deep zone in order to furnish joints with the most outstanding resistance to applied compressive forces.^[31,38]

Calcified cartilage zone: This zone is a thin layer and is the nearest region to the subchondral bone.^[39] Collagen fibrils are incorporated into the calcified cartilage, contributing to a smooth transition between tissues possessing different mechanical properties and securing the cartilage to bone.^[31,40]

2.1. Articular cartilage damage

Cartilage lesions can result from unusual and repetitive loadings, a foreign body intrusion, and misalignment. It can also occur as a consequence of accidents, sports injuries, traumas, diseases, and congenital anomalies. Unfortunately, these injuries can cause severe long-term sequelae.^[41] Additionally, the AC structure presents many changes with aging, and a reduction in the chondrocytes' number can be observed in the superficial layer. In contrast, layers closer to the subchondral bone may indicate an increment in cell density. Chondrocytes can also undergo metabolic changes due to ageing, resulting in an imbalance between the synthesis of the matrix and its degradation. Consequently, ECM usually loses water and an intrinsic gain in stiffness can be observed, thus making the entire OC unit more prone to damage.^[39,42]

OA is a common heterogeneous degenerative joint disease and an irregular tissue remodeling driven by the inflammatory process that can arise from untreated cartilage injuries, accompanied by

pain, limitation of movement, and loss of joint function.^[43,44] In OA, the cartilage response varies because of the tissue's avascular nature, chondrocytes immobility, and the limited proliferation capability of mature chondrocytes.^[45,46]

The Cartilage Lesion Classification System of the International Cartilage Repair Society (ICRS) is a universal standard for chondral lesion severity classification, which focuses on the lesions' depth (**Figure 1.B**). Accordingly, grade 0 lesion is observable when the joint's cartilage surface is regular. Nonetheless, minor indentations and superficial fissures in the AC surface can lead to the development of grade 1 lesions (**Figure 1.B(I)**). In ICRS grade 2 lesions, irregular involvement of the middle zone and less than 50 % of the cartilage's depth are visible (**Figure 1.B(II)**). Grade 3 lesions spread in the cartilage's depth (> 50 %), involving the calcified zone (**Figure 1.B(III)**). Lesions affecting the subchondral bone are identified as grade 4 (**Figure 1.B(IV)**).^[47] Importantly, understanding the mechanism and grade of degeneration is beneficial for controlling and treating the injury.

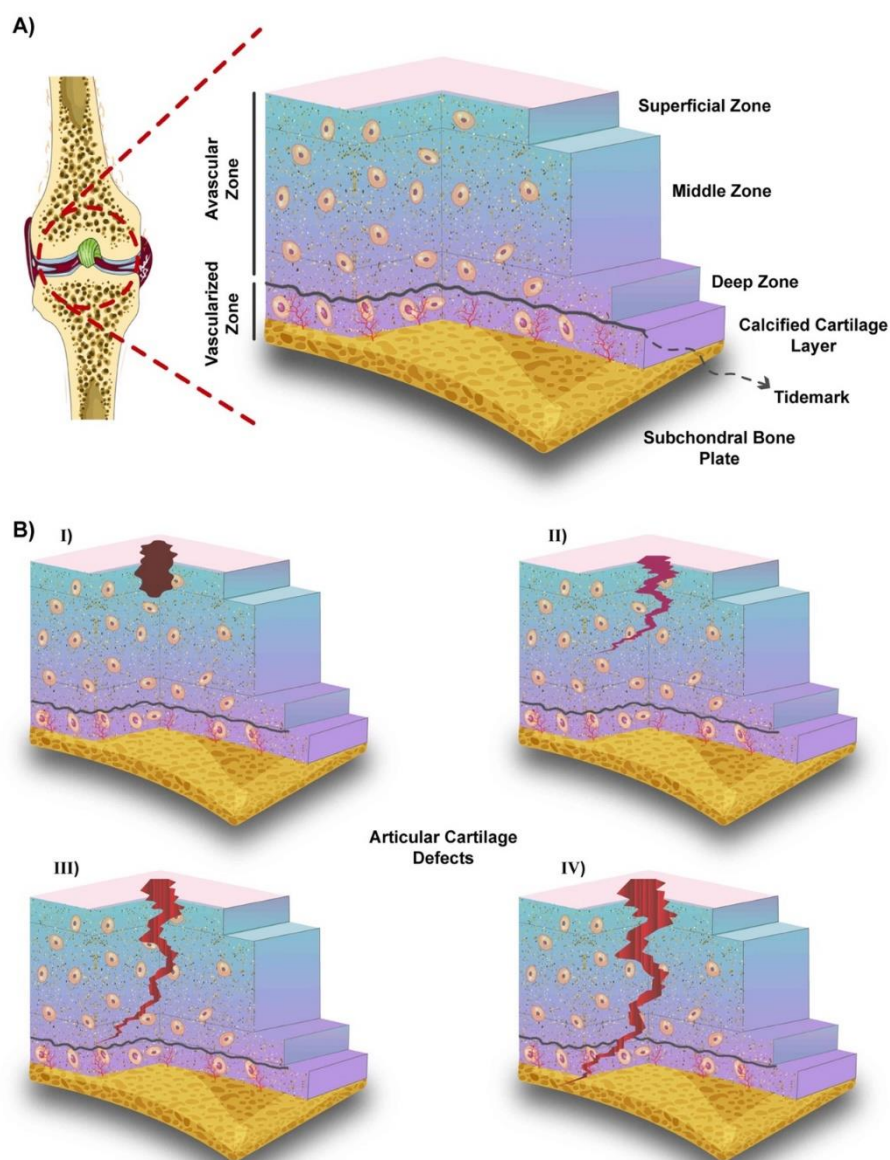


Figure 1. A) Schematic of the AC's macro- and micro-structure. B) Schematic of cartilage lesions based upon the ICRS classification system.

2.2. Strategies for the cartilage treatment

Surgical treatment methods have displayed size-dependent application (2-4 cm cartilage defect) to avoid further degeneration and progression towards OA.^[48,49]

Other approaches, including electrical stimulation, laser, pharmacological agents, tissue grafting, biomaterial replacement, and cell transplantation, have also been employed as replacement or treating methods for the injured AC.^[50]

Bone marrow stimulation methods used for treating minor defects have progressed from open debridement of the damaged cartilage and removal of subchondral bone to the microfracture technique in which the calcified cartilage is removed and the subchondral plate is perforated.^[51] In the microfracture approach, bone marrow is released into the defect and a blood clot is produced that may potentially contribute to the development of fibrocartilage, which is not the same as the native tissue in mechanical and biological characteristics. Thus, unfortunate results like loss of tissue's structure and function, increased pain level, and higher rates of failure as compared to the OC graft therapies are ultimately obtained. In order to circumvent the microfracture method's deficiencies, several approaches have been clinically established for the enhanced bone marrow stimulation, including concomitant molecules injection such as GFs, employment of acellular scaffolds such as collagen membranes or liquid hydrogels, and use of micronized acellular cartilage ECMs from allografts.^[52-58]

OC autograft delivers mature, live cartilage-subchondral bone units to the chondral defects, supporting the applied loads and permitting faster rehabilitation in the early post-operation phase as compared to the other cell-based repair strategies.^[59] Because of the limited autograft sources, it is only clinically viable to repair merely small-sized defects.^[60] By its turn, allografts are immune-privileged due to the cartilage's avascular nature,^[61] opening up the possible use of allogeneic approaches'. However, uncoordinated allografts and native cartilage architecture can generate unbalanced biomechanical loading, which can lead to degenerative joint changes.^[62,63] Cell-based cartilage repair techniques can grow and permit the implant to be shaped according to the defect site, making them desirable for treating larger chondral lesions (3-4 cm) of varying topography.^[64,65]

Despite the fact that the aforementioned strategies allow the management of pain and enhancement of joint functions, AC's native structure and biomechanical properties still cannot be fully restored. Consequently, researchers have concentrated on TE methods for restoring joint

function without eliciting immunological responses in OA and other joint diseases.^[66–69] In this context, biomaterials can act as supporting substrates for cells' attachment, differentiation, proliferation, and migration. It is highly desirable that after particular amounts of time, they are replaced by the ECM secreted from the cells^[70], and neo-tissue is eventually produced.^[71–73] In particular, hydrogels, cross-linked water-swollen networks of hydrophilic polymers^[74], have found growing interest as leading candidates for cartilage regeneration owing to their unique composition and structural similarity to the native ECM.^[75,76]

Currently, existing TE-based treatment methods and products available in the market or that are currently being tested in clinical trials are based on multiple approaches, such as “scaffold-free strategies”, “cell-free strategies”, and “cell-scaffold constructs”.^[77] Acquiring scaffold-free tissue-engineered AC is of particular interest since no ideal scaffolds have been presented yet; nonetheless, most investigations on scaffold-free approaches are still in the preclinical stages. This strategy prepares chondrocytes into spheroids that can surmount scaffold-related issues, reduce the loss of chondrocytes, maintain chondrocyte phenotypes, and provide a natural matrix component. However, this technique has associated technical challenges since it requires a prolonged cultivation time and possess an intricate culturing process, all of which push up the costs. At the moment, few scaffold-free systems are employed in the clinics, being Chondrosphere® (spherox) already licensed for repair of symptomatic AC defects. For developing Chondrosphere®, autologous chondrocytes are cultured *in vitro* for six to eight weeks to proliferate and aggregate into spheroids, followed by implanting them in the defects. Interestingly, the phase III of clinical trial concluded that using Chondrosphere® was at least as efficient as the microfracture technique in patients with small lesions. Conversely, in those individuals with big lesions, groups treated with Chondrosphere® displayed favorable results after follow-up for four years as compared to the baseline parameters.^[78–80]

Cell-free techniques are not completely “cell-free” and usually employ endogenous stem cells indirectly. Developing cell-free tissue-engineered AC based upon MSCs is an emerging idea. In this approach, MSCs or their derivatives are used for the cartilage regeneration without the direct transplantation of MSCs. The mentioned method circumvents several difficulties consist of iatrogenic

trauma happening as a result of harvesting autologous cells, prolonged cultivation time, and considerable costs of *in vitro* cell culture and expansion procedures. Nevertheless, few products, such as TruFit® scaffolds, are currently available on the market. The TruFit® scaffold comprises a poly(lactic-co-glycolic acid) (PLGA) co-polymer, 10 % calcium sulfate, polyglycolide fibers, and surfactant. Early clinical studies with small samples illustrated that employing the TruFit® scaffold could merely lead to the regeneration of fibrocartilage tissue. Furthermore, a recent clinical evaluation with two years of follow-up demonstrated that the treatment process of patients receiving the TruFit® scaffold with the aim of repairing cartilage defects was not fairly effective.^[81–85]

TE approaches on the basis of “cell-scaffold constructs” are the most generally used techniques among all others. In this regard, matrix-induced autologous chondrocyte implantation (MACI) is a regular treatment method based upon the “cell-scaffold constructs” approach. In the MACI, autologous chondrocytes are loaded in a scaffold, and the resultant chondrocyte-scaffold construct is then implanted into the cartilage defect. Similarly, “cell-scaffold constructs” based on MSCs, embryonic stem cells, and induced pluripotent stem cells (iPSCs) can also be fabricated. In recent years, numerous MACI-based commercial products have been developed, like BioSeed®-C and NOVOCART® 3D.^[77,79,81] BioSeed®-C makes use of fibrin glue as the cell carrier to grow cells on the polyglactin 910/poly-p-dioxanone fleece scaffold. This product has been employed in more than 3,000 patients. In this regard, a clinical study (n=79) revealed significant beneficial effects of the BioSeed®-C-based therapy. Within another investigation (n=50) and following four years of treatment, the ICRS scores of patients were noticeably improved. Moreover, the figures of magnetic resonance imaging (n=44) displayed a full defect filling of 72.7 %, a moderate defect filling of 25 %, and a less than 50 % defect filling of 2.2 % of the patients.^[86–88] NOVOCART® 3D is a biphasic scaffold consisting of collagen type I and type III. The evaluation conducted by Müller et al.^[89] showed that the visual analog scale scores at six, twelve, twenty-four, and thirty-six months post-implantation were significantly higher than those in the pre-operation condition. In addition, compared with the patients who were implanted with the scaffold after the failure of bone marrow stimulation method, lower failure rate of operation and more encouraging results were achieved in

those receiving NOVOCART® 3D as the first treatment. Niethammer and co-workers^[90] found that NOVOCART® 3D obviously enhanced post-operative visual analog scale scores in children and adolescents (< twenty years) with AC defects in comparison with adults having the same defects. Research outcomes indicated that utilizing NOVOCART® 3D is a useful technique with the goal of regenerating AC defects, mainly when used as the preferred treatment strategy for children and adolescents. Notably, phase III clinical trials of NOVOCART® 3D are currently underway.

3. Hydrogels in the cartilage repair

3.1. Traditional hydrogels

Hydrogel-based biomaterials have been recognized as promising materials to better support cell growth and promote tissue restoration by means of adequately transport and allow the diffusion essential nutrients, oxygen, and waste by-products. Indeed, these highly hydrated natural- or synthetic-based polymeric networks can provide an environment analogous to the native cartilage for the cells. The selection of the type of hydrogel for the cartilage repair should satisfy several criteria, including swelling ability, porosity-related parameters, permeability, biocompatibility, biodegradability, cell functionality, and required mechanical needs.^[91–95] Of note, the compressive strength of hydrogels is an essential characteristic for scaffolds employed in the cartilage TE to support external forces.^[96] Furthermore, it is widely acknowledged that the matrix stiffness has a significant impact on the differentiation of cells into chondrocytes. Nevertheless, if the hydrogel's mechanical properties are too high, the produced shearing stresses post-implantation will hinder the ingrowth of neo-tissue.^[97–99] Published data on compressive and tensile moduli of AC obtained from human femoral heads are in the range of 0.079-13 MPa and 0.76-54.6 MPa, respectively.^[100] Thus, mechanical characteristics should be firstly considered when developing hydrogel scaffolds for application in the cartilage bioengineering. The most interesting studies involving the use of traditional hydrogels as scaffolds for the cartilage repair are discussed.

Single-component non-hybrid hydrogels contain one kind of cross-linked polymer. By its turn, multi-component hydrogels are referred to hydrogel systems composed of various chemically or physically cross-linked polymers and biological molecules. Several drawbacks of multi-component hydrogels, such as the risk of viscous polymeric solutions' inhomogeneous mixing and the mutual interference among diverse functional groups, are effectively controlled in single-component ones.^[101] In this regard, Chen et al.^[102] fabricated injectable adhesive hydrogels consisting of modified HAs (methacrylated HA (HAMA) and HA modified by aldehyde and methacrylate groups (AHAMA)). Results exhibited high survival of bone marrow MSCs (BMSCs) and improved neo-cartilage/host integration. It was also observed an enhanced cartilage regeneration in a way that O'Driscoll histological scores were 18.3 ± 4.6 in treated rat models with OC defects and 11.5 ± 2.1 in untreated groups, twelve weeks after implantation. After four weeks of cartilage repair, nearly complete chondral defects' filling in the treated groups was observed. Furthermore, tissue indentation on the defect region was apparent, four weeks post-implantation. Twelve weeks later, the groups treated with AHAMA demonstrated a glossier morphology, with no clear boundaries. Several minor defects were noticed in the untreated and HAMA-treated groups. In HAMA-treated groups, several cartilage and fibrous-like tissues were observed with minimal disruption. The groups implanted with the AHAMA scaffold showed enhanced cartilage repair with a smooth surface. After twelve weeks, the formed boundary between the native cartilage and the neo-tissue was yet significant in the HAMA-treated and untreated groups, whereas the AHAMA-treated ones displayed a proper integration of tissue with the native cartilage, and the cells possessed more cartilage-like arrangements (**Figure 2.A**). In another study, collagen type II methacrylamide was prepared as a bioactive mono-component hydrogel. It was proved that this type of hydrogel lead to favorable growth and proliferation of BMSCs, promoted the expression of chondrogenic markers (aggrecan, collagen type II, and Sox9), and allowed differentiated chondrocytes' to maintain their phenotype. Moreover, generation of cartilaginous matrix and cartilage lacuna in the treated male athymic mice were observed. The main challenge in the construction of collagen-based biomaterials is to retain their intrinsic triple helical conformation, which is an essential biological property of collagen as it is functional for binding to the integrin, antibody, and collagenase. Interestingly, the results of this experiment revealed that functionalization

of collagen type II protected its native triple helical conformation, providing cells with a suitable chondro-inductive micro-environment for growth and differentiation.^[103] In another study, elastin-like recombinamers (ELRs) were proposed to be used as injectable hydrogel carriers for the human MSCs' (hMSCs) delivery *in vivo*. Briefly, the ELRs were genetically engineered to contain cell-adhesion sequences in order to provide a cell-friendly environment for hMSCs, followed by self-assembly of ELRs molecules into hydrogels via physical cross-linking at the physiological temperature and encapsulation of hMSCs. The generated OC lesions of male New Zealand white rabbits were filled with cell-encapsulated hydrogels, and the regenerated tissue with a bright and smooth appearance, which integrated well into the surrounding chondral tissue, was observed in the animal models. Moreover, microscopic evaluations showed that the OC defect regions were fully filled with hyaline cartilage with regular surface, having not a single sign of discontinuity, and ossification areas were seen beneath the regenerated AC. All in all, the novel cell-embedded hydrogels can be promising candidates for cartilage bioengineering applications.^[104]

Even though many promising experiments have been carried out on single-component hydrogels, it has been shown that multi-component hydrogels possess the desired mechanical and physicochemical properties as well as biocompatibility. Moreover, multi-component or hybrid hydrogels permit effective interaction with cells and efficient tissue regeneration due to their ECM-like mimetic structure.^[105] Polysaccharides are particularly attractive for cartilage regeneration due to the fact that they can be easily tuned for their hydrophilicity and functionality, and their similarity to the natural ECM. Nevertheless, they should be blended with other components to increase their mechanical strength.^[106,107] For instance, the poor mechanical stability of HA due to the fast degradation rate (4.5 and 8 hours when treated with hydrogen peroxide and hyaluronidase) results in weak cellular interactions which have limited its clinical applications and increased the need for combination with other polymers.^[108,109] In addition, HA-based hybrid hydrogels can enhance the lubrication within the synovial fluid since HA is capable of acting as a boundary lubricant in the cartilage. Indeed, the mechanically trapped HA-based complex slightly reduces the friction force (from 2.1 to 1.2 mN under 12 mN load), and eliminates the wear damage to the shearing/rubbing

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surfaces.^[110] Dialdehyde HA/chitosan hydrogels have also been fabricated and tested for the regeneration of cartilage tissue. The produced hydrogels were non-toxic, stable and biodegradable. Moreover, spherical rabbit chondrocytes' phenotype was preserved *in vitro* when these cells were encapsulated within the gels. Furthermore, collagen type II and aggrecan were expressed by the embedded cells. Following twelve weeks of cell-loaded gels' implantation in the New Zealand white rabbits with OC defects, the regenerated cartilage with smooth texture, that integrated well with the non-injured adjacent cartilage, was observed, and it included a mixture of hyaline and fibrous cartilage.^[111,112] In order to considerably augment the HA/chitosan hydrogels' mechanical properties, the silanized-hydroxypropyl methylcellulose (Si-HPMC) was introduced into these hydrogels. The human chondrocytes were observed in the pores of highly porous hydrogels, suggesting that there might be plenty of possibilities for ensuring cellular attachment and migration. Furthermore, the resultant cell-laden gels could support the cartilaginous tissue formation in Sprague-Dawley rats with cartilage defects. Interestingly, staining results revealed that the regeneration rate of defects in treated animal models reached about nearly 79.5 % at twenty-one days, demonstrating relatively good *in vivo* regeneration. More importantly, in comparison with HA/chitosan hydrogels, Si-HPMC-reinforced HA/chitosan/Si-HPMC hydrogels displayed noticeable enhancement in compressive strength and Young's modulus owing to the higher cross-linking density. In this regard, roughly 3.5-and 7-fold increase was observed for compressive strength and compressive elastic modulus, respectively, by means of introduction of Si-HPMC into the HA/chitosan gels. Moreover, modulation of the hydrogel matrix's stiffness via altering the Si-HPMC concentration in the hydrogel was possible (**Figure 2.B (I,II,III)**).^[113] It is also worthnotice that another ternary hydrogel containing gellan gum (GG), chondroitin sulfate (CS), and silk fibroin (SF) proved to be successful in cartilage TE. In fact, the hydrogels' physicochemical and mechanical features improved with their integration since these ternary matrices could make up for the shortcomings of each biomaterial. It was reported that the degree of swelling for the SF/CS hydrogel was 6 % and increased to 30 % for the GG/CS/SF hydrogel. Furthermore, the elastic modulus augmented from 30 to 630 kPa with the GG content increment. In addition, the 0.75 % (w v⁻¹) GG/3.25 % SF/CS and 1 % GG/3 % SF/CS hydrogels showed a considerably higher ultimate stress with the increase of strain and exhibited significantly

greater strength (**Figure 2.B (IV,V)**). Enhanced rabbit primary chondrocytes' adhesion due to the SF addition and facilitated cell migration owing to the CS integration were observed. Although declaring the suitable degradation rate of scaffolds for cartilage regeneration remains challenging, based upon the subsequent *in vitro* results in which hydrogels did not inhibit cell growth over time, it was believed that 20-30 % degradation of the hydrogels after four weeks may be suitable for application as biomaterials in the cartilage tissue repair.^[96] Collagen is regarded as the most broadly distributed subgroup of proteins within the body, offering substantial benefits in cartilage repair strategies.^[114] In spite of the excellent biological properties of the pure collagen, low water solubility, long gelation time (545 s at 37 °C), low compression modulus (1.9 kPa), and rapid degradation (89 % after incubation with collagenase for twenty-four hours) restrict its applications.^[98,115] Thus, blending collagen with other organic/inorganic biomaterials has been suggested to transcend these limitations. Lu and co-workers^[98] developed cross-linked collagen/genipin/carbon dot nanoparticles (NPs) hydrogels so as to enhance the features of the pure collagen. Cross-linking via genipin and the presence of carbon dot NPs provided a 21-fold increase for the compression modulus (42.8 kPa) as well as 39 % lower degradation rate after twenty-four hours in comparison with the pure collagen gels. Moreover, enhanced proliferation of rat BMSCs by 50.3 %, up-regulation of cartilage-specific genes' expressions in the Sprague-Dawley rats with cartilage-only defects, and improved secretion of glycosaminoglycans (GAGs) by 205.1 % on day twenty-one were observed. In another evaluation, Kontturi and a team of researchers^[116] engineered an *in situ*-forming hydrogel comprising transforming GF- β_1 (TGF- β_1)-loaded collagen type II-HA-polyethylene glycol (PEG) ether tetrasuccinimidyl glutarate for the bovine chondrocytes delivery. The results revealed the formation of a mechanically stable multi-component hydrogel that was capable of both support the viability of cells and release the TGF- β_1 in a sustainable manner.

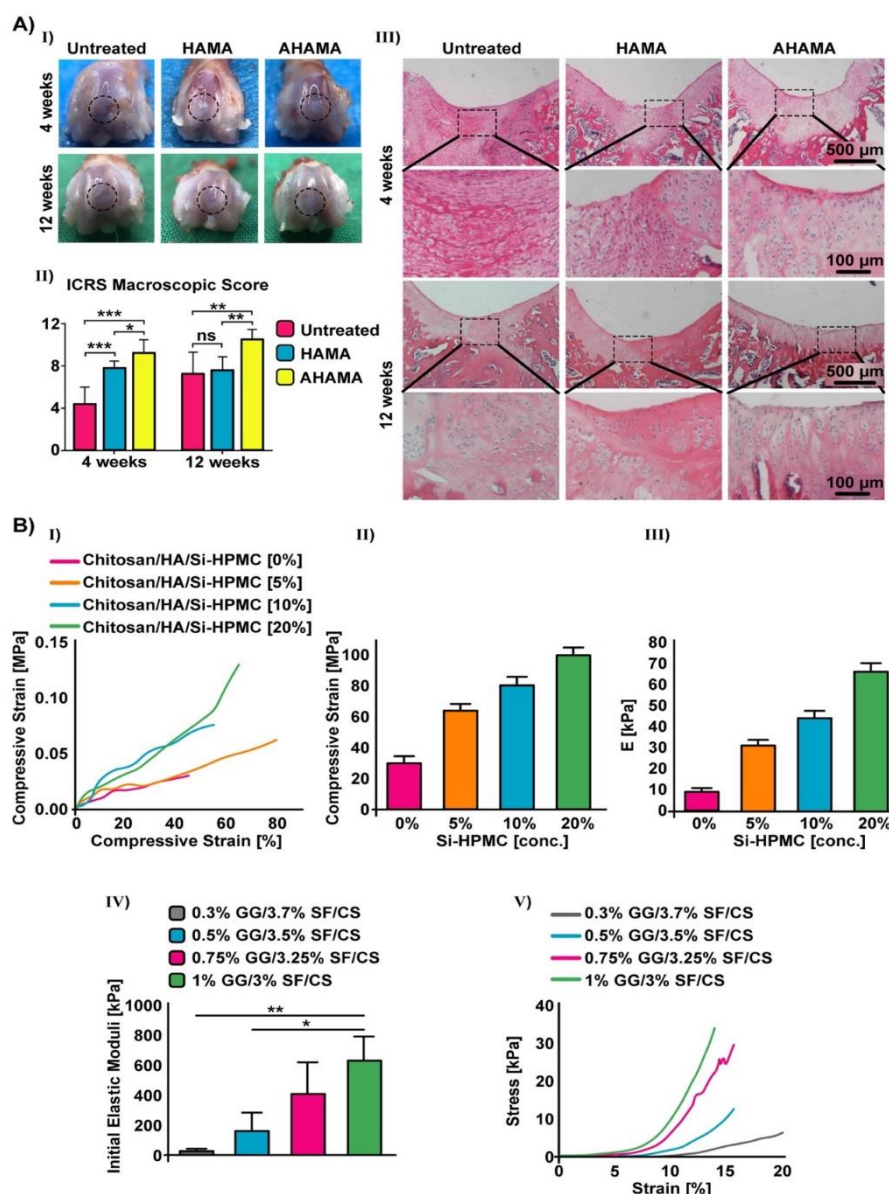


Figure 2. A) The capacity of the HAMA and AHAMA hydrogels in the cartilage regeneration used in rat models possessing OC defects. I) Cartilage defect's macroscopic illustration four and twelve weeks after the implantation. II) The ICRS scores of untreated, HAMA-and AHAMA-treated groups at four and twelve weeks post-implantation. III) Hematoxylin and eosin staining of the repaired cartilage after four and twelve weeks post-surgery. Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[102] Copyright 2020, Elsevier. B) The graphs depicting various cartilage-related hydrogels' mechanical properties: I) The different hydrogels' compressive stress-strain curves. II, III) The compressive strength and compressive elastic modulus of various hydrogels, respectively.

Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[113] Copyright 2021, Taylor & Francis. IV) The initial Young's modulus at 5-10 % strain. V)

The stress-strain curves of the hydrogels illustrating a dramatically greater ultimate stress with the strain enhancement. Reproduced under the terms of the Creative Commons Attribution 4.0

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Synthetic hydrogels have been widely used in repairing cartilage tissue due to their more controllable characteristics than natural polymers. In this regard, polyvinyl alcohol (PVA)-based hydrogels have been extensively investigated for cartilage regeneration owing to their biocompatibility, flexibility, low friction, and high lubricity.^[117] Favorably, the addition of polysaccharides or proteins to the PVA gels can enhance cellular properties.^[118,119] Xie et al.^[117] reported on the multi-component PVA/HA/human-like collagen (HLC) hydrogels presenting excellent rat chondrocytes' viability (> 100 %), great toughness, and high elasticity. The increment of compression modulus up to 5.56 MPa in the utmost strain of 90 % could be observed via blending HLC with PVA-HA possessing 3.34 MPa modulus in the 80 % strain. Furthermore, possessing high porosity and interconnected porous structures are critical parameters for cellular growth and the exchange of nutrients and wastes in an ideal hydrogel scaffold. Notably, a porous structure can facilitate the mechanical interlocking between the surrounding tissue and hydrogel scaffolds, the pores' network structure aids in promoting neo-tissue formation, and hydrogel scaffolds having high porosity enable the biofactors' efficacious release.^[120–123] A porosity of more than 90 % and pore sizes between 150-250 μm are being reported as optimal for the cartilage tissue repair.^[124,125] With an average pore size of 100-300 μm and a porosity of 94.52 %, the PVA/HA/HLC hydrogel was eminently desirable and closely resembled the ECM environment in which chondrocytes are able to grow. Due to the 3D porous structure and uniform cell distribution in the PVA/HA/HLC hydrogel, the cell fluorescence layering was enhanced, indicating that the cultivated cells grew not only on the gel surface but also in its deep layers. Besides, the effective repair of treated New Zealand white rabbits with the AC damage was observed. Within another innovative experiment, Peng and co-workers^[99]

investigated different ratios of PVA/chitosan hydrogels, and although there was a composition with the highest elastic modulus, they chose a hydrogel with 6:4 ratio displaying ≈ 0.06 MPa Young's modulus as the final candidate because they considered the optimality of all characteristics, including adequate swelling, great mechanical features, and accelerated proliferation of BMSCs. The dramatic increase related to the chondrogenesis of BMSCs seeded onto the hydrogel (cultivated with a chondro-induced medium) suggest the BMSCs' strong potential for differentiating into the chondrocytes once mixed with the PVA/chitosan hydrogel. Finally, the selected hydrogel positively influenced the *in vivo* treatment of OC defects in male mature New Zealand rabbits (**Figure 3.A, B**).

Stimuli-responsive hydrogels can exhibit distinctive alterations in their properties, such as swelling, degradation, rheological features, release behaviors, and mechanical characteristics^[126–128] when exposed to external and internal stimuli such as the metabolite concentration, pH, light/ultraviolet (UV) source, enzymes, osmotic pressure, magnetic/electrical fields, temperature, redox, and ultrasound irradiation.^[129–132] The functional groups present within the smart hydrogel and the cross-linking agent undergo phase transition after being exposed to the triggers, resulting in the alteration of cross-linking density and hydrogel's properties. By realizing these responses and controlling them, scholars are able to fabricate stimuli-responsive hydrogels with tunable features.^[133] In this regard, Saghebasl et al.^[134] fabricated thermo-sensitive gelatin/(poly(*N*-isopropyl acrylamide) (PNIPAAm)-polycaprolactone (PCL)-PEG-PCL-PNIPAAm) hydrogels. The study revealed that hydrogels with a compression strength of 0.25 MPa displayed a pore size of 129 ± 47 μm , showed a porosity of 85 %, and exhibited a phase transition conversion near 34 °C that was in the suitable range for cell culture and cartilage TE applications. Additionally, cultured human chondrocytes had higher survival rate, enhanced cellular adhesion, and increased expression of cartilage-related genes in the fabricated hydrogels compared to the control groups. In another study, Giammanco and co-workers^[135] synthesized photo-responsive acrylamide-alginate gels encapsulating ferric ions, and the hydrogels' characteristics, such as mechanical features, chemical composition, and micro-structure were tuned via visible light irradiation. Cultured ATDC5 cells, a chondrogenic cell line derived from mouse teratocarcinoma cells, had favorable viability (80-85 %) after one day of cultivation,

illustrating the hydrogels' cytocompatibility. Moreover, cells displayed better adhesion interaction with the irradiated gels' surface compared with the non-irradiated hydrogels (2.95×10^4 versus 1.53×10^4 cells cm^{-2}). Finally, significantly higher production of sulfated GAGs, a hallmark of chondrogenesis, in cells cultivated on irradiated gels than non-irradiated ones was observed. Bahney and a group of scholars ^[136] developed enzyme-responsive hydrogels sensitive to the matrix metalloproteinase-7 (MMP-7) for the cartilage TE. Briefly, MMP-7 substrates (PLE, VPLS, and a scrambled control (sc) of VPLS) were incorporated into the backbone of PEG diacrylate (PEGDA), a minimally degradable biomaterial, to obtain bioresponsive hydrogels named PLE-PEGDA, VPLS-PEGDA, and sc-PEGDA. The PEGDA-based semi-interpenetrating network (sIPN) hydrogel was used as the control group. The authors concluded that PLE-PEGDA and VPLS-PEGDA hydrogels displayed a dose-dependent response to MMP-7, with PLE-PEGDA having a more rapid degradation. However, the sc-PEGDA hydrogel was non-degradable owing to showing no degradation upon exposure to MMP-7. Following photo-encapsulating hMSCs into hydrogels and *after in vitro* culture for six and twelve weeks, collagen type II deposition was found to be limited to the pericellular domain in all of the non-degradable hydrogels. Conversely, interterritorial collagen deposition in both PLE-PEGDA and VPLSL-PEGDA hydrogels was observed, with the PLE-PEGDA hydrogel exhibiting greater and earlier deposition of collagen type II as compared to the VPLSL-PEGDA hydrogel (**Figure 3.C**). Interestingly, the PLE-PEGDA scaffold's degradation led to an enhanced total accumulation of collagen in comparison with that of the sc-PEGDA hydrogel (**Figure 3.D**). Together with the alteration in the distribution of collagen, this resulted into a dramatically augmented dynamic compressive modulus after twelve weeks of cultivation (**Figure 3.E**).

In **Table 1**, experiments investigating various traditional hydrogels for application in cartilage TE are summarized.

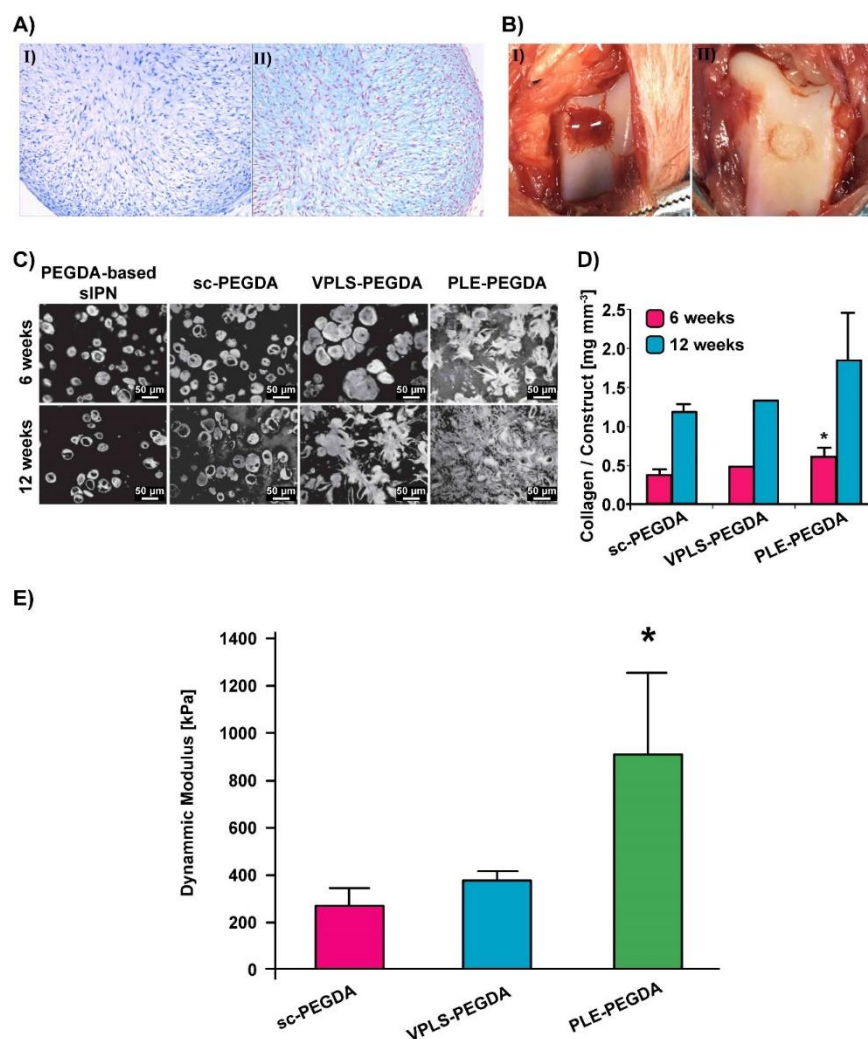


Figure 3. A) The morphology of BMSCs that were seeded on PVA/chitosan hydrogel in: I) the typical cell culture, and II) chondro-induced medium. B) The OC defect: I) prior to; and II) after treatment with the BMSCs-laden PVA-chitosan hydrogel. Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[99] Copyright 2019, Springer Nature. C) Immunohistochemistry of collagen type II following six weeks and twelve weeks of in vitro culture. D) Total content of collagen in hydrogels at six weeks (gray bars) and twelve weeks (black bars). E) Compressive elastic modulus of hydrogels after twelve weeks of culture (*p < 0.05). Reproduced under the terms of the FASEB license.^[136] Copyright 2011, Wiley.

Table 1. Summary of various traditional hydrogels' investigations in the cartilage repair.

Hydrogel composition	Cell source/Animal model	Outcomes	Ref.
HAMA and AHAMA	BMSCs/Rat models with OC defects	<p>-Exhibited high cell survival</p> <p>-<i>In vivo</i> improvement in the integration between neo-cartilage and host tissues</p> <p>-Significantly enhanced cartilage regeneration (O'Driscoll histological scores: 18.3 ± 4.6 in treated groups and 11.5 ± 2.1 in untreated ones twelve weeks after implantation)</p> <p>-Observed nearly complete chondral defects' filling in the treated groups in comparison with the untreated ones, and apparent tissue indentation on the defect region four weeks post-implantation</p> <p>-Formation of a glossier morphology, with no clear boundaries, in the groups treated with AHAMA, and observed several minor defects in the untreated and HAMA-treated groups after twelve weeks</p> <p>-Observed several cartilage and fibrous-like tissues with minimal disruption in HAMA-treated groups, and enhanced cartilage repair with a smooth surface in groups implanted with the AHAMA scaffold</p> <p>-Significant formed boundary between the native</p>	[102]

		cartilage and the neo-tissue in the HAMA-treated and untreated groups after twelve weeks, and fabulous integration of tissue with the native cartilage with cells possessing more cartilage-like arrangements in the AHAMA-treated ones	
Collagen type II methacrylamide	BMSCs/Male athymic mice	<p>-Favorable growth and proliferation of BMSCs</p> <p>-Promoted expressions of chondrogenic markers (aggrecan, collagen type II, and Sox9)</p> <p>-Retention of the differentiated chondrocytes' phenotype</p> <p>-<i>In vivo</i> generation of cartilaginous matrix and cartilage lacuna</p> <p>-Protected collagen type II's native triple helical conformation after employing the method of functionalized modification, providing cells with a suitable micro-environment for growth and the key chondro-inductive matrix for their differentiation</p>	[103]
ELRs	hMSCs/Male New Zealand white rabbits with OC lesions	<p>-Provided hMSCs' delivery <i>in vivo</i></p> <p>-Observed regenerated tissue with a bright and smooth appearance, which integrated well into the surrounding chondral tissue, in the animal models</p> <p>-Fully filled OC defect regions with hyaline cartilage with regular surface, having not a single sign of discontinuity</p>	[104]

		-Seen ossification areas beneath the regenerated AC	
Dialdehyde HA/Chitosan	Rabbit chondrocytes/ New Zealand white rabbits with OC defects	<p>-Fabrication of non-toxic, stable, and biodegradable hydrogels</p> <p>-In vitro preservation of embedded spherical cells' phenotype</p> <p>-Expression of collagen type II and aggrecan by the encapsulated cells</p> <p>-Observed regenerated cartilage with smooth texture, that integrated well with the non-injured adjacent cartilage, including a mixture of hyaline and fibrous cartilage following twelve weeks of cell-loaded gels' implantation in animal models</p>	[111,112]
HA/Chitosan/Si-HPMC	Human chondrocytes/Sprague- Dawley rats with cartilage defects	<p>-Presence of cells in the pores of highly porous hydrogels, suggesting the existence of plenty of possibilities for ensuring cellular attachment and migration</p> <p>-Supported cartilaginous tissue formation in the animal models</p> <p>-Nearly 79.5 % regeneration rate of defects in treated animal models at twenty-one days, demonstrating relatively good <i>in vivo</i> regeneration</p> <p>-Roughly 3.5-and 7-fold increase for compressive strength and compressive elastic</p>	[113]

		<p>modulus, respectively, by the introduction of Si-HPMC to the HA/chitosan gels</p> <p>-Possible modulation of the hydrogel matrix's stiffness via altering the Si-HPMC concentration in the hydrogel</p>	
GG/CS/SF	Rabbit primary chondrocytes/-	<p>-Improved physicochemical and mechanical features of hydrogels with their integration</p> <p>-Degree of swelling: 6 % and 30 % for SF/CS and GG/CS/SF hydrogels, respectively</p> <p>-Augmented elastic modulus from 30 to 630 kPa with the GG content increment</p> <p>-A considerably higher ultimate stress with the increase of strain and significantly greater strength in 0.75 % (w v⁻¹) GG/3.25 % SF/CS and 1 % GG/3 % SF/CS</p> <p>-Enhanced cellular adhesion due to the SF addition and facilitated cell migration owing to the CS integration</p> <p>-Observed 20-30 % degradation of the hydrogels after four weeks, that may be suitable for application as biomaterials in the cartilage tissue repair</p>	[96]
Collagen/Genipin/Carbon dot NPs	Rat BMSCs/Sprague-Dawley rats with cartilage-only defects	<p>-Observed 21-fold increase for the compression modulus (42.8 kPa) as well as 39 % lower degradation rate provided by cross-linking via genipin and the presence of carbon dot NPs in</p>	[98]

		<p>the multi-component hydrogels after twenty-four hours in comparison with the pure collagen gels</p> <p>-Enhanced proliferation of cells by 50.3 %, up-regulation of cartilage-specific genes' expressions in the animal models, and improved secretion of GAGs by 205.1 % on day twenty-one</p>	
<p>Collagen type II/HA/PEG ether tetrasuccinimidyl glutarate loaded with TGF-β_1</p>	Bovine chondrocytes/-	<p>-Formation of a mechanically stable multi-component hydrogel</p> <p>-Supported viability of cells</p> <p>-Sustained release of TGF-β_1</p>	[116]
PVA/HA/HLC	Rat chondrocytes/New Zealand white rabbits with the AC damage	<p>-Excellent cell viability (> 100 %), great toughness, and high elasticity of the multi-component hydrogels</p> <p>-Observed increment of compression modulus up to 5.56 MPa in the utmost strain of 90 % via blending HLC with PVA-HA possessing 3.34 MPa modulus in the 80 % strain</p> <p>-Displayed an average pore size of 100-300 μm and a porosity of 94.52 %, closely resembling the ECM environment in which chondrocytes grow</p> <p>-Enhanced cell fluorescence layering due to the 3D porous structure and uniform cell distribution in the hydrogel, indicating growing of the cultivated cells not only on the gel</p>	[117]

		<p>surface but also in its deep layers</p> <p>-Effective repair of treated animal models' AC damage</p>	
PVA/Chitosan	<p>BMSCs/Male mature New Zealand rabbits with OC defects</p>	<p>-Selected hydrogel (PVA/chitosan = 6:4 ratio) displaying ≈ 0.06 MPa Young's modulus with superior characteristics, including good swelling, great mechanical features, and accelerated proliferation of cells, compared to others</p> <p>-A dramatic increase related to the chondrogenesis of BMSCs seeded onto the hydrogel (cultivated with a chondro-induced medium), illustrating the BMSCs' strong potential for differentiating into the chondrocytes once mixed with the PVA/chitosan hydrogel</p> <p>-Positive influence of the chosen hydrogel on the treatment of OC defects in animal models</p>	[99]
<p>Gelatin/(PNIPAAm-PCL-PEG-PCL-PNIPAAm)</p>	Human chondrocytes/-	<p>-Observed compression strength of 0.25 MPa, a pore size of 129 ± 47 μm, a porosity of 85 %, and a phase transition conversion near 34 °C for produced hydrogels that was in the suitable range for cell culture and cartilage TE applications</p> <p>-Higher cell survival rate, enhanced cellular adhesion, and increased expression of cartilage-related genes in the fabricated hydrogels compared to the control groups</p>	[134]

Acrylamide/Alginate encapsulated with ferric ions	ATDC5 cells/-	<p>-Tuned hydrogels' characteristics, such as mechanical features, chemical composition, and micro-structure via visible light irradiation</p> <p>-Favorable cell viability (80-85 %) after one day of cultivation, illustrating the hydrogels' biocompatibility</p> <p>-Better adhesion interaction of cells with the irradiated gels' surface compared with the non-irradiated hydrogels (2.95×10^4 versus 1.53×10^4 cells cm^{-2})</p> <p>-A significantly higher production of sulfated GAGs in cells cultivated on irradiated gels as compared to non-irradiated ones</p>	[135]
PEGDA-based sIPN, PLE-PEGDA, VPLSL-PEGDA, and sc-PEGDA	hMSCs/-	<p>-A dose-dependent response of PLE-PEGDA and VPLSL-PEGDA hydrogels to the MMP-7, with PLE-PEGDA having a more rapid degradation</p> <p>-Nondegradability of the sc-PEGDA hydrogel owing to showing no degradation upon exposure to MMP-7</p> <p>-Observed collagen type II deposition limited to the pericellular domain in all of the nondegradable hydrogels following photo-encapsulating cells into hydrogels and after <i>in vitro</i> culture for six and twelve weeks</p> <p>-Illustrated interterritorial collagen deposition in both PLE-PEGDA and VPLSL-PEGDA</p>	[136]

		<p>hydrogels, with the PLE-PEGDA hydrogel exhibiting superior and earlier deposition of collagen type II as compared to the VPLSL-PEGDA hydrogel</p> <p>-Enhanced total accumulation of collagen due to PLE-PEGDA scaffold's degradation as compared to that observed for the sc-PEGDA hydrogel</p> <p>-A dramatically augmented dynamic compressive modulus of the PLE-PEGDA hydrogel after twelve weeks of cultivation</p>	
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3.2. Microfluidic hydrogel-based systems

Despite several advances, the fabrication of complex functional tissues and capillary-sized structures' manufacture employing traditional hydrogels that comprise single-component, multi-component, and stimuli-responsive hydrogels (**Figure 4.A**) are still challenging. Therefore, the convergence of the microfluidic technology and TE can sort out the major problem of mass transfer and diffusion in the hydrogels while allowing mimicking the native ECM's performance. Hydrogel-based microfluidic platforms incorporating hydrogel systems (**Figure 4.B**), and microfluidic hydrogel-based scaffolds comprising hydrogels prepared by microfluidic devices as well as hydrogels with microfluidic channels (**Figure 4.C**), are considered promising alternatives for traditional hydrogels employed in the cartilage TE.

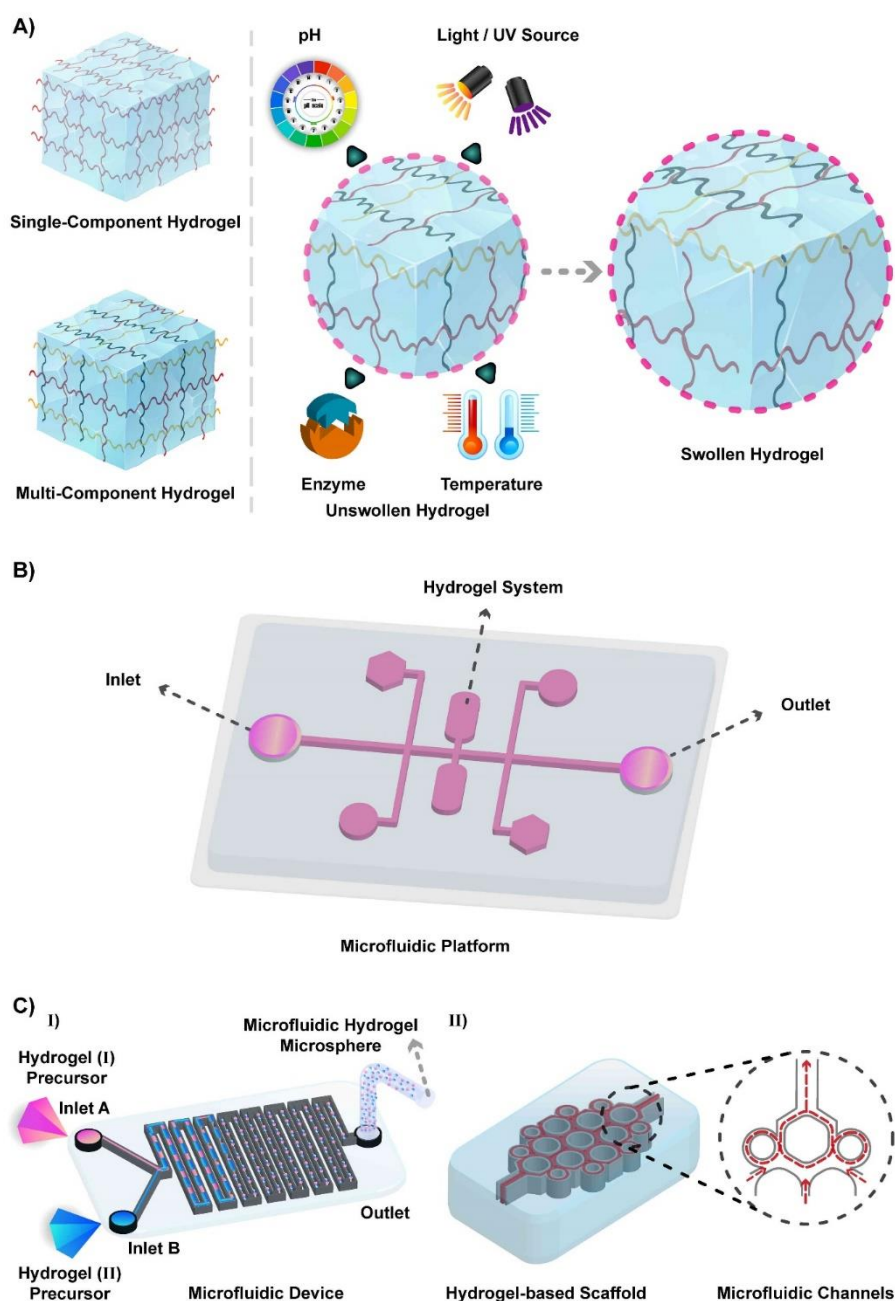


Figure 4. A) Schematic representation of traditional hydrogels comprising single-component, multi-component, and stimuli-responsive hydrogels along with several stimuli. B) Image displaying a hydrogel-based microfluidic platform incorporating a hydrogel system. C) Scheme showing microfluidic hydrogel-based scaffolds, including: I) an example of hydrogels prepared by microfluidic devices and II) an instance of hydrogels with microfluidic channels.

The precise control and utilization of tiny amounts of fluids, commonly nano-or micro-liters, based upon fluid flow regulation within artificial micro-structures fall into the realm of microfluidic technology.^[137–139] Microfluidic systems have exhibited satisfactory portability, low consumption of reagents, inexpensiveness, high resolution, rapid response, fast mixing, and great sensitivity in screening drugs due to their small scale. Given the microfluidics' versatility and the hydrogels' superior performances, when microfabrication techniques are utilized to manufacture hydrogel materials, endless possibilities can be opened up, resulting in a synergic mutual-promoting effect. In essence, the microfluidic/hydrogel combination has several unique advantages over traditional hydrogels used for cartilage repair.^[140–142] Firstly, since microfluidic hydrogel-based scaffolds are inherently capable of maintaining fluid flow, they possess instant perfusion, facilitating nutrients delivery and metabolites elimination when cells are embedded in these structures. Secondly, adjusting cells' and substances' transport to and from the hydrogel became possible by means of changing the geometry of the microfluidic network. Third, the microfluidic geometry within the hydrogel-based scaffold is reproducible and is well-controlled because micro-channels' locations and sizes can be selected via design, thereby making flow distribution foreseeable and enabling computational modeling for the solute transport. Another significant advantage is that the microfluidic hydrogel-based structures can precisely control injectability, one of the critical requirements that needs to be addressed for the successful use of hydrogels in cartilage TE. Furthermore, as illustrated in various studies, microfluidic technology provides a golden opportunity to fabricate various shapes of hydrogel-based scaffolds, such as microgels, cylindrical and hollow gel fibers which are not producible in other conventional approaches.^[143–146]

In the context of hydrogel-based microfluidic cell culture platforms^[147], multiple benefits may be achieved, such as decreased contamination risk, reduced reagents consumption, high-quality experimentation, and recapitulating specific tissues functions. It is also more flexible than typical tissue culture well plates due to the possibility of carrying out studies that involve single cell up to millions of cells. Within the channels incorporated in these microfluidic systems, interactions between cells can be controlled by regulating various factors, such as shear strain and stress. Indeed, spatial

control over cell behavior can be provided via designing structural features in hydrogel-based microfluidic platforms. Hydrogels possessing controlled signaling molecules' gradients can be constructed in the microfluidic device to generate concentration gradients of two or even more inlets. Besides, these cell culture systems can be employed for drug discovery and toxicity investigations. In these advanced devices, drugs can be evaluated more quickly and at a more extensive range of conditions. Other key advantages can be gained utilizing those systems, including diminished dependence on the animal models, reduced final cost, eliminated species variation, as well as enhanced assay throughput and overall testing efficiency. Interestingly, the assessment of stem cell expansion and differentiation has also been investigated using such systems. Indeed, high-fidelity screening of stem cell culture is possible because of these systems' advantageous capabilities comprising continuous fluid flow and high parallelization.^[148–152] **Figure 5** illustrates the microfluidic hydrogel-based systems' major excellence, including adequate mass exchange, adjustable interactions of cell populations, controllable injectability, gradient profile generation, perfusion capability, production of hydrogel-based scaffolds with various shapes, enhanced drug discovery/toxicity investigations, and diminished reagents consumption. The pros and cons of traditional hydrogels and microfluidic hydrogel-based scaffolds are summarized in **Table 2**.

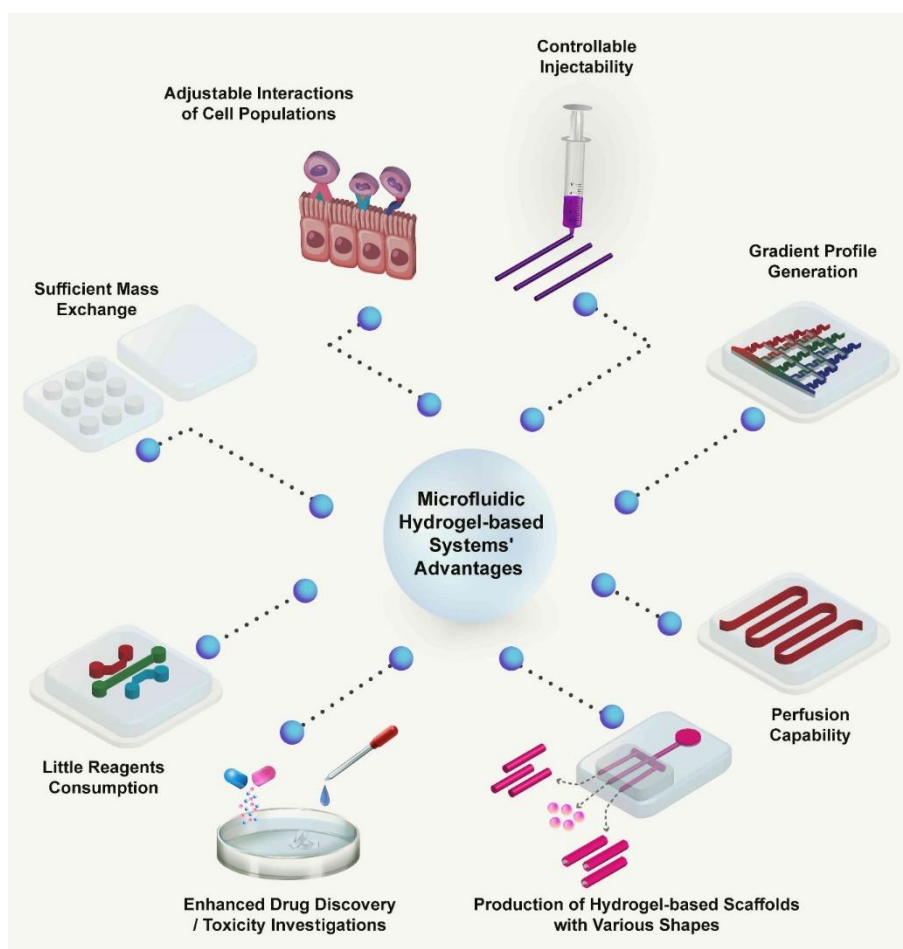


Figure 5. Microfluidic hydrogel-based systems' unique benefits.

Table 2. Summary of advantages and disadvantages of different hydrogels employed in the cartilage TE.

Type of hydrogel	Advantages	Disadvantages	Ref.
Single-component and multi-component hydrogels	<ul style="list-style-type: none"> -Production by many facile and cost-effective methods -Displaying versatility in various fields -Possessing properties analogous to the characteristics of the native ECM 	<ul style="list-style-type: none"> -Require a surgery to be placed in the desired region -Require mixing with other components for better performance of single-component hydrogels 	[101,107]

	<ul style="list-style-type: none"> -Having high water content, softness, flexibility, biocompatibility, permeability to oxygen and nutrients, and porous structure 	<ul style="list-style-type: none"> -Being usually uncontrollable in the aperture size, mechanical strength, swelling capacity, and sensitivity to the external/internal stimulations, a problem associated with single-component hydrogels -Structural inhomogeneity of multi-component hydrogels -Challenge of mutual interference among diverse functional groups in multi-component hydrogels 	
Stimuli-responsive hydrogels	<ul style="list-style-type: none"> -Forming in situ through stimuli-responsive effects -Having injectability with minimal invasiveness -Usability for irregularly shaped sites -Better bio-mimicking of the ECM structure compared with the single-component and multi-component hydrogels -Altering their features with employing external and internal stimuli 	<ul style="list-style-type: none"> -Problem of limited employment, restricted to the aqueous solutions -Require external/internal sources that can cause cytotoxicity (<i>e.g.</i>, light) -Dependence of the response time on the sample size -Exhibit high possibility of deformation 	[107,153]
Microfluidic hydrogel-based scaffolds	<ul style="list-style-type: none"> -Possessing instant perfusion -Facilitating nutrients delivery and metabolites elimination when cells are embedded in these structures 	<ul style="list-style-type: none"> -Bubble formation in the channels -Possible clogging of channels by cells -Complex and high-cost manufacturing methods 	[142–146,154,155]

	<ul style="list-style-type: none"> -Enabling adjustment of cells' and substances' transport to and from the hydrogel via changing the geometry of the microfluidic network -Achieving reproducible and well-controlled microfluidic geometry within the hydrogel-based scaffold owing to the selection of micro-channels' locations and sizes via design -Making flow distribution foreseeable and enabling computational modeling for the solute transport -Controlling injectability -Production of various shapes of hydrogel-based scaffolds, such as microgels, cylindrical and hollow gel fibers -Modular design and adjustable porosity 	<ul style="list-style-type: none"> -Inability to generate large-scale tissue constructs comprising micro-vascularized network channels -Lack of controlling over long-term cellular function and survival 	
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4. Microfluidics fabrication methods

4.1. Microfluidic hydrogel-based scaffolds' manufacturing approaches

Over the past few years, different approaches have been devised in order to produce microfluidic hydrogel-based scaffolds. Herein, spatial resolution time to manufacture ratio, processability, cost-

effectiveness, time consumption, scalability, and reproducibility are effective parameters for selecting a proper microfluidic hydrogel-based scaffolds' fabrication technique.^[156]

A practical methodology to achieve the most desirable operating parameters related to the manufacture of microfluidic hydrogel-based scaffolds, especially polymeric ones, is computational fluid dynamics employment. The method utilizes data structure and numerical analysis to investigate fluid flows and model flow behavior within micro-channels. In this regard, Mahmoudi et al.^[157] engineered alginate nanogels encapsulating transforming GF- β_3 (TGF- β_3) for promoting the chondrogenesis of hMSCs. In that study, the effect of flow rate ratio on nanogel sizes and simulated fluid flow using the finite element method, a discretization technique in computational fluid dynamics, was evaluated. It was reported that for the flow ratios of 0.01, 0.05, 0.1, 0.15, and 0.2, the nanogel diameters were 43 ± 4 , 58 ± 5 , 76 ± 5 , 94 ± 7 , and 125 ± 7 nm, respectively. Additionally, the nanogels' average diameter for the bulk approach was 137 ± 22 nm. Hence, this microfluidic technique allowed generating nanogels with smaller diameters compared to the bulk procedure. The simulation by finite element method followed the same pattern, i.e. the higher the flow rate ratios, the lower the sheath flow rate; thus, the larger the diameter of the nanogel. Furthermore, the nanogels had high encapsulation efficiency, supporting better differentiation of hMSCs. Interestingly, larger nanogels allowed a faster release of the GF, and presented a lower cell viability. Smaller-sized microfluidic nanogels possess a superior performance in respect to the chondrogenic differentiation. Consequently, the computational fluid dynamics technique can aid in designing microfluidic hydrogel-based scaffolds with superior properties in cartilage TE applications.

In recent years, the microfluidic hydrogel-based scaffolds' fabrication methods have greatly evolved and are briefly overviewed.

Molding: Molding is a relatively simple and traditional manufacturing process that involves shaping pre-gel solutions via employing a fixed frame, known as a mold. Herein, various templates like microneedles, degradable fibers, and patterned polymers (mostly polydimethylsiloxane (PDMS)) fabricated by lithographic approaches can be utilized to form microfluidic hydrogel-based

scaffolds.^[158] Concerning this subject, Goldman and co-workers^[15] designed a microfluidic hydrogel-based scaffold via molding to obtain full-thickness and strong cartilage tissues. The cell-agarose solution was injected between an acrylic casing and a PDMS mold to form a microfluidic cell-laden gel. Each target region could be independently cast in this method, and independent networks were formed between molded parts in the acrylic casing (**Figure 6.A(I)**).^[159] Therefore, such type of technique is of great value in OC tissue regeneration since the MSCs-laden gel could be split into two sections that were perfused by chondrogenic and osteogenic media, leading to the OC construct's formation. That type of structure also made it possible to study the impact of culture conditions and different signals such as chemical and mechanical cues on cell functions. As shown in **Figure 6.A(II)**, the effect of cytokine supplementation on deoxyribonucleic acid (DNA) content using this structure is evidenced.^[159]

A facile molding technique to fabricate microfluidic hydrogel-based scaffolds is casting hydrogel precursors around microneedles or elastomeric rods (**Figure 6.B**). With respect to this matter, a group of researchers^[158] cast the hepatic cell-laden hydrogels around the micro-channels via flowing sucrose crystals and a cell-contained agarose solution through a cylindrical PDMS-based mold with an implanted microneedle. Nonetheless, manual removal of the needles after the molding process posed several obstacles. To address this issue, Jiménez-Torres et al.^[160] used an iron-capped mold that could be removed magnetically (**Figure 6.C**). In another strategy, glass and polymer fibers were introduced as alternatives for needles. In that study, fibers could be eliminated by means of self-degradation or dissolving within a proper solvent in the absence of manual handling (**Figure 6.D**). Nevertheless, the solvents' toxicity could negatively influence cell viability. In that regard, Nazhat and co-workers^[161] presented soluble phosphate glass fibers in a compressed collagen gel. That advantageous approach provided micro-channels of variable diameters due to the phosphate glass fibers degradation.

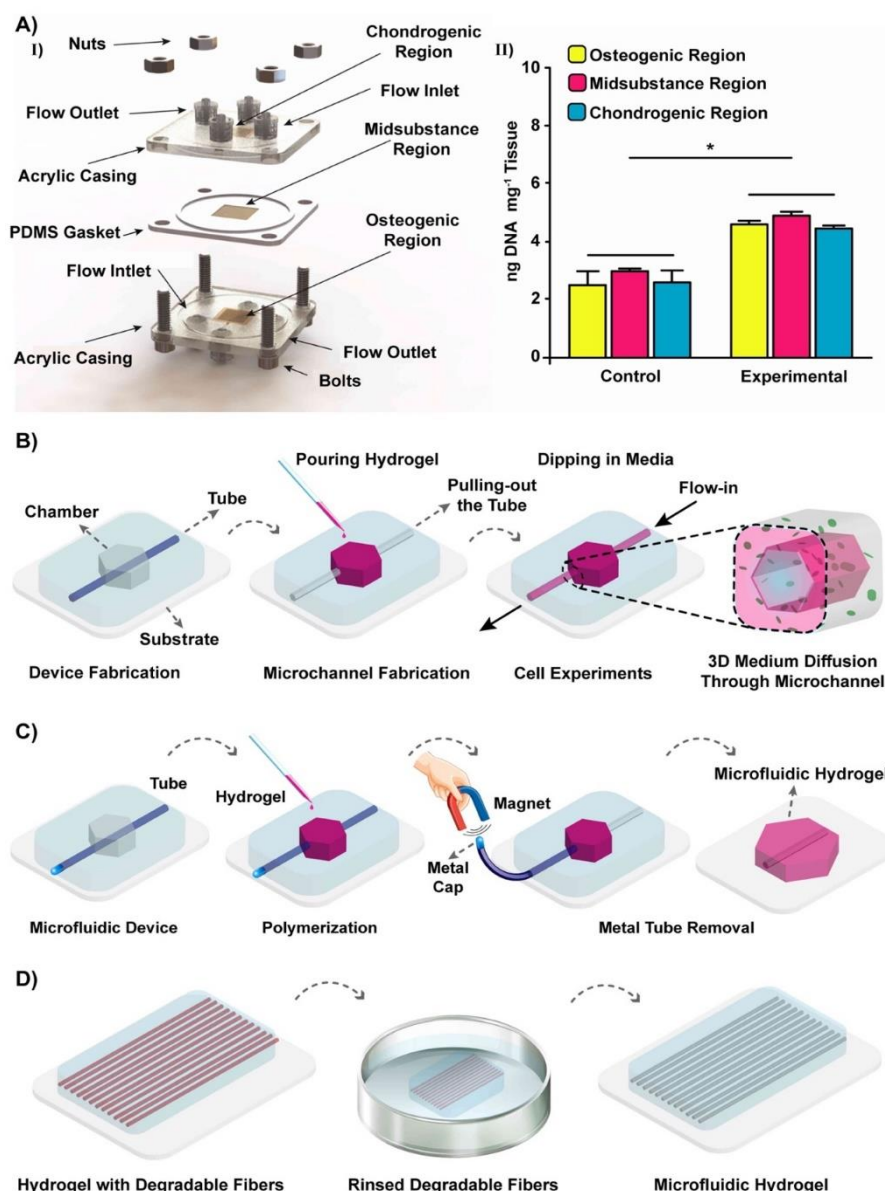


Figure 6. A) I) The construction procedure of the microfluidic OC graft. II) The DNA content was remarkably higher within the experimental group, which got cytokine supplementation in comparison with the control group after two weeks of culture. Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[159] Copyright 2016, Mary Ann Liebert, Inc. B) Schematic displaying the fabrication method for microfluidic hydrogel-based scaffolds. C) Representation of iron-capped rods' magnetic extraction. D) Schematic showing the fabrication of microfluidic hydrogel-based scaffolds via fibers degradation.

Additive manufacturing: Additive manufacturing methods are another subset of microfluidic fabrication techniques that can allow the fabrication of layer-by-layer structures employing cell-free or cell-laden hydrogels.^[162,163] Such type of leading-edge technology has emerged as one of the most desirable fabrication approaches due to its capability to generate sophisticated and reproducible tissue-specific constructs containing biological components or living cells.^[164,165]

One strategy, introduced by Lee et al.^[166], refers to the layer-by-layer patterning approach employing surface tension forces and open microfluidic-based principles in order to shape layers of hydrogel in given patterns to ultimately design a 3D structure. In fact, they employed the stereolithography technique to make a series of patterns as a tool which played the role of a mold. In taht study, a patterning tool allowed the pre-gel solution to readily flow through capillary action in the absence of external pressure to generate each of the layers in a regulated shape. The method's ability to generate geometries with overhanging functions was demoisntrated by means of designing an agarose cylinder in a double helix form in thirty layers. Other fabrication techniques use a sacrificial material or a casting to build fluidic channels throughout a 3D hydrogel construct. It is noteworthy that such type of approach can create 3D fluidic channels in a hydrogel complex without dependence on a post-fabrication step or a sacrificial material. Moreover, the layer-by-layer patterning method is relatively low-price and straightforward, thereby allowing to save material and is suitable for shaping expensive chemicals.^[166–168]

3D printing method, a state-of-the-art additive manufacturing technology, has several benefits in manufacturing complicated designs with broad applications. Bioprinting that utilizes a bioink, an ink formulation permitting the live cells' printing^[169], has a significant potential to advance medicine, especially the TE. Generally, as shown in **Figure 7**, 3D printing techniques include inkjet printing (**Figure 7.A**), extrusion printing (**Figure 7.B**), stereolithography printing (**Figure 7.C**), laser-assisted printing (**Figure 7.D**), two-photon lithography (TPL) printing (**Figure 7.E**), and volumetric printing (**Figure 7.F**).^[170–176]

In general, two of the most popular and used printing strategies are inkjet- and extrusion-based printing techniques.^[177,178] Inkjet printing is a non-contact layer-by-layer fabrication method transferring digital patterns via uniformly tiny ink drops onto a substrate.^[179,180] This indicates that only the ink droplets are required to contact the printed surface for the inkjet printing to take place. In fact, this type of method involves the spray of the ink onto a substrate via a nozzle at a very close distance rather than printing it onto the substrate.^[181] Such technology has the advantage of being a non-contact method, which reduces the wastage of expensive materials and allows for highly repeatable printing at high deposition speeds on dynamic, uneven, or sensitive substrates with precise details.^[182] Inkjet printing is categorized into drop-on-demand and continuous inkjet. Within the drop-on-demand technique, as a response to a digital waveform or signal, liquid drops are produced with an average diameter of 15-55 μm . Conversely, a harmonic modulation is utilized to break a constant stream of liquid into 80-100 μm droplets in the continuous inkjet mode.^[183] This developing printing method has several benefits over traditional methods. Interestingly, it is applicable for various substrates due to its non-contact nature, and besides, cross-contamination from the substrate is less likely to affect biological components.^[184] Furthermore, exerting low shear stress on cells enhances cell viability as compared to the extrusion-based printing technique.^[185,186] In addition to the key benefits inkjet printing brings, several drawbacks hinder its application in some cases. The method is not able to print high cell densities, meaning that printer's nozzle can be clogged by an accumulation of cells or cell clumps. Furthermore, melting or cold materials may jam the print head. In most cases, issues are typically brought on by a high nozzle length or room temperature. Additionally, the nozzle can become clogged due to the abundance of fibers in composite inks as well as viscous or non-homogeneous components. Nevertheless, the poor construction of vertical printed parts is considered as another obstacle of this manufacturing approach.^[187-191] In an disruptive work, a group of scholars^[192] utilized inkjet printing along with a sacrificial strategy to fabricate vascular micro-channels within collagen hydrogels. The channels' dimension possessed a broad range (0.5-1.2 and 0.7-1.5 mm for height and width, respectively). It was concluded that the vascular micro-channel inside hydrogels was able to support the tissue viability with 5 million cells mL^{-1} density up to 5 mm in distance under the physiological perfusion condition.

Extrusion printing involves inks' extrusion through one or multiple nozzles to produce 3D structures.^[193,194] The extrusion strategies are sorted into coagulation bath printing, free-form reversible embedding, direct ink writing, and coaxial printing.^[195,196] The advantages of this technique rely on the possibility to print hydrogels with different viscosities and the use of high cell densities. For all the benefits it provides, it is only suitable for viscous gels, thereby hampering components' selection and damaging the cells.^[197,198] Within an innovative study, Zhang et al.^[199] produced hollow hydrogel filaments made up of sodium alginate using a coaxial extrusion-based manufacturing device. It was proved that the bovine cartilage progenitor cells-loaded microfluidic printable channels, in the shape of hydrogel filaments with an average inner diameter of 135 μm , not only supported the cellular environment's mechanical integrity in 3D, but also enabled the media transport to the cell assembly. Furthermore, the microfluidic channels displayed superior flexibility in comparison with the previously reported microfluidic channel manufacturing approaches^[200–202], and no post-processing was required subsequent to the fabrication.

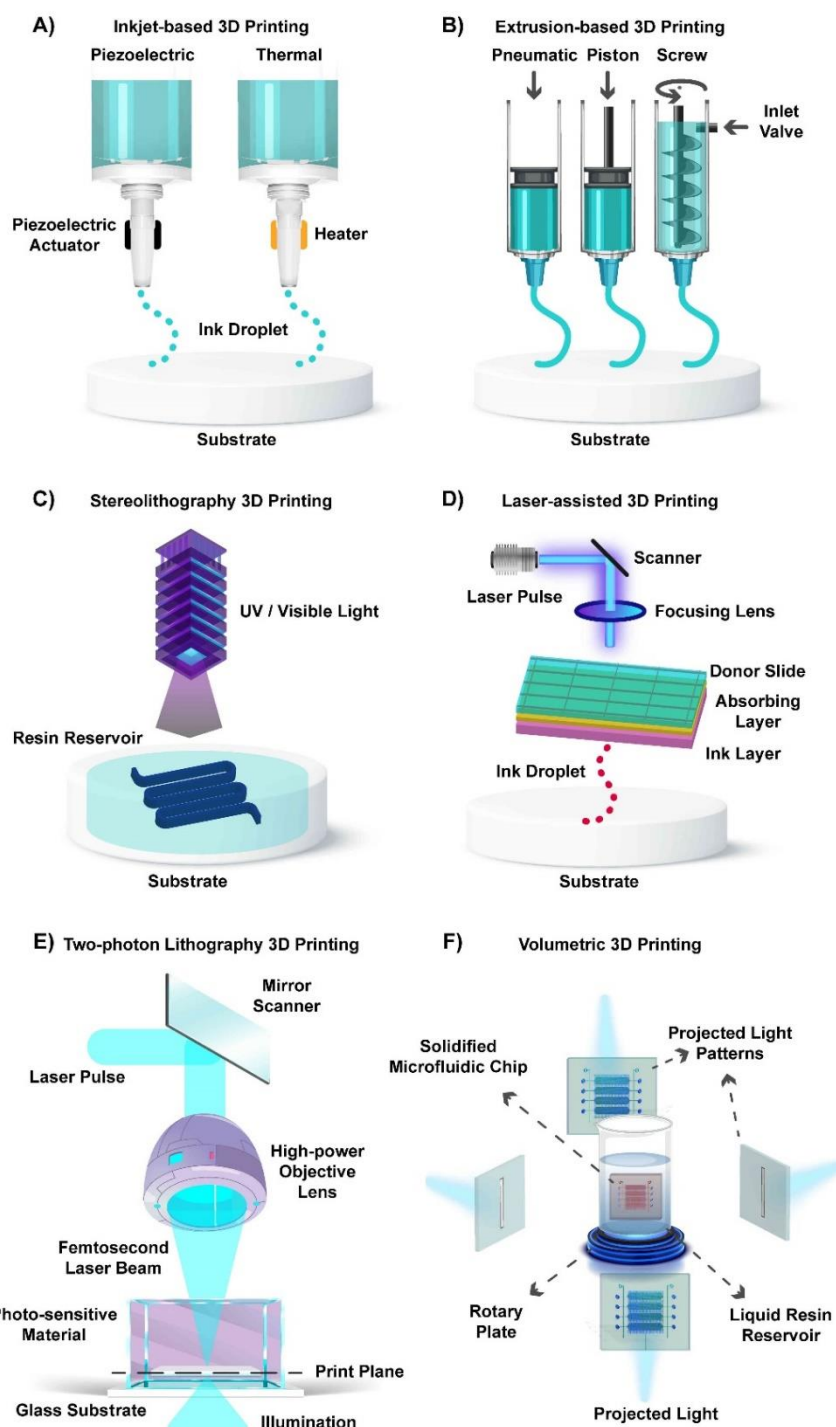


Figure 7. Schematic of the various printing approaches: A) An inkjet printer depositing tiny bioink's droplets to build a tissue in a layer-by-layer manner. B) An extrusion printer depositing cell-laden solutions or hydrogels by pneumatic or manual force. C) Stereolithography printing. D) Laser-assisted printing. E) TPL printing. F) Volumetric printing.

Multiple experiments have been focusing on tissue regeneration by means of employing a combination of printing and microfluidics technologies. Developments in the 3D printing methods have permitted the use of the microfluidic technology in an increasing number of fields and have excited the biologists' interest. The integration of these two technologies can increase the resolution and cell viability as compared to other single technologies, such as rapid prototyping, molding, and electrospinning. Therefore, their combination has superior advantages over single manufacturing approaches. Extrusion-based printing is regarded as an ideal bioprinting technique as it is cell-friendly and enable multi-material deposition. Nevertheless, since biologically-relevant constructs usually should be of superior complexity and high resolution, novel approaches are required in order to provide an effective control over the deposited materials. Indeed, a combination of these two abovementioned state-of-the-art technologies can open up interesting possibilities in multi-phase and multi-material printing. However, it is presently restricted to the extrusion printing and encounters obstacles in expanding to other printing approaches. In this regard, scholars are putting effort to modify and enhance the existing methods via designing innovative nozzles or including other components.^[203–205]

Notably, several microfluidic chips can be directly integrated in the print heads of nozzles utilized for the 3D printing, and fluid bioinks flow through the micro-channels of these chips. Such type of chips, employed as print heads, can be modular microfluidic devices with connected microfluidic discrete units such as L junction, T junction, mixer modules, straight channel modules, and reaction chambers, which are assembled to fabricate fully-functional microfluidic hydrogel-based scaffolds. The micro-concentrators' integration into the print heads enables a remarkable enhancement in the cellular concentration in the process of bioprinting. Furthermore, the rapid microfluidic switching's addition and enhanced resolution through flow focusing are reported.^[205] The benefit associated with the printed hydrogel partitioning chip design is that researchers are able to readily change the hydrogel biomaterial, leading to the change in the diffusion barrier's pore size and allowing various solutes' selective transport across the barrier.^[205,206] Other advantages include the possibility to control

the overflow, combining cells with bioinks in a controllable and reproducible manner, generating decreased shear stresses during the procedure owing to the sheath flow surrounding the laminar core, and possessing rapid and simple assembly of individual units into a distinctive configuration.^[196,207]

In this regard, Zhao and a team of reserachers^[208] presented an airflow-assisted bioprinting approach by which versatile spiral micro-architectures within hydrogel microspheroids could be printed, enabling one-step bioprinting of extraordinary hydrogel-based structures. In brief, a microfluidic nozzle was developed in order to enhance the capability of complex cell encapsulation with heterotypic contact. The authors chose the microfluidic PDMS chip, as the dispensing nozzle's body, and multiple metal capillaries were inserted at the inlet/outlet orifices. In that chip, Y-shaped micro-channels permitted extrusion of two or three parallel bioinks from a nozzle with rapid response and high resolution. Intricate structures, such as a Tai chi pattern, a rose, and single-cell line could be readily printed in the spheroids. As proof-of-concept, a human multi-cellular organoid of spirally vascularized ossification was reconstructed employing such approach, showing the ability of the method in building mini-tissues on microspheroids. By its turn, Attalla and co-workers^[209] developed a novel microfluidic nozzle which enabled multi-axial extrusion to pattern and print hollow bi-and tri-layered channel structures. In that work, the nozzle allowed embedding of these structures within gels' layers in a low-cost, rapid, and simple manner via varying printing speeds ($1\text{-}16\text{ m min}^{-1}$) and flow rates ($1\text{-}6\text{ mL min}^{-1}$). Specifically, the scalable design permitted for versatility in the material incorporation so as to generate heterogeneous structures and enhance long-term cellular viability. In a pioneering investigation, a novel BMSCs-encapsulated bioink composed of gelatin methacryloyl (GelMA)-CS-amino ethyl methacrylate (AEMA) was used in a coaxial printing system, including a microfluidic chip and a standard printing needle. This precise method permitted the manufacture of complex solid hydrogel geometries with extraordinary shape fidelity.^[210] Two bioinks embedded with human articular chondrocytes (HACs) and human BMSCs (hBMSCs) were formulated for chondral defect regeneration in an evaluation. The microfluidic extrusion system utilized in this experiment comprised a coaxial adapter and a microfluidic printing head compatible with different cell-laden hydrogel bioinks. In this regard, a microfluidic mixer forced the loaded bioinks to the coaxial

extrusion system's internal region, accompanied by the adapter's injection of calcium chloride, a cross-linking agent. Although that method had limited mixing efficiency, increased resolution and high cell viability were its main advantages.^[18] Within an innovative investigation, a 3D bio-mimetic hydrogel platform was produced for the calcified cartilage TE. The bioink and cross-linker were dispensed by the microfluidic pumps in a core-shell method via a coaxial needle system, and that system enabled printing bioinks and numerous additives with different chemical and rheological characteristics.^[211]

Soft-lithography: Molding techniques are simple, accessible, and cost-effective. Nevertheless, it presents important limitations as it do not allow producing specific and accurate geometries. Furthermore, manual handling should be performed after molding, in some particular situations. As a result, soft-lithography, a subgroup of additive manufacturing considered as a precise and reproducible method, has progressed to fabricate templates required for microfluidic systems. This method employs soft and elastomeric materials as well as hydrogels to manufacture nano-and micro-scale 3D structures (with a width and depth below 100 μm)^[212,213] for finding biological applications based on printing and molding.^[214,215] Sealed cell-laden agarose hydrogels with heating molded gel-surface microfluidic channels were produced with soft-lithographic approaches (**Figure 8.A**).^[202] Interestingly, Wang et al.^[168] developed a strategy to generate 3D micro-channels making use of a soft-lithography method. A single-layer sodium alginate was formed as a sacrificial microfluidic patterned template, and after embedding the template in the hydrogels, it was dissolved in ethylenediaminetetraacetic acid solution to create microfluidic channels. Notably, when combined with the advantages of the microfluidic device, this method offers a simple, quick, affordable, and highly controlled technique for creating single-layer or 3D networks.

Although soft-lithography offers considerable advantages, one disadvantage is that a stamp master must be made using a separate lithography technique, such as photo-lithography. Nevertheless, this step only should be done once because the master may then be employed to make stamps again. Therefore, this process is accompanied by templates' production, stamping steps related to patterning,

and bonding procedures between individual layers, increasing its difficulty level to a greater extent.^[216,217] Hence, the primary focus is lately shifting to other methods such as bioprinting.

Photopatterning: Photopatterning methods are subtractive manufacturing approaches which involve fabricating customized structures and user-defined micro-channels employing light or laser to cross-link polymeric pre-gel solutions. In the laser-induced process, laser pulses are utilized to degrade the hydrogel and form two-dimensional (2D) or 3D micron and sub-micron scale channels with high resolution. Regarding this matter, 3D channels of cell-laden PEG hydrogels^[218] and PEGylated fibrinogen hydrogels^[219] are good examples of photo-patterned structures that can be obtained by laser degradation (**Figure 8.B(I)**). Although such type of approach is reproducible and accurate, it is categorized as a costly and time-consuming method that can induce cellular damage if cell-laden hydrogels are envisioned. To overcome its limitations, it is necessary to regulate the scan speed, laser source, laser power, pulse duration, wavelength, and repetition rate.^[220] Besides, in order to remove other constraints such as the impossibility to create multiple microfluidic networks within a single structure, Slater et al.^[221] merged the laser-based degradation of a hydrogel with the image-guided laser to create an intricate 3D structure having microfluidic networks in PEGDA hydrogels (**Figure 8.B(II)**). In that study, it was shown the possibility of obtaining two independent and firmly intertwining channels which are capable of transporting fluids that filled the PEGDA hydrogel.

Table 3 presents a summary of fabrication methods' investigations for microfluidic hydrogel-based scaffolds.

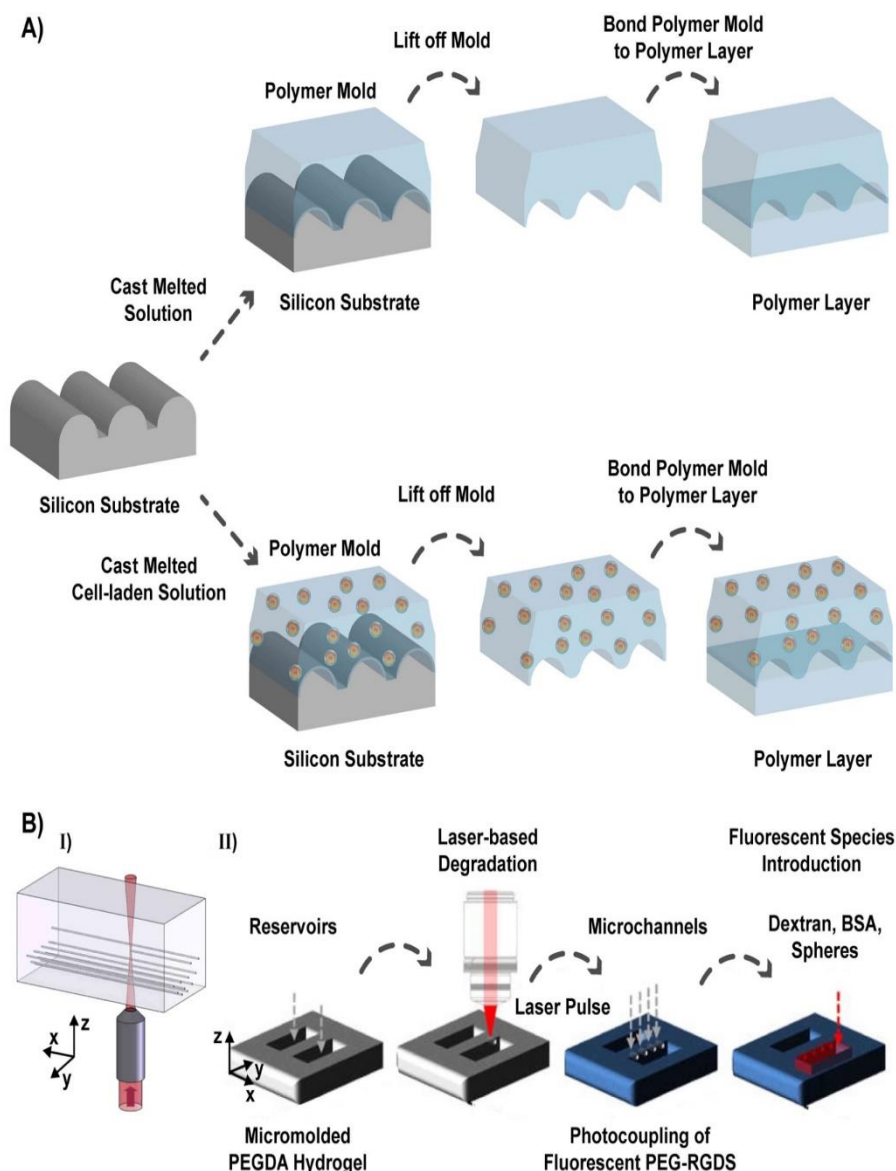


Figure 8. A) Schematic of the soft-lithography method for manufacturing microfluidic cell-laden and cell-free agarose hydrogel-based scaffolds. B) I) An illustration of patterning micro-channels in the 3D hydrogels employing laser degradation. Reproduced under the terms of the Elsevier user license.^[219] Copyright 2009, Elsevier. II) Scheme exhibiting the production of microfluidic channels in the PEGDA hydrogels via the laser-induced degradation technique. Reproduced under the terms of the Creative Commons John Wiley and Sons license.^[221] Copyright 2016, Wiley.

Table 3. Summary of fabrication methods' investigations for microfluidic hydrogel-based scaffolds.

Method	Material/Cell	Advantages and disadvantages	Fabrication cost	Fabrication speed	Resolution accuracy	Cell compatibility	Ref.
Molding (Microneedle; Degradable fiber template)	PDMS	-Able to produce full-thickness constructs	Low	Median	Low	Median	[15,158,160,161]
	Agarose	-Improvement of mass transfer					
	Hepatocellular carcinoma cells	-Simplicity					
	PDMS	-Low cost					
	Polystyrene	-Specific equipment requirement	Low	Median	Low	Median	[15,158,160,161]
	Collagen	-Demand for manual handling					
	Human umbilical vein endothelial cells	-Limited to simple geometries					
	Agarose	-Capability to affect cellular viability due to the organic solvent's presence					
	PDMS						
	MSCs						

Layer-by-layer patterning	Agarose	-Minimal waste production	Low	High	Median	High	[166]
	PEG	-Capable of creating overhanging and microfluidic structures					
Printing	Collagen type I	-Able to pattern hydrogels that cannot be printed because of their viscosity	High	Median	High	Median	[18,165,166,198,199]
	Human fetal lung fibroblasts	-Relatively time-consuming					
Printing	GelMA	-Multi-material printing capability	High	Median	High	Median	[18,165,166,198,199]
	AHAMA	-High scalability and reproducibility					
Printing	CS	-High potential for the complex networks' fabrication	High	Median	High	Median	[18,165,166,198,199]
	Alginate BMSCs	-Minimal waste production					
Printing	Sodium alginate	-Specific equipment requirement	High	Median	High	Median	[18,165,166,198,199]
	Bovine cartilage progenitor cells	-Cell damage as a function of high viscosity and					

		injection -Merely suitable for viscous printable materials					
Combination of 3D printing and microfluidics	Sodium alginate Human umbilical vein endothelial cells MSCs	-Multi-phase and multi-material printing -Mainly restricted to the extrusion-based printing -Remarkable enhancement in the cellular concentration	High	Median	High	High	[209,210]
	GelMA						
	CS						
	AEMA BMSCs	-Enhanced resolution -Specific equipment requirement					
Soft-lithography	Agarose Murine hepatocytes	-Reproducibility -Accuracy -The heating	Median	Median	Low	Median	[168,202]

Photo patterning	PDMS	requirement for					
	Alginate	sealing, capable of					
	Gelatin	damaging cells					
	Collagen	-Limited to soft and					
	type I	elastomeric materials					
	Human umbil						
	ical						
	vein endothel						
	ial cells						
	PEGylated	-High resolution					
	fibrinogen	-Programmable					
	PEGDA	-Reproducibility					
	Ganglion	-Limited scale	High	Low	High	Median	[218–221]
	cells	-Time-consuming					
	PEGDA	-Cell damage due to					
	Endothelial	laser emission					
	cells						

4.2. Microfluidic chips' manufacturing approaches

Within the field of OoCs, a proper fabrication approach can allow enhancing the interactions between cells and tissues, address biocompatibility requirements, and determine a specific application or an organ's micro-structure.^[222,223] Furthermore, a suitable technique should be one that provides high resolution, short processing time, and superb control over spatial arrangement.^[224] Concerning

this subject, many diverse OoCs' fabrication methods have been developed so far, and the subsequent paragraphs discuss these techniques as well as their relevant original research.

Molding: As mentioned, molding is one of the oldest and simplest fabrication approaches within the TE field. Considering the processing time requirements and the importance of manufacturing detailed micro-structures, novel techniques have been proposed, which are often combined with the molding method to design OoCs with the best possible features. For instance, 3D printing or soft-lithography is employed to prepare the chip mold, and then the chip is molded via the molding method. Recently, Shrestha et al.^[225] utilized 3D printing for the PDMS molds' rapid fabrication. In fact, one of the challenges of PDMS molding is the adhesion of PDMS to the mold due to its unsuitableness, which was successfully overcome in this study by means of performing a surface treatment on the printed molds. In this regard, the printed molds (printed files that were drawn with computer-aided design (CAD)) were washed, dried with isopropanol, post-cured with UV for three minutes, soaked in the ethanol for the complete removal of monomers, treated with oxygen plasma, and finally silanized under the vacuum (**Figure 9.A**).

Lithography: Previous to employing 3D printing techniques, lithography was the most common approach for preparing microfluidic chips. Soft-lithography, a subset of the lithography method, employs these substrates as molds to construct micro-devices when 3D micro-patterns made of photo-resist materials attached to the top of silicon wafers are created.^[226] Employing this technique, a microfluidic device with gradient generating patterns for evaluating biomolecular and shear stress gradients' impacts on the various properties of hMSCs has been developed.^[227] Briefly, an elastomer base named Sylgard® 184 was mixed with the curing agent to create PDMS, and the obtained material was poured onto silicon-based wafers with photo-resist patterns. Subsequent to the degassing process under vacuum, the abovementioned mixture was cured overnight at 80 °C, and in order to form the device, the cured PDMS-based membranes were connected to a slide following the oxygen plasma treatment. Tuning resolution and good integrity is possible by means of using the replica molding technique, an approach suitable for manufacturing microfluidic chips embedded into microgels^[228], that includes several fabrication steps: 1) A silicon-based wafer is spin-coated with a

photo-resist material, which is then overlaid by a photo-mask substrate. 2) The wafer gets exposed to the UV light to be cross-linked or uncross-linked, while the photo-mask substrate only allows the photo-resist's desired regions to be illuminated. 3) The patterned mold is prepared when the pattern is moved to the silicon mold. 4) As the final step, the liquid biomaterial (generally PDMS) is poured on the fabricated mold and polymerized after baking, followed by peeling off the PDMS stamp.^[229] When glass-based materials are employed as molding substrates, the adopted technique is capillary molding^[230] in which the PDMS layer's patterns must be brought into contact with a substrate (*e.g.*, a glass slide). Indeed, the capillarity leads to the formation of a liquid polymer's shape analogous to the cavities' pattern on the PDMS layer. The PDMS layer is used as a stamp which is then soaked into the molecular ink and pressed on the substrate in a molding method named microcontact printing. In addition, within the microtransfer molding approach, the PDMS layer can be filled with a liquid polymer and then transferred onto the substrate while the extra polymer is eliminated. The main types of soft-lithography techniques are depicted in **Figure 9.B**.^[223,231]

It is worthnotice that photo-lithography transfers the microfluidic chip pattern onto a substrate by the selective induction of a light beam or a laser. Interestingly, Liu and co-workers^[232] could effectively solve the clogging and deformation issues of a chip's micro-channels by means of utilizing the laser ablation method mixed with low-temperature bonding of optically clear adhesive film and liquid optically clear adhesive. In another valuable study, Occhetta et al.^[233] developed a novel OA-induced cartilage-on-a-chip model with the aim of screening an array of etiologically targeted agents, also known as disease-modifying OA (DMOA) drugs.^[234] In that study, the master molds were obtained employing the photo-lithography technique in a cleanroom environment and then polymerized two layers of PDMS in casts at 65 °C for at least three hours, followed by the production of a membrane (750 µm thickness), which separated the top and bottom layers via precisely dosing the PDMS that was poured onto a substrate.

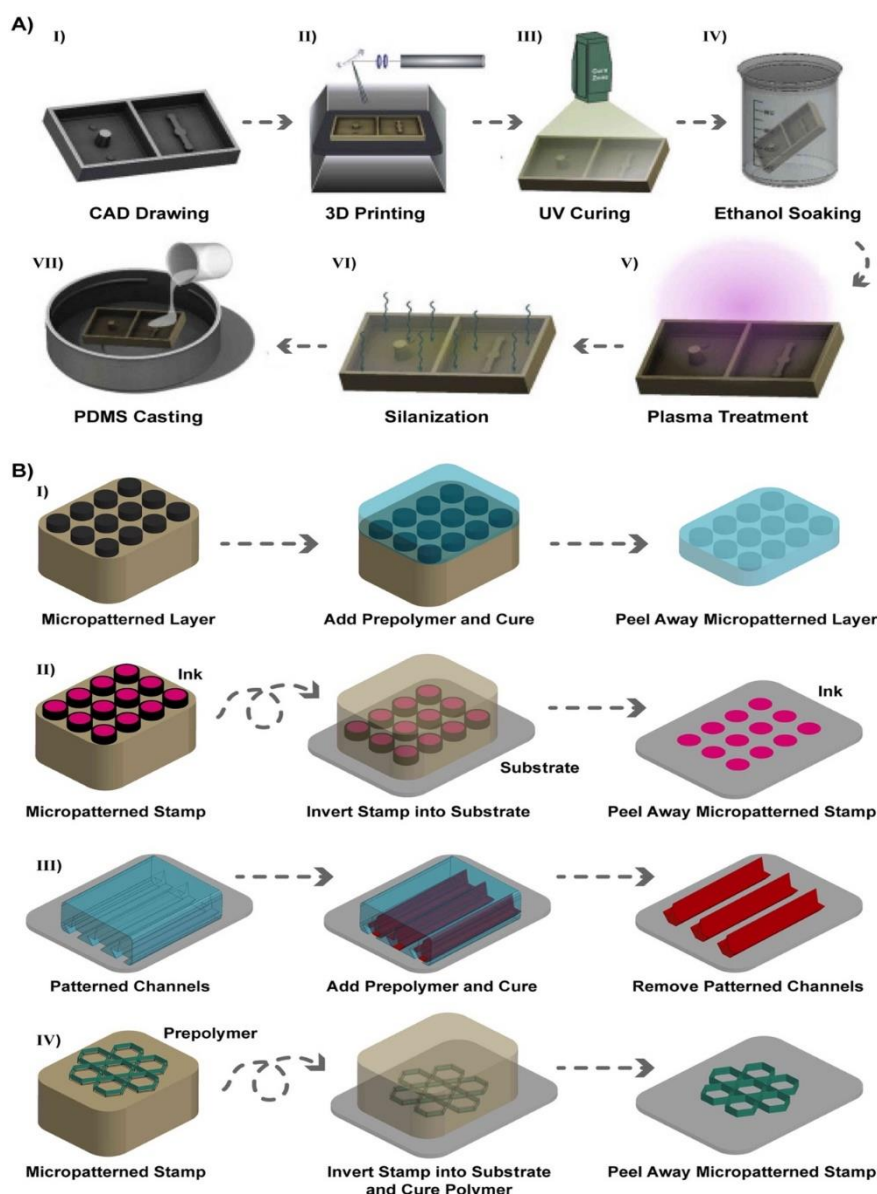


Figure 9. A) A figure depicting the procedure for the printed molds' surface treatment: I) The desired mold's CAD drawing employing Solidworks®. II) The 3D printer was used to print the finalized files. III) Subsequently, the mold was rinsed with isopropanol, followed by the UV post-curing process. IV, V) Dipping the mold into the ethanol before the plasma treatment was performed. VI, VII) The mold was silanized before casting PDMS. Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[225] Copyright 2019, Elsevier. B) A schematic showing the various soft-lithography approaches: I) Replica molding. II) Capillary molding. III) Microcontact printing. IV) Microtransfer molding.

3D printing: It is rather difficult to fabricate an OoC with complete control over its materials' structures in a one-step process unless utilizing 3D printers, devices capable of providing prolonged cell culture, controlled simulation of 3D architectures, and rapid prototyping.^[235–237]

Inkjet 3D printing combined with microfluidics has displayed considerable potential in diminishing the microfluidic chips' fabrication cost and spatially controlled printing of various cell types without inflicting damage.^[238] In this context, Zhang et al.^[239] employed a home-made inkjet bioprinter for cell patterning in microfluidic devices with the aim of co-culturing, stimulating, and analyzing the hepatoma and glioma cells for the drug metabolism and diffusion tests. The printer was utilized to print multiple-cell-encapsulating sodium alginate matrix and form a meticulous arrangement of cell arrays on glass slides, covered by a PDMS layer with connected micro-channels. One of the ongoing developments of the OoCs is the integration of their membranes with sensors so as to monitor the variables in an online manner. Moya and co-workers^[240] fabricated amperometric oxygen sensors within an ultrathin and porous cell culture membrane of a liver-on-a-chip system via an inkjet printer to achieve *in situ*, simultaneous, and real-time monitoring of the oxygen along with the cell culture microfluidic chamber.

Bearing in mind the possibility of merging extrusion-based 3D printing and microfluidics principles, Ozbolat and a group of researchers^[241] designed a human bone marrow endothelium cells (BMECs)-and human umbilical vein endothelial cells-loaded microfluidic chip by extrusion printing. In this context, Carbopol ink could enable the channel templates' extrusion printing, which were then overlaid with the PDMS to produce a microfluidic device upon PDMS curing and the sacrificial ink's removal. The samples had the lowest channel thickness (around 180 μm) with uniform circularity and higher resolution as compared to those manufactured via the lithography technique. Moreover, it was revealed that the cells displayed a good response to the fluid flow, and their adherence to the microfluidic channel edges was not observed.

Stereolithography 3D printing, a traditional printing technology invented in the 1980s, uses UV or laser light to selectively photo-polymerize a photo-sensitive resin as a layer of a microfluidic chip.^[242] The method employs materials to create microfluidic structures with typical properties $< 50\ \mu\text{m}$. Feasibility to use a wide range of materials, achieving incredibly high feature resolutions, and producing high-quality surface finishes are some of the advantages this approach offers.^[243–245] An example is the work of Nour Goushki and co-workers^[246] which reported to enhance the integrity of their fabricated bone-on-a-chip by the stereolithography method. The advantage is that the anisotropic structure was able to be kept while assembling nanopatterns on the microfluidic chip. Furthermore, they reported to achieve a large surface area as well as a high flow rate owned by the 3D architecture.

In the laser-assisted 3D printing method, a thermoplastic sheet is induced via a laser beam which then turns into droplets constructing microchips. Of note, several drawbacks of inkjet- and extrusion-based 3D printing are eliminated employing laser-assisted printing. For instance, this technique provides the greatest possible droplet resolution due to the laser pulse's precision.^[247,248] It is important to state that various parameters, such as laser power and the distance between the ribbon and the receiving substrate, may alter the resolution of laser-assisted 3D printing.^[248,249] Utilizing a novel version of this approach named direct laser plotting, Wang et al.^[250] produced polymer-based microfluidic chips. It was utilized a carbon dioxide laser plotter in order to generate the microstructures. The favorable patterns could be directly plotted onto the PDMS substrates and polymer films via the manipulation of laser pulses' density and strength. Using this technique, microfluidic chips possessing five layers were obtained within a few minutes, without the need of employing a cleanroom. Besides, by exploiting the laser impulsion's interference effect, a feature size of $50\ \mu\text{m}$ or even smaller was obtainable for the microfluidic channels.

TPL is a one-step 3D printing technique using ultra-short laser beam (femtosecond pulsed laser with wavelength around $800\ \text{nm}$) which can provide a high level of control for optional printing in scales less than a micrometer. By means of using this method, laser energy can be concentrated on a specific spot in desired time with remarkable accuracy. Succinctly, the primary process involves the polymerization of a photo-sensitive material as a result of two photons' coincidence absorption in a

nonlinear manner, with each providing half of the energy. Manufacturing intricate 3D micro-structures, obtaining high resolution, improved scalability, and the elimination of layer-by-layer process are few of the benefits this printing strategy can provide.^[251] Due to the low feature size resolution, TPL has made feasible the fabrication of complex nano- and micro-scale structures such as microfluidic chips.^[252] Mandt et al.^[175] successfully developed a high-resolution gelatin-based placenta-on-a-chip by means of using this method. They generated the 3D gelatin-based structures by TPL within the customized microfluidic chip' intersection which separated the X-shape chamber into two compartments used for cell culture. In this regard, a TPL printer having a laser pulse with a length of 70 femtosecond and 80 MHz repetition rate at 800 nm was employed. It was revealed that the layer distance in the printing process should be kept at 0.8 μm in order to guarantee dense cross-linking. By association of CAD models, the accurate structural adaptability of a specific geometry was enabled.

In recent years, volumetric 3D printing has captured considerable attention in the field of TE. Unfortunately, there are several problems with conventional printing procedures, including the inability to preserve cell-cell interactions and the extended printing time for printing large organs. To surmount these obstacles, volumetric printing is the introduced alternative used to generate entire cell-laden tissue constructs in the preferred time period. This method, inspired by computer tomography, utilizes the visible light laser to cross-link the construct.^[176] Short building time (a few tens of seconds in comparison with tens of minutes for the sequential approaches) and capability to print intricate hollow structures without requiring support struts are some of this technique main advantages.^[253] Indeed, in volumetric 3D printing, the complete 3D structure is synchronously generated via scattering a container of photo-sensitive resin from various angles. The Radon transform is used to calculate a succession of 2D light patterns, which are then projected simultaneously onto a revolving resin container. The intended 3D structure may be created in a single step by superimposing many 2D photos of the same item seen from different angles, which considerably reduces printing time.^[254]

Volumetric 3D printing, which completely abandons the conventional layer-by-layer stacking, can pass through structural components and enable printing with quite viscous fluids. A limitation is that only transparent resins may be utilized with volumetric printing. As a result, there will be less

diffraction and a uniform light dosage regardless of penetration depth. Furthermore, the object's printed size is rather small compared to other methods.^[255] It is of critical importance for suitable object fabrication in volumetric printing that each light pattern cover the entire build volume. The concentration of the photo-initiator is regulated by this function. In other terms, while creating a resin formulation for volumetric printing, the initiator proportion should be both sufficient to enable the gel to polymerize and low enough to let the light pass over the entire volume.^[176] Due to the novelty of this technique, few experiments have employed this approach, including the work by Bernal and co-workers^[256] in which the volumetric bioprinting of intricate organoid-laden constructs capturing human liver tissue's essential functions was introduced. A group of researchers^[176] carried out the study to print cell-loaded auricle constructions with high survival using volumetric printing. Further the evaluation carried out by Toombs et al.^[257] assessed developing perfusable branched 3D microfluidics with low wall thickness (85 μm) and channel diameter (150 μm) by employing this technique. In the context of using the volumetric 3D printing for fabrication of microfluidic chips, a group of scholars^[258,259] revealed that volumetric 3D printing can be a revolutionary method for rapidly fabricating microfluidic chips with high accuracy and transparency. In addition to its speed and accuracy, it allowed overcoming common challenges such as easily remove the support from the system.

In line with the improvements in volumetric printing, controlling light scattering is of high importance especially when printing constructs with sizes larger than the scattering mean free path, since optical distortion can result in scrambled spatial information, poor printing fidelity, and resolution loss. In an attempt to overcome this challenge, Madrid-Wolff et al.^[253] first experimentally characterized the light propagation through the scattering resin and iteratively corrected the projected patterns shown onto a micromirror digital device. By analyzing images, they found that the high spatial frequencies' poor transmission owing to the light scattering by the photo-curable resin. Therefore, a numerical correction was made in order to accentuate the highest spatial frequencies' features. Favorably, results showed the enhanced contrast of light dose improved resolution and printing fidelity of printed geometries, and maintained cellular viability one-hour post-printing.

Electrospinning: Electrospinning, a well-known method for producing micro/nanofibers, has been widely used and developed in TE applications. The electrospun fibers allowing for matrix remodeling, cellular migration, and ECM synthesis can provide a suitable platform for the production of soft tissues' equivalents. In electrospinning, as shown in **Figure 10.A**, by applying a high voltage to the needle and the collecting plate, a strong electric field is generated. When the electric charge overcomes the solution's surface tension, a Taylor cone is formed, followed by pulling the polymer jet toward the collecting plate. The surface charge density's increment and the solvent evaporation can lead to the generation of micro/nanofibers network on the collecting plate. Due to their unique properties, these fibers can be suitable candidates for placing in the OoCs' chamber and acting as substrates for seeding and culturing different cells. It is noteworthy to mention that the fibers' diameter depends upon the method's parameters, especially voltage, needle diameter, and solution viscosity. It is possible to employ this simple and inexpensive approach with a wide range of polymers. Nevertheless, since the used polymer must be in the solution form, some toxic organic solvents are introduced to the complex, thus negatively affecting the fibers' cytocompatibility.^[253,260–264]

Recently, Tuerlings et al.^[265] designed a dual-tissue microfluidic device as a bio-mimetic model to study OA disease (**Figure 10.B**). In this regard, they first produced PCL nanofibers and microfibers using an electrospinning machine and then placed them within a molded PDMS chip consisting of a chondrogenic channel and an osteogenic channel in order to separate the channels via the electrospun PCL fibers. In another investigation, Kim et al.^[266] succeeded in producing PCL fibers (100 μm thickness) with 600–6000 nm diameter via the electrospinning technique and put these fibers within the microfluidic chip chamber as a proper substrate for cell cultivation. The results revealed that the 3D culture of cells and monitoring cells' conditions in real-time for fourteen days were possible by means of employing this fibers-embedded chip. Furthermore, as shown in **Figure 10.C**, high biocompatibility and cellular viability above 95 % were achieved.

Table 4 presents a summary of current fabrication methods for manufacturing microfluidic chips with their relevant original research.

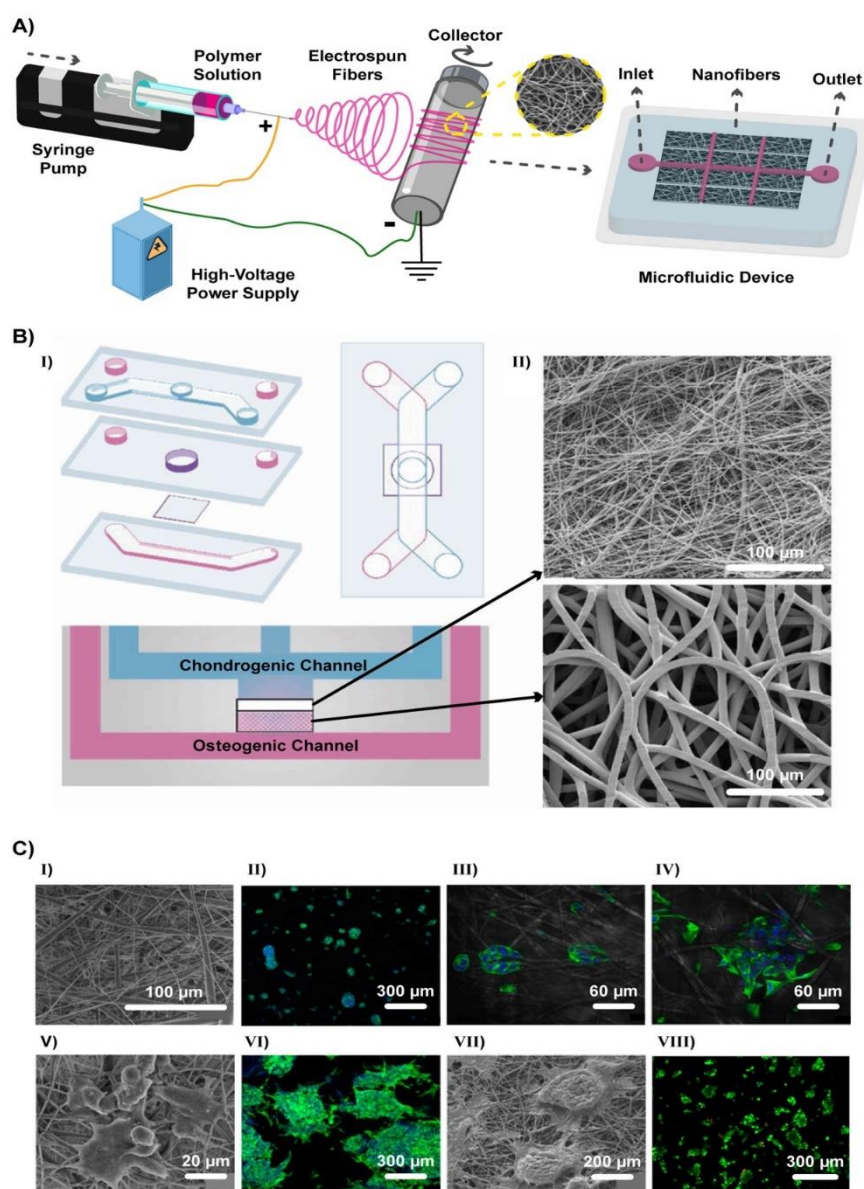


Figure 10. A) Schematic of the electrospinning technique for developing OoCs. B) The OC unit-on-a-chip model: I) A figure illustrating the microfluidic chip's design. II) The electrospun PCL matrix's scanning electron microscope images with nanofibers (top) and microfibers (bottom). Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[265] Copyright 2022, Wiley. C) I) Electrospun nanofiber scaffold. II, III, IV) The scanning electron microscope and confocal microscopy images of cells cultivated onto the nanofiber compartment with the microfluidic-based system for one day and V, VI, VII) fourteen days. VIII) Live & dead assay outcomes related to the designed chip (day one).

Table 4. Summary of current fabrication methods for manufacturing microfluidic chips with their relevant original research.

Main Method	Supplemental technique	Material/Cell source	Industrial points	Ref.
Molding	3D printing	PDMS and Polyester/ Human airway epithelial cell line	-Low-cost fabrication -Simple method -Time-efficient	[225]
Lithography	Soft-lithography	PDMS/hMSCs	-Amenable to cell culture -Capable of producing gradient generating patterns within PDMS	[227]
Lithography	Photo-lithography (laser ablation method mixed with low- temperature bonding)	Optically clear adhesive film and Liquid optically clear adhesive/-	-Solved clogging and deformation issues of the chip's micro-channels -Achieved 99 % bonding area rate of chips	[232]
Lithography	Photo-lithography	PDMS/HACs	- Relatively costly -Time-consuming -Complicated procedure	[233]

			-High resolution	
Molding	Inkjet printing	PDMS and Sodium alginate/Human hepatoma cell line and Human glioma cell line	-Able to co-pattern multiple cell liens -Automated manufacturing of complex OoCs -Capable of spatially controlled printing	[239]
Molding	Inkjet printing	Polymethylmethacrylate, Gold NPs, and Silver NPs/Hepatocytes	-Capable of integrating electrochemical sensors in the OoCs membrane -Cost-efficient	[240]
Printing	Extrusion printing	PDMS and Carbopol ink/Human umbilical vein endothelial cells and BMECs	-Affordable cost -Possibility of high-temperature printing -Employment of materials with suitable viscosity	[241]
Printing	Stereolithography/ TPL	PDMS and Acrylate-based resin/hMSCs	-Reasonable accuracy and resistance in 3D structure -Long printing time -Expensive -High-quality surface finishes	[246]
Printing	Laser-assisted printing (direct laser	PDMS and Polymer-based films/-	-Direct plotting of favorable patterns on the	[250]

	plotting)		<p>substrates via the manipulation of laser pulses' density and strength</p> <p>-Clean room-free</p> <p>-Time-efficient</p> <p>-Obtained feature size of 50 μm or even smaller for the microfluidic channels by exploiting the laser impulsion's interference effect</p>	
Molding	Electrospinning	<p>PDMS and PCL/ Human primary articular chondrocytes</p>	<p>-Potential of producing fibers on micro-and nano-scale</p> <p>-Capable of controlling the percentage and porosity size</p>	[265]
Molding/Soft-lithography	Electrospinning	<p>PDMS and PCL/ Human liver hepatocellular carcinoma cells</p>	<p>-Ability to provide fibers with desired, suitable thickness and diameter for different applications</p> <p>-Capable of controlling cell behaviors such as migration by fiber diameter and porosity</p>	[266]

TPL	Molding	Gelatin, PDMS and PEGDA/Human umbilical vein endothelial cells	-Precise structural adaptability -High-resolution structure -Specific equipment requirement -Expensive -Time-efficient	[175]
Printing	Volumetric 3D printing	GelMA/Human liver cancer cells	-Support-free -Very short fabrication time -Specific equipment requirement -Expensive -High resolution -Without waste material -Optically smooth surface	[256,259]

5. Microfluidic hydrogel-based scaffolds' preclinical and clinical achievements in the cartilage repair

5.1. Preclinical evaluations

Naturally derived and synthetic-based polymeric hydrogel-based scaffolds have been extensively tested in order to evaluate their efficiency in the AC regeneration field.^[267,268] Microfluidic hydrogel-

based scaffolds, as a novel subgroup, have recently captured a great deal of attention within this research area due to their abovementioned major benefits. Besides utilizing different types of materials to form these structures, various parameters have been closely investigated in experiments to obtain the optimum repair of the AC unit.

An emerging rapid prototyping technique for manufacturing microfluidic structures, microfluidic hydrogel-based scaffolds in particular, is 3D printing as mentioned.^[207] Concerning this subject and in order to compensate for the restricted chondrocytes' supply, Costantini et al.^[210] generated ECM-bio-mimetic hydrogels embedded with hBMSCs, as an alternative source of cells with high availability, by microfluidic-based coaxial printing. GelMA/CS AEMA/AHAMA, GelMA/CS AEMA, and GelMA were devised as bioinks with alginate as a fixed component used for instantaneous gelation. In terms of the compression modulus, they possessed values of 100.1 ± 10.2 , 59.7 ± 5.4 , and 48.1 ± 8.3 kPa, respectively. All of them displayed hBMSCs viability ($> 85\text{-}90\%$) and morphology maintenance. Besides, the synergistic effect of bio-mimetic matrix and chondrogenic media ultimately led to aggrecan and collagen type II expression. Conversely, collagen type X and type I expression were confined to three weeks. Alginate/GelMA/CS AEMA hydrogel was the best candidate for cartilage formation since it had the highest collagen type II/collagen type X and collagen type II/collagen type I ratios. Nonetheless, no fluorescence signal appeared in the intercellular space, which may be harmful for an engineered neo-cartilage's *in vitro* fabrication. To address this obstacle, future experiments should concentrate on enhancing cell density during bioprinting, via tailoring the hydrogels' diffusion and transport features, or via bioprinting a co-culture of chondrocytes and MSCs.

Another important subject in microfluidic constructs that should be meticulously examined is the porosity-related factors, namely pore size and porosity.^[269] Scaffold pore size is a determining parameter affecting the efficiency of cartilage regeneration.^[270–272] According to the previous studies, scaffolds with 150–500 μm pore size can considerably enhance chondrocytes' adhesion, proliferation, and ECM production^[125], with 150–250 μm being the optimum value.^[273] For instance, the pure bacterial cellulose's (pure BC) pore size is 0.05–10 μm , relatively smaller than the mammalian cells' dimensions. Therefore, the undesired pore size may hinder cells from migrating and penetrating

through the material. Yin et al. ^[274] tackled the aforementioned challenge by engineering agarose particle-templated bacterial cellulose hydrogel microparticles (pBC-M) via a microfluidic technique, and successfully creating a porous scaffold with a range of 300-500 μm pore size. As a result, the cellular features were significantly improved due to the appropriate pore size of the structures. Indeed, human P1 chondrocytes cultured on pBC-M showed higher viability (85-99 %) and proliferation after fourteen days of culture in comparison with those cultivated on the pure BC (**Figure 11.A(I, II)**). Cells invaded and dispersed throughout the pBC-M scaffolds, whereas on the pure BC substrates, they grew merely on the surface (**Figure 11.B**). In fact, they were not capable of penetrating into the pure BC scaffolds due to their pore size. Besides, cells illustrated a 3D distribution extending across the pBC-M scaffold while their distribution was restricted to the pure BC gel's top surface (**Figure 11.C**). One limitation of the presented evaluation was that the cell density reduced with increment of the pBC-M substrate depth, which can be overcome by optimizing the cell seeding procedure. Furthermore, the porosity of constructs has shown a significant impact on TE, and researchers have always sought to reach the optimum percentage suitable for each tissue formation.^[275] They have proposed brand-new materials and innovative techniques so as to obtain the proper total porosity for cells attachment and matrix deposition. In the study carried out by Jiménez et al. ^[276], the polyacrylate hydrogels prepared by a microfluidic approach had 91 % porosity with highly-interconnected pores, HACs successfully attached to the scaffolds after twenty-one days of culture. The generation of a hyaline-like ECM was observed in treated immunodeficient mice models. In this context, some scholars consider that the best porosity for chondrocytes' adhesion and ECM deposition should be higher than 90 % ^[124], and the experiment mentioned earlier could reach the optimum level.

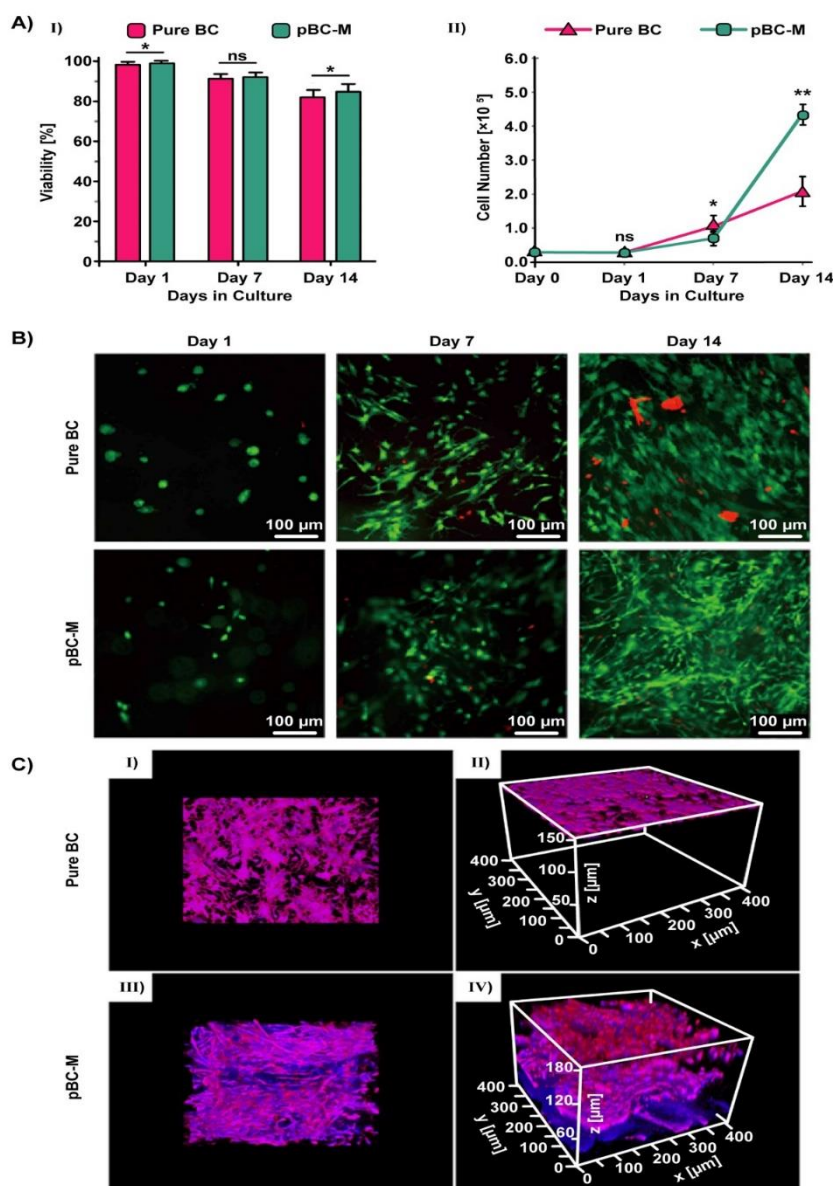


Figure 11. A) The assessment of cellular viability on the scaffolds: I) The viability graph of seeded cells in the pBC-M and the pure BC scaffolds within the cultivation periods of one, seven, and fourteen days. II) The cells' number after their proliferation on the pBC-M and the pure BC scaffolds following one, seven, and fourteen days of culture. B) The cells were stained for live & dead fluorescence labeling. C) The distribution of cells seeded on the pBC-M and the pure BC scaffolds after fourteen days: Fluorescence micrographs' I) top-down and II) oblique view of cells cultured on the pure BC substrates for fourteen days and fluorescence micrographs' III) top-down

and IV) oblique view of cells cultivated on the pBC-M scaffolds for fourteen days.

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Moreover, an essential factor illustrating an effect on cells' functions is the architecture of the networks within the hydrogel scaffolds.^[277] Regarding this subject, microfluidic networks are key parameters in microfluidic hydrogel-based scaffolds, enabling better mimicry of the tissue environment ^[278], and allowing nutrient delivery as well as waste disposal.^[279] In essence, the development of hydrogels embedded with microfluidic channels has troubleshooted the problem of poor mass transfer associated with traditional tissue-engineered constructs, resulting in insufficient mechanical functionality and large gradients in ECM deposition. In this context, a group of scholars ^[15] devised novel bovine articular chondrocyte-seeded agarose-based hydrogel containing microfluidic networks. It was shown that a microfluidic structure could be produced by casting the agarose/cell solution between the PDMS mold and the acrylic casing, permitting gelation at room temperature. Favorably, assays performed on a 5 mm microfluidic construct displayed that GAGs and collagen contents increased at the one-week culture period (nearly 2-and 4-fold increment than the control group, respectively). The cellular proliferation was enhanced owing to the microfluidic network presence. However, the newly produced GAGs were eventually lost after two weeks because of rather high velocities in the microfluidic channels. Besides, multiple fluidic networks' presence negatively affected the mechanical characteristics of the microfluidic construct (roughly 2-fold reduction in storage modulus compared to the control group). These phenomena were because of the diminished construct mass after two weeks. Indeed, this is a critical issue that can be solved if extra factors are manipulated, such as investigation of other material platforms, evaluation of micro-channeled structure's mechanics, and assessment of longer culture periods.

In TE, a pre-formed scaffold with seeded cells or an injectable mixture of cells and gelation precursor components can be effectively used.^[280] Injectable hydrogels, as bioactive molecules' and

cells' carriers, are attractive options thanks to offering several advantages: possessing the ability to be injected directly into the defect area in a non-invasive or minimally invasive manner, enabling the uniform incorporation of molecular signals and cells across the scaffold, and being capable of high targeting, to name a few.^[281,282] More importantly, traditional bulk hydrogels self-assemble through a top-down approach, making them difficult to apply in *in situ* TE via a minimally invasive manner. This constraint is addressed by employing injectable hydrogels due to their self-assembly into 3D scaffolds based upon the bottom-to-up strategy. Moreover, microfluidic technique is superior to other methods owing to its controllable and convenient micrometer-scale droplet production procedure resulting in the generation of monodisperse-sized hydrogels and its mild production conditions that retain the drug activity.^[281,282] Interestingly, Lin et al.^[283] manufactured injectable rabbit primary chondrocyte-laden chitosan/PEGDA hydrogel microspheres (CP-MS) for cartilage bioengineering applications. The water-in-oil technique followed by a cross-linking procedure in a capillary microfluidic chip was performed to fabricate cell-embedded hydrogels (**Figure 12.A**). During a seven-day culture period, cells cultivated on the scaffolds (550 ± 9 mm diameter) were all around these microspheres' 3D surface, had high viability (80 %) and proliferation rate that were similar to those of the cells cultured in the control group (tissue culture polystyrene) (**Figure 12.B**). When the cell-loaded CP-MS were injected into a mold capable of emulating a cartilage defect, no considerable decrease in cellular survival was observed after injection and also subsequent to seven days of culture due to the microspheres' protection. Following the one-week culture, bottom-to-up self-assembly occurred for the CP-MS in the mold (**Figure 12.C**), showing their potential to form a cartilage-like scaffold eventually. In another pioneering experiment, Li et al.^[284] fabricated injectable cell-laden gelatin microgels for cartilage repair. The hBMSCs were encapsulated in the gelatin microgels cross-linked with 4-arm poly(ethylene glycol)-*N*-hydroxysuccinimide through a microfluidic device. Based on multiple evaluations, it was concluded that the microgels were effectively fixed in an *in vitro* tissue-mimic adhesion model, were stable up to three weeks in *in vitro* culture, ensured high cellular viability (88 ± 5 %), guaranteed active migration of hBMSCs, led to enhanced GAGs production (140 % of the control) and increased aggrecan expression (> 100-fold), as well as greater collagen type II expression (150,000-fold increment) compared to the control bulk gels, indicating chondrogenic

differentiation and hyaline-like cartilage formation in the injectable microgels. Several matters should be taken into account before translating the presented system into the clinic: 1) designing *in vivo* experiments to specify the microgels' degradation rate with the presence of proteolytic enzymes and inflammatory cells, 2) assessing other factors serving roles in guiding chondrogenesis, and 3) evaluating the functionality of the reconstructed tissue by biomechanical testing. In a nutshell, these injectable cell-laden hydrogels with improved cellular functions can provide the ground for future *in vivo* applications.

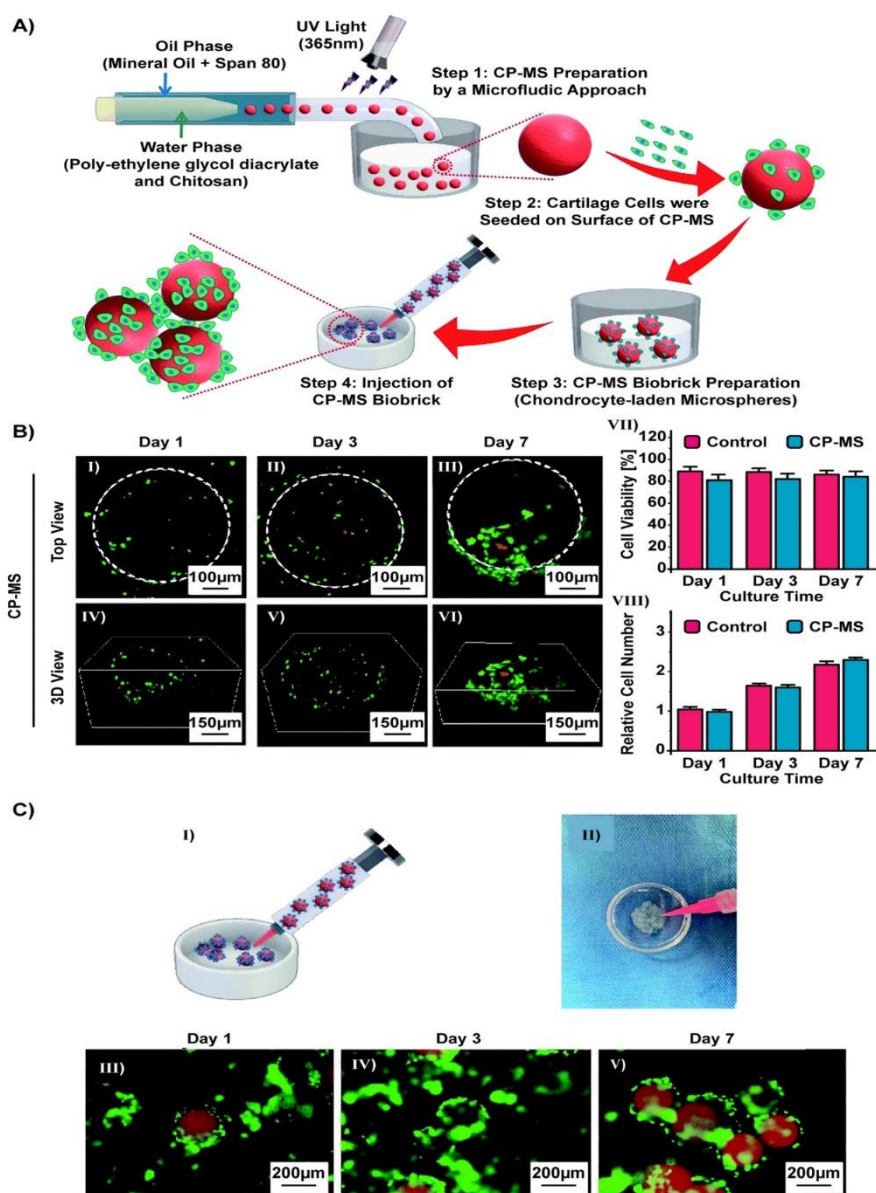


Figure 12. A) Scheme displaying the microsphere fabrication based upon the CP-MS's manufacture by a microfluidic core-shell chip, and preparation of the injectable cell-laden CP-MS scaffold by employing a bottom-to-up approach. B) The cellular survival and proliferation of the cell-laden CP-MS: the confocal images' (I–III) top view and (IV–VI) 3D view of cells cultivated on the CP-MS after one, three, and seven days. VII) The viability graph for control and CP-MS groups after culturing for one, three, and seven days. VIII) The cells' number in control and CP-MS groups after cultivation for one, three, and seven days. C) The cell-laden microspheres' injection and self-assembly in vitro: I) The injection procedure of cell-laden microspheres. II) The optical images of cell-laden CP-MS's injection into a petri dish and cell-laden microspheres' fluorescent images after

injection and cultivation for (III) one, (IV) three, and (V) seven days, where cells were labeled with calcium acetoxymethyl (green), and microspheres were labeled with Nile red dye (red). Reproduced under the terms of the Creative Commons Attribution-NonCommercial 3.0 Unported (CC BY-NC 3.0) license.^[283] Copyright 2020, Royal Society of Chemistry.

Other types of microfluidic hydrogel-based scaffolds include constructs with the ability for the controlled release of different materials. These advanced constructs are essentially engineered to release drugs or biological factors such as GFs over a specific amount of time for various aims.^[285] In this context, Witte and co-workers^[286] designed cell-laden alginate-fibronectin microgels, known as chondrobags, to promote hyaline cartilage regeneration by employing the microfluidic approach. The mixture of alginate with skeletal stem cells (SSCs)/HACs was combined with fibronectin, followed by the production of 3D microgels via a co-flow focusing device (**Figure 13.A**). As a consequence of loading the microgels with TGF- β_1 or TGF- β_3 and conducting multiple tests, improved HACs' numbers were seen in the chondrobags embedded with TGF- β_3 possessing an average of 11.8 cells cluster⁻¹ in comparison with 8.3 and 1.8 cells cluster⁻¹ observed in the TGF- β_1 -loaded and the control groups, respectively (**Figure 13.B(I)**). Besides, GF release experiments showed that microgels exhibited a 4-fold higher affinity for the TGF- β_1 compared to the TGF- β_3 . The up-regulated expression of Sox9 (4.1-fold change for TGF- β_1 and 3.6-fold change for TGF- β_3), aggrecan (8.5-fold change for TGF- β_1 and 8.1-fold change for TGF- β_3), as well as collagen type II (111.4-fold change for TGF- β_1 and 252.7-fold change for TGF- β_3) for HACs, along with HACs' phenotype maintenance, were confirmed in the hydrogels loaded with GFs. Besides, only the expression of Sox9 was highly up-regulated following the SSCs treatment with TGF- β_1 (4.7-fold change) and TGF- β_3 (4.6-fold change). On the one hand, expression of micro ribonucleic acid-140-3p (miR-140-3p), a chondrogenic regulator, was up-regulated (TGF- β_1 chondrobags: 61.8-and 1.7-fold change for HACs and SSCs, respectively, and TGF- β_3 chondrobags: 81.2-and 3.4-fold change for HACs and SSCs, respectively) (**Figure 13.B(II, III)**). On the other hand, the expression of micro ribonucleic acid -146b (miR-146b), a negative modulator of chondrogenesis, was down-regulated (TGF- β_1 chondrobags: 0.8-and 0.1-fold

change for HACs and SSCs, respectively, and TGF- β_3 chondrobags: 0.5-and 0.2-fold change for HACs and SSCs, respectively) (**Figure 13.B(IV, V)**) in the cell-laden scaffolds due to the GFs' addition. Eventually, histological analysis revealed that the chondrobags encapsulating cells in the presence of both GFs displayed enhanced cellular proliferation, ECM formation, nuclei distribution, and absence of bone production, with more intense Alcian blue staining for TGF- β_3 chondrobags compared to TGF- β_1 ones (**Figure 13.C**). Another comprehensive study conducted by Dehghan-Baniani et al. ^[287] focused on the preparation of a dual-function chitosan-based thermogel capable of co-delivering chondro-inductive and anti-inflammatory biomolecules. In that investigation, a hydrophobic chondro-inductive drug, named kartogenin (KGN), was loaded in the starch microspheres by a droplet-based microfluidic chip, and diclofenac sodium (DS), an anti-inflammatory drug, was embedded within halloysite nanotubes, followed by mixing these two with *N*-(β -maleimidopropoxy) succinimide ester-modified chitosan to obtain the desired hydrogel. Notably, in comparison with the conventional approaches, microfluidic approaches can cause uniform distribution of drugs in the particles, thereby leading to a diminished early burst release and a more sustained delivery of drugs over time. It was revealed that the KGN and DS were released continuously for over thirty and forty days, respectively, and both drug carriers were suitably dispersed in the hydrogel, indicating homogenous drug release. Besides, the developed hydrogel's proper time of gelation (5 ± 0.5 min) at 37 °C can enhance the clinical viability compared with the previously prepared hydrogels with gelation times of 10 to more than 60 min. The shear modulus of hydrogel was 167 ± 5 kPa, which was comparable to the amount reported for the AC (50-250 kPa). Furthermore, the fabricated hydrogel had no detrimental impact on the human adipose-derived MSCs. After twenty-one days, the expressions of Sox9, collagen type II-alpha I, and aggrecan were up-regulated in the hydrogel compared to the control group, and GAGs DNA⁻¹ content enhanced up to 2 times when cells were cultivated on the hydrogel in comparison with the control group. Although the fabricated chitosan-based hydrogel's chondro-inductivity and ability to release biological cues for a long-term were proved in that experiment, further evaluations are required in order to also verify its anti-inflammatory action *in vivo*.

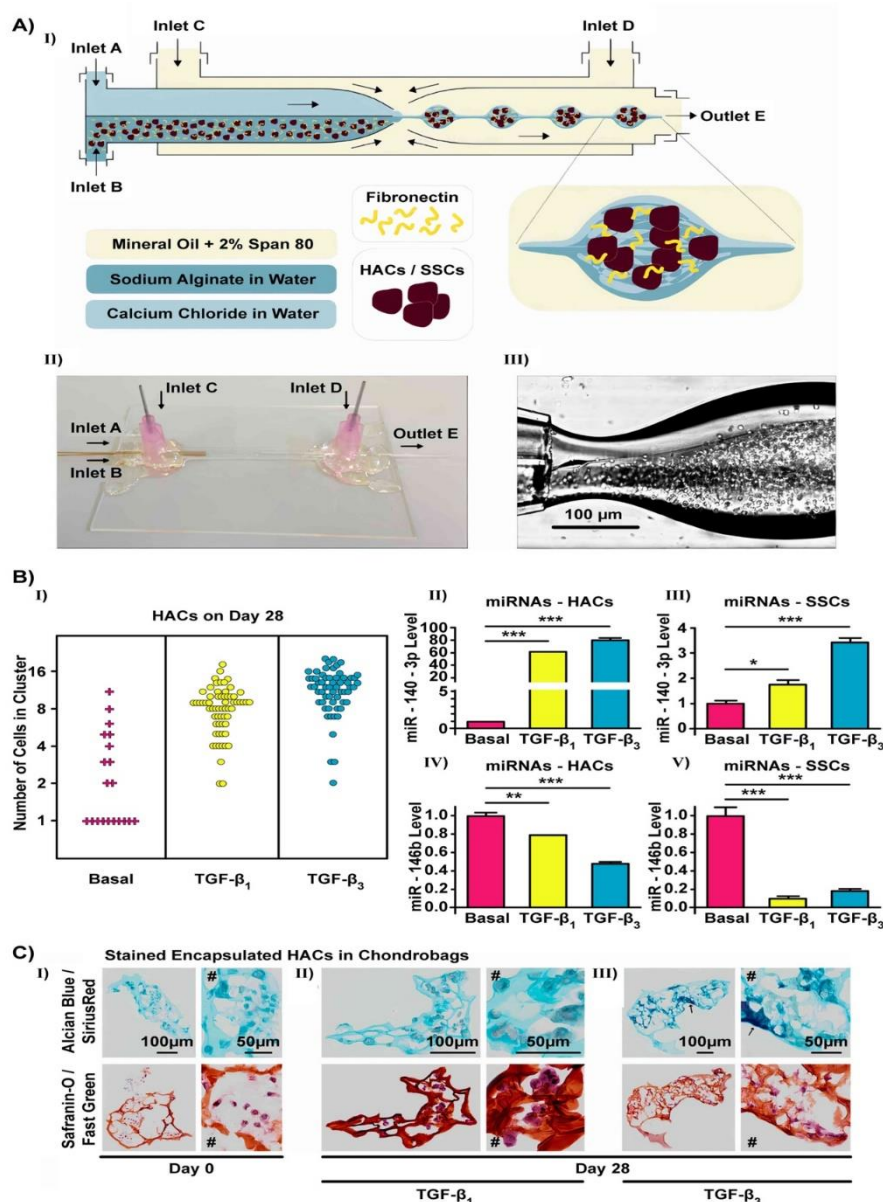


Figure 13. A) The setup of the droplet-based microfluidic device, encapsulation process, and mono-dispersity: I) Schematic display of the cells' encapsulation and the microfluidic device. II) A figure showing the microfluidic capillary-based instrument. III) A snapshot exhibiting cells' encapsulation employing the microfluidic device. B) I) The graph with cells' numbers within the clusters comprising HACs after twenty-eight days. II, III) The miR-140-3p expressions in HACs and SSCs, respectively and IV, V) The miR-146b expressions in HACs and SSCs, respectively, with all of them cultivated in the TGF- β_1 or TGF- β_3 presence for twenty-eight days compared to the untreated control group (* p < 0.05, ** p < 0.01, *** p < 0.001). C) The stained encapsulated HACs within

chondrobags. Histological sections of cell-embedded chondrobags: I) Basal culture was employed for day zero. II) Cells were cultivated in the TGF- β_1 presence (Figure depicting day twenty-eight). III) Cells were cultivated in the TGF- β_3 presence (Figure depicting day twenty-eight). # represents the larger magnification of the respective images (right images). Furthermore, arrows point to the darker Alcian blue staining in comparison with the lighter staining in the background. Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[286] Copyright 2020, IOP Publishing.

5.2. Clinical evaluations

In order to benefit from the many advantages microfluidic technology offers for drug delivery, such as enhanced pharmacokinetics and improved therapeutic outcome,^[288] an innovative study focused on embedding KGN into microfluidic GelMA/liposome hybrid microgels for the treatment of OA. In that experiment, the drug was loaded in the liposomes, followed by the generation of liposomes-in-GelMA-in-oil emulsions by a microfluidic device and the cross-linking process to finally obtain microgels. Since liposomes were anchored well in the microgels via non-covalent interaction and physical network hindrance, the sustained and long-term release of KGN within the degradation period (more than three weeks) was achieved. Nevertheless, the drug encapsulation efficiency was lower in the hybrid microgels (43.8 ± 16.6 %) as compared to the liposomes (83.5 ± 8.1 %) owing to the drug leakage happening in the oil-removing step of the manufacture and small amounts of liposomes present on the outer layer that diffused out during this step. BMSCs-seeded microgels could highly express collagen type II-alpha I, aggrecan, and Sox9. Moreover, the complex could successfully promote the differentiation of stem cells into chondrocytes. The therapeutic effect of the drug-loaded structure was also evaluated in the rat OA model. The results demonstrated the 72 % reduction of total osteophyte volume, the decreased depth of cartilage erosion (75 % reduction), and the enhanced expression levels of GAGs, collagen type II, and aggrecan as compared to the

phosphate buffer saline (PBS) group, thus indicating the suitability of the proposed microgels for the OA treatment.^[289]

A great deal of progress has been achieved when employing microfluidic hydrogel-based scaffolds as bioactive molecules' carriers to treat and regenerate cartilage defects by co-delivery of cells and these molecules or cell-free co-delivery of components, both *in vitro* and *in vivo*.^[290] In the context of using co-delivery systems and MSCs-mediated treatments, dental MSCs-laden alginate hydrogels as co-delivery systems were examined. In the manufacturing process, a two-channel microfluidic device was used to prepare arginylglycylaspartic acid-coupled TGF- β_1 -loaded alginate hydrogel microspheres embedded with periodontal ligament stem cells (PDLSCs) or gingival MSCs (GMSCs). Furthermore, alginate hydrogels encapsulating hBMSCs and alginate hydrogels without cells were utilized as the positive and negative controls, respectively. An sustained release of TGF- β_1 was observed for fourteen days. The hBMSCs showed high cellular viability after two weeks of cultivation (nearly 80 %), and collagen type II, as well as Sox9, were highly expressed after two weeks, with hBMSCs and GMSCs displaying lower expression levels of these genes as compared to the PDLSCs. For *in vivo* studies, cell-laden scaffolds were implanted subcutaneously into the Beige nude XID III (NU/NU) mice's dorsal surface. The results revealed the differentiation of all cells into round-shaped chondrocytes as well as chondrogenic tissues formation. Contrarily, the negative control failed to display any positive signs for chondrogenesis. Moreover, basic fibroblast GF (bFGF) and bone morphogenetic protein-4 GFs were observed in the early stages of chondrogenic differentiation, proposing the TGF- β_1 's essential role within chondrogenesis. As a result, a suitable micro-environment and the presentation of signaling biomolecules, namely TGF- β_1 , are fundamental prerequisites for the viability and chondrogenic differentiation of the stem cells in an alginate-based microencapsulation system. Future experiments should investigate the possibility of employing GMSCs and PDLSCs in the regeneration of cartilage as well as disk defects in temporomandibular joint within more suitable animal models.^[291] Another pioneering investigation evaluated the synergistic effect of stromal cell-derived factor-1 (SDF-1), a chemokine, and KGN for healing AC defects. The PLGA microspheres encapsulated with SDF-1 and KGN were manufactured via the

capillary microfluidic device, followed by incorporating them into the HA injectable hydrogel. In vitro tests indicated the sustained release profiles of two model drugs representing SDF-1 and KGN for more than two months. The benefit of this co-encapsulation was that with the SDF-1 release, BMSCs could be recruited from the marrow clot generated during the microfracture process to the region where the hydrogels were located, and this site was also able to release KGN concomitantly, which was capable of inducing chondrogenic differentiation of the homed BMSCs. Thereafter, the hydrogels were injected into the rabbit models possessing full-thickness cartilage defects, and at the eight-week evaluation, cartilage-like tissue with cell clusters and lacunas was formed and integrated well with its nearby cartilage and subchondral bone. Nonetheless, minor defects were still observed. At the twelve-week assessment, neo-cartilage was more mature, and its thickness was more even than before, demonstrating the effectiveness of the proposed cell-free co-delivery hydrogel systems for the AC defects' repair. More importantly, extensive *ex vivo* cell culture and extra *in vitro* chondrogenesis step were avoided in this innovative experiment. Of note, important issues should be addressed in the upcoming evaluations. First, a longer follow-up is still required to ascertain that the neo-tissue will not experience fibrous degradation, as it is typically the case with some ongoing clinical treatments, namely microfracture. Secondly, additional confirmation regarding the neo-tissue's resemblance to the normal AC tissue's histology is needed, which can be of enormous help to detect the homed cells, track their origin, and study the quantitative correlation between KGN, SDF-1, and cell status.^[292]

In the stimuli-responsive hydrogels serving as powerful delivery matrices for drugs, the drugs' release can be modulated via biological stimuli like temperature or external triggers.^[293] Employing these structures, Yang et al.^[294] designed temperature-responsive, hydrogel-based injectable microcarriers loaded with DS for the OA treatment. The fabrication process included several steps: 1) Utilizing a microfluidic device to create silica-based beads for the pre-gel solutions' infiltration, 2) UV-triggered cross-linking of PNIPAAm pre-gel solution that incorporated the silica-based beads and achieving opal particles, and 3) Encapsulating DS-doped AHAMA pre-gel solution into the particles' nanopores. Simulation experiments revealed that when the temperature was raised to 39 °C analogous to the temperature of the joint cavity during OA, the loaded drug could be released due to the

temperature sensitiveness and the resultant shrinkage of PNIPAAm hydrogel particles. Conversely, the microcarriers' drug-release switches could be locked once the inflammation was diminished, leading to the prevention of drug waste and improvement in the OA treatment's efficiency. Besides, the employed drug had no toxic effects on cultured HACs after three days. Eventually, OA was induced in the Sprague-Dawley rats by destabilization of their medial meniscus, and after injecting hydrogels into these models, results after six weeks showed improved synovial hyperplasia, enhanced amount of synovial fluid, increased collagen type II content (5-fold change), and decreased expression of tumor necrosis factor- α (TNF- α), an OA-related proinflammatory cytokine (1.5-fold change) as compared with the control group, illustrating the potential application of these scaffolds in the OA therapies.

Combining 3D printing with microfluidic technology, Idaszek et al. ^[18] devised bioprinted cell-laden microfluidic hydrogel-based scaffolds made up of two sets of bioinks via an extrusion printer with a microfluidic printing head, that could mimic the ECM of AC (hyaline and calcified zones). The bioinks' compositions were as follows: 1) 4 % w v⁻¹ alginate + 6 % w v⁻¹ GelMA + 4 % w v⁻¹ CS AEMA loaded with 10×10^6 cell mL⁻¹ hBMSCs (coded as HC1), 2) the same composition as the previous one except the bioink was encapsulated with 7.5×10^6 cell mL⁻¹ hBMSCs and 2.5×10^6 cell mL⁻¹ HACs (coded as HC2), and 3) 4 % w v⁻¹ alginate + 6 % w v⁻¹ GelMA + 4 % w v⁻¹ CS AEMA + 0.5 % w v⁻¹ AHAMA + 0.5 % w v⁻¹ β -tricalcium phosphate (β -TCP) loaded with 10×10^6 cell mL⁻¹ hBMSCs (coded as CC). Indeed, the first two bioinks were fabricated for the hyaline cartilage, and the third one was designed for the calcified cartilage, all of which demonstrated high shape fidelity and open porosity. Mixing index, an essential factor for microfluidic mixers, was evaluated and proved to be lower in comparison with other investigations, illustrating the restricted mixing efficiency of the proposed microfluidic system. In the case of cellular viability, the HC1, HC2, and CC bioinks had great cell survival (92 ± 5 %, 92 ± 2 %, and 88 ± 4 %, respectively) after extrusion, leading to the conclusion that the shear stress created in the microfluidic channels during the bioinks' flow could not exert damaging effects on cellular viability. After two weeks of culture, there were no important differences between the three bioinks and all still had high cellular viability. Further experiments

confirmed that CC bioink possessed the highest expressions of the assessed genes, including collagen type II- α I, aggrecan, collagen type I- α I, collagen type X- α I, and alkaline phosphatase (ALP). Besides, the HC2 bioink yielded more hyaline phenotype than the other two, and interestingly, the introduction of β -TCP and AHAMA into the CC bioink promoted the hypertrophic chondrocytes' development by up-regulating the expressions of collagen type X- α I and ALP. Twelve weeks after hydrogels' implantation in the male Wistar rats with surgically damaged trochlea, differentiating cartilaginous cells were seen in rats' trochlear groove, the region of implantation, which were absent within the control group. Moreover, collagen type II and tenascin, potential cartilage regeneration markers, were present in the treated rats; therefore, these novel bioinks printed via a microfluidic printing head can enable the heterogeneous tissue's fabrication with high cell survival and resolution.

Traditional hydrogels comprising a single polymer network typically possess poor mechanical characteristics that are inferior to those of the normal cartilage tissue. In contrast, double-network (DN) hydrogels, consisting of two intertwined cross-linked networks, have been developed as smart biomaterials and carriers with tunable and superior mechanical features, thus revealing great promise in the hydrogels' design for the cartilage regeneration.^[295,296] Interestingly, the use of microfluidic alginate-polyacrylamide DN hydrogels embedded with TGF- β_3 -loaded PLGA NPs for the cartilage repair was reported by a team of researchers.^[297] The loaded NPs' size was 126 ± 4.52 nm with polydispersity index of 0.137, indicating that the microfluidic approach can enable the manufacture of small NPs with narrow size distribution. The sustained release of the GF (encapsulation efficiency: 77.7 %) over sixty days and high compression modulus (59.79 ± 1.58 kPa) of the hydrogels were the main interesting results. Concerning cell viability, both L929 fibroblasts and differentiated chondrogenic cell line derived from mouse teratocarcinoma cells displayed good survival and high proliferation after three days of cultivation. *In vivo* experiments involved generating cylindrical cartilage defects in the trochlear groove's center of male Sprague-Dawley rats and implanting the hydrogels to indicate their impact on the repair process. A new smooth tissue surface with a hyaline matrix combined with fibrocartilage and fibrous tissue was observed, twelve weeks post-surgery. By its turn, the control group had disorganized fibrous neo-tissue with an irregular surface. Moreover,

several tests proved the enhanced cartilage repair and the generation of chondrocyte clusters with columnar structure as well as thicker neo-cartilage in the treated rats in comparison with the control group. These *in vivo* outcomes could demonstrated that there was consistency between mechanically enhanced hydrogels' degradation profile and cartilage repair, thus suggesting the promise of the novel DN hydrogels for finding applications in cartilage TE.

Improving joint lubrication along with anti-inflammatory treatment^[298] can be considered an innovative and efficacious approach for treating OA with decreased lubrication as one of its main features and the resultant joint inflammation.^[299,300] In this regard, hydrogel microspheres are capable of resembling ball bearings to diminish friction at the sliding interfaces by a rolling mechanism, turning these structures into ideal candidates for biolubricants.^[301] Concerning these matters, ball-bearing-inspired DS-loaded injectable hydrogel microspheres named DS-loaded poly (dopamine methacrylamide-to-sulfobetaine methacrylate)-grafted microfluidic GelMA spheres (MGS@DMA-SBMA@DS) were prepared by means of employing the microfluidic technology to treat the progressive OA. This experiment confirmed an excellent lubricating effect owing to the firm hydration layer production, and the sustained drug release. Culturing microspheres with rat chondrocytes and examining them revealed good cell viability (nearly 3.75×10^4 viable cells) and anti-inflammatory effect of the complex due to the DS presence. Indeed, this phenomenon may have emerged because of the complex that shielded the cells from inflammatory factor-induced degeneration via the catabolic proteases' down-regulation (thrombospondin type 1 motif 5 (ADAMTS5) and matrix metalloproteinase-13 (MMP-13)) and up-regulation of the anabolic molecules (collagen type II and aggrecan). Additionally, the hydrogel could alleviate the patients' pain suffering from OA since it diminished tachykinin-1 expression. The microspheres' therapeutic effect was also investigated by intra-articularly injecting the structures into the rat OA models. Decreased total osteophyte volume (53 % reduction) and increased expression of collagen type II (128 % increment) were observed *in vivo*. Furthermore, there was no sign of acute inflammation in the rats at one- and eight-weeks post-surgery. In addition, an increased joint space widths compared to the control group were ultimately observed. Thus, the ball-bearing-inspired hydrogel microspheres could

positively affect OA in two ways: 1) improved lubrication related to microfluidic GelMA spheres coated with poly (dopamine methacrylamide-to-sulfobetaine methacrylate), and 2) the anti-inflammatory effect of the employed DS, which was desirable for the OA therapy.^[302] In a recent study, DS-loaded injectable hydrogel microspheres titled DS-loaded poly (dopamine methacrylamide-to-2-methacryloxyethyl phosphorylcholine)-coated microfluidic GelMA spheres (GelMA@DMA-MPC@DS) were fabricated by Han and a group of researchers.^[303] It was revealed that the copolymeric coating, with hydration lubrication, enhanced the lubrication ability by reducing the coefficient of friction from almost 0.026 in GelMA to 0.019 in poly (dopamine methacrylamide-to-2-methacryloxyethyl phosphorylcholine)-coated microfluidic GelMA spheres (GelMA@DMA-MPS), and also enabled the sustained drug release via controlling the burst release (66 % in GelMA versus 12-20 % in GelMA@DMA-MPS). Furthermore, the generated microspheres exerted no detrimental impact on the rat chondrocytes at different time points (**Figure 14.A**). These cells were treated with interleukin-1 β (IL-1 β) (5 nM) for twelve hours to induce their degeneration, and afterwards, the expressions of several OA-related genes were measured in several groups. The GelMA@DMA-MPC@DS group displayed a drastic increase in aggrecan and collagen type II-alpha I expression levels in comparison with the blank group (the induced cells without microspheres), but there was a limited impact for the GelMA@DMA-MPC and GelMA groups (**Figure 14.B(I, II)**). This result suggest that GelMA@DMA-MPC@DS could relatively protect the rat chondrocytes. In the case of MMP-13 expression, although there were no considerable alterations for GelMA@DMA-MPC and GelMA groups as compared to the blank group, the GelMA@DMA-MPC@DS group illustrated significant MMP-13 down-regulation (**Figure 14.B(III)**). This result suggest that the complex's can have an anti-inflammatory effect. Ultimately, the rat OA models received intra-articular injections of the mentioned compositions for a total of eight weeks, and the increment of articular space width in GelMA@DMA-MPC@DS group compared to the other compositions and PBS groups was clearly evident. Further staining's showed that the PBS group had OA common features, and the GelMA group exhibited no remarkable difference from the PBS one. Nonetheless, GelMA@DMA-MPC and GelMA@DMA-MPC@DS groups demonstrated considerable enhancement in tidemark integrity, tissue cloning, and morphology change, with the second group

having the most intense positive staining (**Figure 14.C**). The best GAGs content and the least depth of lesion belonged to the GelMA@DMA-MPC@DS group, with a 50.5 % increase and 34.9 % decrease compared to the PBS group, respectively. Therefore, considering all of the outcomes, the designed DS-loaded hydrogel microspheres could inhibit the OA progression at the highest level via employing the synergistical effect of the sustained drug release and improved lubrication.

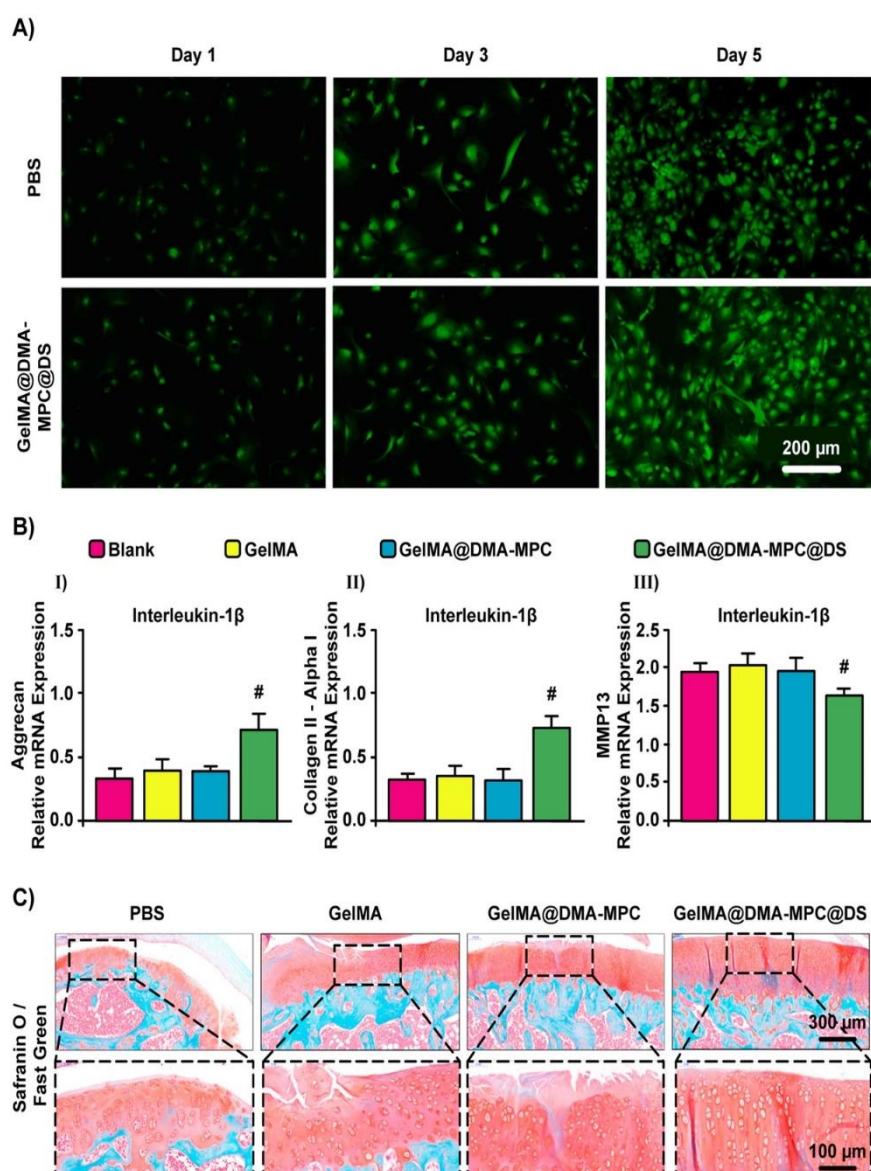


Figure 14. A) The fluorescence images illustrating the survival of cells that were co-cultured with GelMA@DMA-MPC@DS hydrogel microspheres in comparison with the PBS group for one, three,

and five days. B) The relative messenger ribonucleic acid expressions of: I) aggrecan; II) collagen type II- α I; and III) MMP-13 in the IL-1 β treated cells that were cultivated with the GelMA@DMA-MPC@DS, GelMA@DMA-MPC, and GelMA microspheres for twenty-four hours (# presents $p < 0.05$ compared with the blank group). C) The histological staining analysis revealing the histological alterations in the cartilage section's different degrees for various rat groups at eight weeks post-surgery. Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[303] Copyright 2021, Elsevier.

Enhancing biolubrication while preserving cellular homeostasis is another effective method that can be considered to treat OA disease.^[304] In this context, Lei et al.^[305] engineered injectable liposome-incorporating hydrogel microspheres with the capability of forming self-renewable hydration layers by means of employing the microfluidic technology to both maintain the cellular homeostasis and address the bottleneck of damaged hydration layers on hydrogel microspheres lacking the ability of self-renewal, with the purpose of delaying the OA progression. Briefly, cationic liposomes loaded with rapamycin, an autophagy activator, were incorporated in the HAMA matrix by a flow-focusing microfluidic device, and the resultant hydrogel microspheres were further investigated via various experiments. At first, the cartilage-targeting ability of liposomes was confirmed since the cationic liposomes could target the negatively-charged GAGs chains within the C-28/I2, an immortalized human chondrocyte cell line. It was revealed that the hydrogel microspheres could expose the liposomes on their outer surface, leading to the generation of hydration layers with self-renewing capability in response to the frictional wear. Nevertheless, the drug encapsulation efficiency was lower in the loaded hydrogel microspheres ($67.39 \pm 4.06\%$) as compared to the liposomes ($87.55 \pm 6.49\%$) owing to the drug leakage happening in the oil-removing step of the manufacture and exposed liposomes on the outer layer that diffused out during this step. Furthermore, rapamycin had a more prolonged release from the liposome-loaded hydrogel microspheres than the sole liposomes (28 days versus 14 days) (**Figure 15.A(I)**). The biocompatibility of the biolubricant was tested in a three-day cultivation period, demonstrating that the number of cells enhanced over

time in each group (control, liposome-loaded microspheres, and rapamycin/liposome-loaded microspheres) without noticeable differences between them and approximately all of the cells survived (**Figure 15.A(II, III)**). The accumulation of reactive oxygen species (ROS) occurs in OA, and autophagy can preserve cellular homeostasis via diminishing ROS generation; thus, in the next step, the ROS level was analyzed, and the intercellular ROS content and apoptotic cells' number were considerably lower in the rapamycin/liposome-loaded microspheres in comparison with the other groups (**Figure 15.A(IV, V)**), indicating the ability of the proposed hydrogel microspheres in reducing ROS generation and inhibiting apoptosis via delivering rapamycin. Eventually, the rat OA models received intra-articular injections of drug-free and drug-loaded microspheres, and as a result, multiple outcomes were achieved: 1) The PBS group had ~ 27.7 % greater osteophyte volume compared to the rapamycin/liposome-loaded microspheres (**Figure 15.B(I)**), indicating the fabricated microspheres' ability to alleviate the joint injury, 2) The joint space width was significantly increased in the rapamycin/liposome-loaded microspheres in comparison with the other groups (**Figure 15.B(II, III)**), illustrating that the produced hydrogel microspheres could effectively act as a biolubricant, and 3) The PBS group had disorganized chondrocytes as well as severe surface abrasion, and the liposome-loaded microspheres group showed relative integrity and regular AC structure compared to the PBS group, whereas no observable degenerative alterations were detected in the rapamycin/liposome-loaded microspheres group (**Figure 15.C**). Hence, the designed novel injectable hydrogel microspheres could provide effective lubrication by forming hydration layers with self-renewal features and simultaneously preserving chondrocytes' homeostasis.

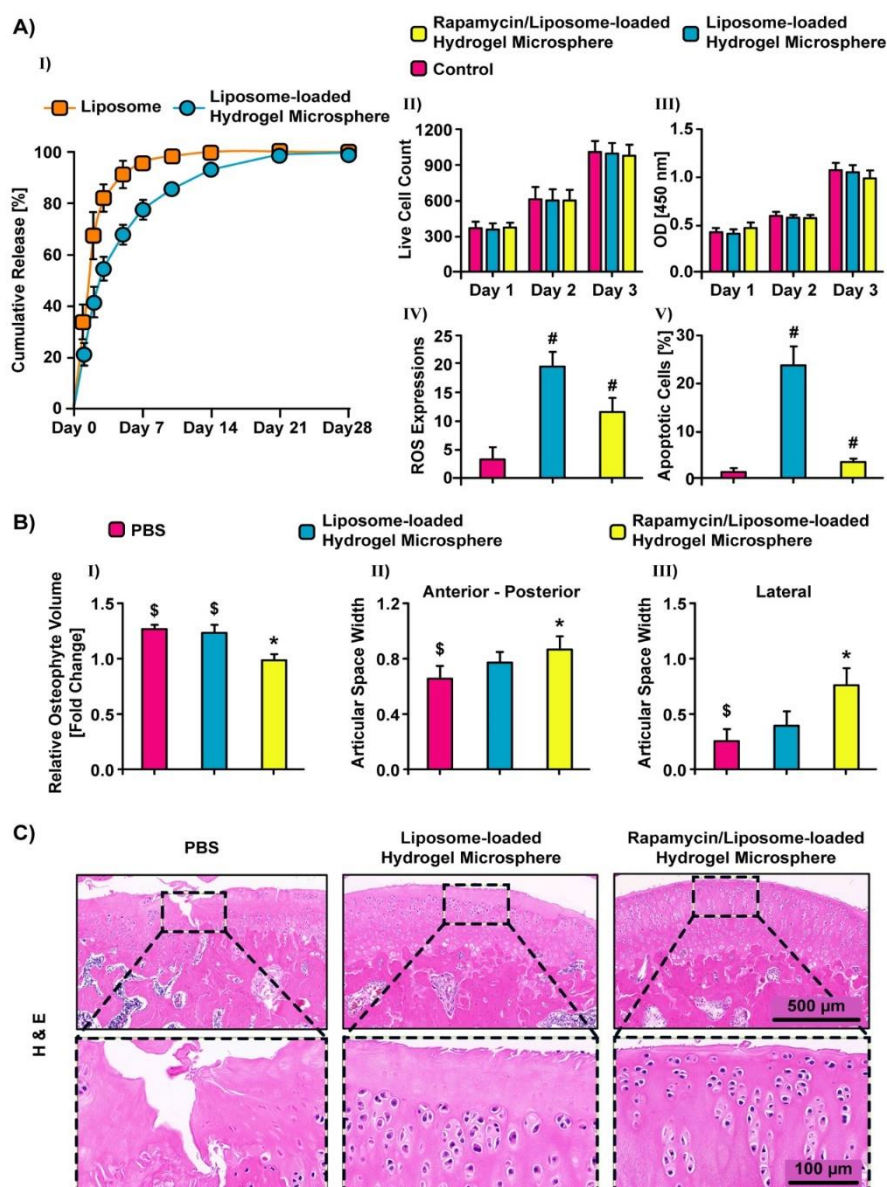


Figure 15. A) I) The release curves of rapamycin which was released from the liposome-loaded hydrogel microspheres and sole liposomes. II) The live cell count achieved from the live & dead staining analysis. III) The cell counting kit-8 assay outcomes on one, two, and three days. IV) The ROS expressions' quantitative analysis based upon the dichlorofluorescein fluorescence intensity. V) The apoptotic cell rate's quantitative analysis based upon the TUNEL assay (# indicates $p < 0.05$ compared to the control group). B) I) The osteophyte volume calculated from the micro-computed tomography images. II) The widths of joint space that were calculated from the anterior-posterior images. III) The widths of joint space that were calculated from the lateral images (\$ and * indicate $p < 0.05$ compared to the rapamycin/liposome-loaded hydrogel microspheres and PBS groups,

respectively). C) The representative figures of hematoxylin and eosin staining from each group.

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Recently, gene therapy has come to the spotlight as a novel approach for treating OA via delivering micro ribonucleic acids (miRNAs).^[306] It has been reported that micro ribonucleic acid-140 (miR-140), which serves an essential role in chondrocytes' homeostasis and development, can retard the degeneration of cartilage and alleviate the OA.^[307,308] Common systemic delivery methods employed for gene therapy possess several disadvantages, such as the risk of off-target effects, and the inability to sustain an adequate therapeutic effect.^[309] Thus, the local delivery approach has grabbed much attention. In this context, microfluidic hydrogel microspheres with the benefits of excellent injectability, decreased possible side effects, and enhanced transfection efficiency are being used nowadays.^[310–312] In a recent investigation, Li et al.^[313] fabricated innovative injectable microfluidic hydrogel microspheres with the combined nano-micron structure that embedded miR-140-loaded NPs. Succinctly, miR-140s were loaded into G5-polyamidoamine dendrimers modified with arginine, histidine, and phenylalanine-modified generation 5 polyamidoamine (G5-AHPs), and then the G5-AHP/miR-140 NPs were incorporated in the lyophilized GelMA hydrogel microspheres prepared previously by a microfluidic flow-focusing device (**Figure 16.A**). Primary mouse chondrocytes treated with hydrogel microspheres were nearly all alive and had increased cell density after five days. To test whether the gene cargo's release rate could be regulated via MMP activity at OA's various phases, microspheres were incubated with 10 ng mL⁻¹ MMP solution; thereafter, it was revealed that the miR-140 was almost entirely released after five days and GelMA microspheres disappeared because their cargos were lost on the seventh day. Subsequently, the complexes were administered by intra-articular injection to the mice that underwent destabilized medial meniscus surgery. Osteophyte formation, wider articular space, excellent morphological integrity, improved tissue cloning, and diminished surface denudation were observed twelve weeks after surgery in the treated group. By its turn, the PBS group possess OA's common structural properties such as denudation erosion,

deformities, and surface discontinuity (**Figure 16.B**). Furthermore, reduced depth of cartilage lesions (48.2 % decrease) and higher collagen type II content were obtained in the treated mice in comparison with the PBS group. Consequently, the engineered gene-hydrogel microspheres delaying the OA progression may be used as an innovative regional gene therapy strategy, in a near future.

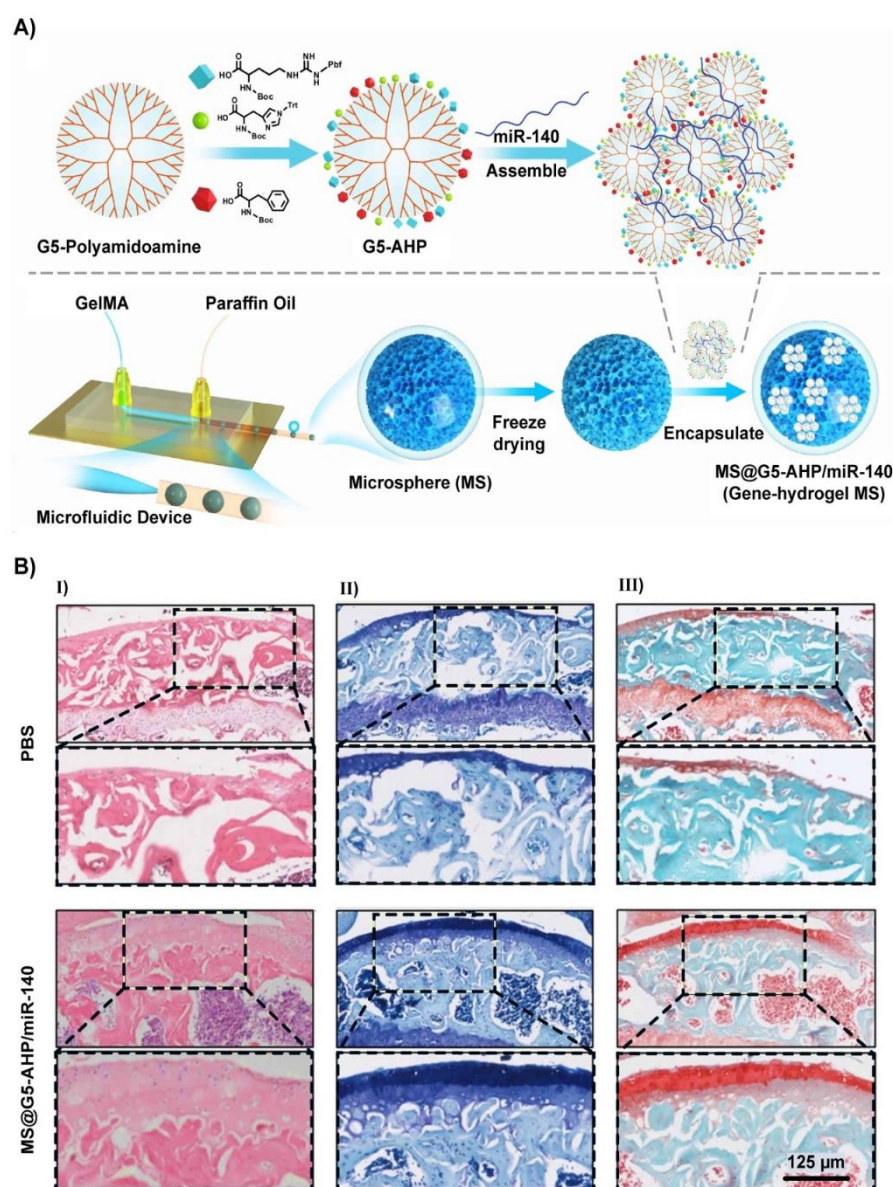


Figure 16. A) Schematic representation of the hydrogel microspheres' fabrication process. B) The cartilage sections' representative: I) Hematoxylin and eosin; II) Toluidine blue, and III) Safranin-O stainings after the treatment of OA employing PBS and gene-hydrogel microspheres at twelve weeks

post-surgery. Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[313] Copyright 2022, Springer Nature.

Table 5 summarizes microfluidic hydrogel-based scaffolds' preclinical and clinical applications in cartilage repair. The simultaneous utilization of hydrogels and microfluidics has assisted scientists in fulfilling fundamental biological requirements in the ever-growing field of TE.^[137] Since the introduction of this unique combination, many preclinical and clinical trials have taken place in order to explore its properties and applications for cartilage bioengineering. Although microfluidic hydrogel-based scaffolds have proven successful in various experiments, some key challenges should still be overcome in order to effectively employ them in the human body. The original research projects mentioned above, alongside the accomplished results, will hopefully provide a comprehensive roadmap to move the field forward.

Table 5. Summary of preclinical and clinical applications of microfluidic hydrogel-based scaffolds in cartilage repair.

Scaffold composition	Cell source/Animal model	Results	Ref.
ECM-bio-mimetic hydrogel bioinks:	hBMSCs	-Achieved compression moduli in order: 100.1 ± 10.2 , 59.7 ± 5.4 , and 48.1 ± 8.3 kPa	[210]
1) Alginate/GelMA/CS AEMA/AHAMA		-High hBMSCs viability (> 85-90 %) and morphology maintenance in all of the bioinks	
2) Alginate/GelMA/CS AEMA		-Expressions of aggrecan and collagen type II due to the synergistic effect of the bio-mimetic matrix and the chondrogenic media	
		-Confined expressions of collagen type X and type I with a three-	

3) Alginate/GelMA		<p>week duration</p> <p>-The highest collagen type II collagen type X⁻¹ and collagen type II collagen type I⁻¹ ratios in Alginate/GelMA/CS AEMA hydrogel bioink, making it the best candidate for the cartilage formation</p> <p>-Appearance of no fluorescence signal in the intercellular space</p>	
Agarose pBC-M	Human P1 chondrocytes	<p>-Production of a porous scaffold with 300-500 µm pore size</p> <p>-Enhanced cellular viability (85-99 %) and proliferation after a fourteen-day culture in the pBC-M scaffold compared to the pure BC scaffold</p> <p>-Invasion and dispersion of cells throughout the pBC-M scaffold and the mere growth of cells on the surface of the pure BC substrate</p> <p>-Cells' 3D distribution extending across the pBC-M scaffold and their restricted distribution on the pure BC gel's top surface</p> <p>-Reduction of the cell density with increment of the pBC-M substrate depth</p>	[274]
Polyacrylate hydrogel	HACs/ Immunodeficient mice models	<p>-Production of a scaffold possessing 91 % porosity with highly-inter-connected pores</p> <p>-Successful attachment of HACs to the scaffold after twenty-one days of cultivation</p> <p>-Observation of generated hyaline-like ECM in treated animal models</p>	[276]
Cell-seeded agarose-based hydrogel	Bovine articular chondrocytes	<p>-Increment of GAGs and collagen contents at the one-week duration (nearly 2-and 4-fold increase than the control group, respectively)</p>	[15]

		<p>-Enhancement of cellular proliferation due to the microfluidic network presence</p> <p>-Loss of newly produced GAGs after two weeks because of rather high velocities in the microfluidic channels</p> <p>-Negative effect of multiple fluidic networks' presence on the mechanical characteristics of the microfluidic construct (roughly 2-fold reduction in storage modulus as compared to the control group) owing to the diminished construct mass after two weeks</p>	
Injectable cell-laden CP-MS	Rabbit primary chondrocytes	<p>-Microspheres' 3D surface filled with cells</p> <p>-High cellular viability (80 %) and proliferation during a one-week culture period, similar to those of the cells cultured in the control group</p> <p>-No considerable decrease in cell survival after the injection of cell-loaded CP-MS into a mold imitating cartilage defect due to the microspheres' protection (immediately after injection and also after seven days of culture)</p> <p>-Microspheres bottom-to-up self-assembly in the mold after one week resulting in the cartilage-like scaffold formation</p>	[283]
Injectable cell-laden gelatin microgel	hBMSCs	<p>-Effective fixation of microgels in an in vitro tissue-mimic adhesion model</p> <p>-Stability of microgels up to three weeks in vitro culture</p> <p>-High cellular viability (88 ± 5 %) and active migration of cells</p> <p>-Enhanced GAGs production (140 % of the control), increased aggrecan expression (> 100-fold), and superior collagen type II expression (150,000-fold increment) as compared to the control</p>	[284]

		bulk gel, indicating chondrogenic differentiation and hyaline-like cartilage formation	
Cell-laden alginate-fibronectin microgel loaded with TGF- β_1 /TGF- β_3	SSCs and HACs	<p>-Improved HACs' numbers in the chondrobags embedded with TGF-β_3 possessing an average of 11.8 cells cluster⁻¹ in comparison with 8.3 and 1.8 cells cluster⁻¹ in the TGF-β_1-loaded and the control groups</p> <p>-The 4-fold increased affinity for the TGF-β_1 as compared to the TGF-β_3 displayed by the microgels</p> <p>-Up-regulated expression of Sox9 (4.1-fold change for TGF-β_1 and 3.6-fold change for TGF-β_3), aggrecan (8.5-fold change for TGF-β_1 and 8.1-fold change for TGF-β_3), as well as collagen type II (111.4-fold change for TGF-β_1 and 252.7-fold change for TGF-β_3) for HACs, along with HACs' phenotype maintenance, in the hydrogels loaded with GFs</p> <p>-Highly up-regulated expression of Sox9 following the SSCs treatment with TGF-β_1 (4.7-fold change) and TGF-β_3 (4.6-fold change)</p> <p>-Up-regulation of miR-140-3p expression (TGF-β_1 chondrobags: 61.8-and 1.7-fold change for HACs and SSCs, respectively and TGF-β_3 chondrobags: 81.2-and 3.4-fold change for HACs and SSCs, respectively) and down-regulation of miR-146b expression (TGF-β_1 chondrobags: 0.8-and 0.1-fold change for HACs and SSCs, respectively, and TGF-β_3 chondrobags: 0.5-and 0.2-fold change for HACs and SSCs, respectively) in the cell-laden</p>	[286]

		<p>scaffolds due to the GFs' addition</p> <p>-Enhanced cellular proliferation, ECM formation, nuclei distribution, and absence of bone production in the chondroblasts containing cells in the presence of both GFs, with more intense Alcian blue staining for TGF-β_3 chondroblasts compared with the TGF-β_1 ones</p>	
Dual-function chitosan-based thermogel	Human adipose-derived MSCs	<p>-Continuous release of KGN and DS for over thirty and forty days, respectively</p> <p>-Proper dispersion of the two drug carriers in the hydrogel, indicating homogenous drug release</p> <p>-Suitable time of gelation (5 ± 0.5 min) at 37 °C that can enhance the clinical viability as compared with the gelation times of 10 to more than 60 min reported for previously prepared hydrogels</p> <p>-Displayed shear modulus of 167 ± 5 kPa, being comparable to the amount reported for the AC (50-250 kPa)</p> <p>-No detrimental impact on the human adipose-derived MSCs by the fabricated hydrogel</p> <p>-Up-regulation of Sox9, collagen type II-α I, and aggrecan expressions after twenty-one days compared to the control group</p> <p>-Enhancement of GAGs DNA⁻¹ content up to 2 times when cultivating cells on the hydrogel in comparison with the control group</p>	[287]
KGN-loaded GelMA/liposome hybrid microgel	BMSCs/Rat OA models	<p>-Sustained and long-term release of KGN within the degradation period (more than three weeks) due to the good anchorage of liposomes in the microgels via non-covalent interaction and</p>	[289]

		<p>physical network hindrance</p> <p>-Lower drug encapsulation efficiency in the hybrid microgels ($43.8 \pm 16.6 \%$) compared to the liposomes ($83.5 \pm 8.1 \%$) owing to the drug leakage happening in the oil-removing step of the manufacture and small amounts of liposomes present on the outer layer that diffused out during this step</p> <p>-High expressions of collagen type II alpha I, aggrecan, Sox9, indicating the chondrogenic differentiation of BMSCs</p> <p>-Reduction of total osteophyte volume (72 % decrease), decreased depth of cartilage erosion (75 % reduction), and enhanced expressions of GAGs, collagen type II, and aggrecan compared to the PBS group in the rat OA model</p>	
<p>Dental MSCs-laden arginylglycylaspartic acid-coupled TGF-β_1-loaded alginate hydrogel microsphere</p>	<p>PDLSCs, GMSCs, and hBMSCs/Beige nude XID III (NU/NU) mice</p>	<p>-Sustained release of TGF-β_1 for fourteen days</p> <p>-High cellular viability of hBMSCs after two weeks of cultivation (nearly 80 %)</p> <p>-High expressions of collagen type II and Sox9 after two weeks, with hBMSCs and GMSCs displaying lower expression levels of these genes compared to the PDLSCs</p> <p>-Differentiation of all cells into round-shaped chondrocytes as well as chondrogenic tissues formation, and failure of the negative control in displaying any positive signs for chondrogenesis in the animal model</p> <p>-In vivo observation of bFGF and bone morphogenetic protein-4 GFs in the early stages of chondrogenic differentiation, proposing the TGF-β_1's essential role within the chondrogenesis</p>	[291]

HA injectable hydrogel encapsulated with SDF-1/KGN-loaded PLGA microspheres	-/Rabbit models possessing full-thickness cartilage defects	<p>-Sustained release profiles of two model drugs representing SDF-1 and KGN for more than two months indicated by in vitro tests</p> <p>-In vivo formation of cartilage-like tissue with cell clusters and lacunas, as well as its well integration with the nearby cartilage and subchondral bone, with observable minor defects at the eight-week evaluation</p> <p>-In vivo production of a more mature neo-cartilage with a more even thickness than before at the twelve-week assessment</p>	[292]
Temperature-responsive, hydrogel-based injectable PNIPAAm microcarriers loaded with DS-doped AHAMA	HACs/Rat OA models	<p>-Release of the loaded drug at 39 °C (analogous to the temperature of the joint cavity during OA) due to the temperature sensitiveness and the resultant shrinkage of PNIPAAm hydrogel particles</p> <p>-Locked microcarriers' drug-release switches with the decrease of the inflammation, leading to the prevention of drug waste and improvement in the OA treatment's efficiency</p> <p>-No toxic effects of the employed drug on cultured HACs after three days</p> <p>-Improved synovial hyperplasia, enhanced amount of synovial fluid, increased collagen type II content (5-fold change), and decreased expression of TNF-α (1.5-fold change) in comparison with the control group after six weeks in the animal model</p>	[294]
Bioprinted cell-laden hydrogels: 1) 4 % w v ⁻¹ alginate + 6 % w v ⁻¹ GelMA + 4 % w v ⁻¹ CS AEMA loaded	hBMSCs and HACs/ Male Wistar rats with the surgically damaged trochlea	<p>-Bioinks' high shape fidelity and open porosity</p> <p>-Lower mixing index in comparison with other investigations, illustrating the restricted mixing efficiency of the proposed microfluidic system</p> <p>-Great cell survival after extrusion in the HC1, HC2, and CC</p>	[18]

with hBMSCs (coded as HC1), 2) the same composition as the previous one except with hBMSCs and HACs (coded as HC2) 3) 4 % w v ⁻¹ alginate + 6 % w v ⁻¹ GelMA + 4 % w v ⁻¹ CS AEMA + 0.5 % w v ⁻¹ AHAMA + 0.5 % w v ⁻¹ β -TCP loaded with hBMSCs (coded as CC)		<p>bioinks (92 ± 5 %, 92 ± 2 %, and 88 ± 4 %, respectively), indicating the absence of damaging effects exerted by shear stress on cell viability</p> <p>-High cellular viability in all bioinks even after two weeks of cultivation</p> <p>-The highest expressions of collagen type II-alpha I, aggrecan, collagen type I-alpha I, collagen type X-alpha I, and ALP in the CC bioink</p> <p>-More hyaline phenotype observed in the HC2 bioink compared with the other two</p> <p>-Promoted hypertrophic chondrocytes' development by up-regulating the expressions of collagen type X-alpha I and ALP after the introduction of β-TCP and AHAMA into the CC bioink</p> <p>-Observation of differentiating cartilaginous cells at rats' trochlear groove after twelve weeks of implantation, being absent within the control group</p> <p>-Presence of collagen type II and tenascin in the treated animal models</p>	
Alginate-polyacrylamide DN hydrogel embedded with TGF- β_3 -loaded PLGA NPs	L929 fibroblasts and differentiated chondrogenic cell line derived from mouse teratocarcinoma cells/Male Sprague-Dawley rats with cylindrical cartilage	<p>-Reported NPs' size of 126 ± 4.52 nm with polydispersity index of 0.137, indicating capability of the microfluidic system in enabling the manufacture of small NPs with narrow size distribution</p> <p>-Sustained release of the GF (encapsulation efficiency: 77.7 %) over sixty days and high compression modulus (59.79 ± 1.58 kPa) of the hydrogels</p> <p>-Displayed good survival (above 70 %) and high proliferation</p>	[297]

	defects in the trochlear groove's center	<p>after three days of culture</p> <ul style="list-style-type: none"> -Generation of a new smooth tissue surface with a hyaline matrix combined with fibrocartilage and fibrous tissue after twelve weeks post-surgery in the treated rats and the observed disorganized fibrous neo-tissue with an irregular surface in the control group -Enhanced cartilage repair, thicker neo-cartilage formation, and generation of chondrocyte clusters with columnar structure in the treated animal models in comparison with the control group -Achieved consistency between mechanically enhanced hydrogel's degradation profile and cartilage repairment 	
<p>Ball-bearing-inspired injectable MGS@DMA-SBMA@DS</p>	<p>Rat chondrocytes/Rat OA models</p>	<ul style="list-style-type: none"> -Excellent lubricating effect owing to the firm hydration layer production -Sustained release of the drug -Good cell viability (nearly 3.75×10^4 viable cells) -Anti-inflammatory effect of the complex due to the DS presence and generally the complex protection of cells from inflammatory factor-induced degeneration via the catabolic proteases' down-regulation (ADAMTS5 and MMP-13) and up-regulation of the anabolic molecules (collagen type II and aggrecan) -Alleviation of patients' pain suffering from OA by diminishing the tachykinin-1 expression -Decreased total osteophyte volume (53 % reduction), enhanced expression of collagen type II (128 % increment), and increased joint space widths in the rats compared to the control group 	<p>[302]</p>

		-No observation of post-surgery acute inflammation in rats after one and eight weeks	
Ball-bearing-inspired injectable GelMA@DMA-MPC@DS	Rat chondrocytes/Rat OA models	<p>-Enhanced lubrication ability by the co-polymeric coating with hydration lubrication (reduced friction coefficient from almost 0.026 in GelMA to 0.019 in GelMA@DMA-MPS)</p> <p>-Enabled sustained drug release via controlling the burst release (66 % in GelMA versus 12-20 % in GelMA@DMA-MPS) due to the co-polymeric coating presence with hydration lubrication</p> <p>-No detrimental impact by generated microspheres on the rat chondrocytes at various time points</p> <p>-Drastic increase in aggrecan and collagen type II-alpha I expression levels within treated cells in the GelMA@DMA-MPC@DS group compared to the blank group and an observed limited impact for the GelMA@DMA-MPC and GelMA groups, indicating the relative protection ability of GelMA@DMA-MPC@DS</p> <p>-No considerable alterations in MMP-13 expression for GelMA@DMA-MPC and GelMA groups compared to the blank group and significant down-regulation of MMP-13 expression in the GelMA@DMA-MPC@DS group, demonstrating the complex's anti-inflammatory effect</p> <p>-Animal models' outcomes:</p> <p>•Increment of articular space width in GelMA@DMA-MPC@DS</p>	[303]

		<p>group compared to the other compositions and PBS groups</p> <ul style="list-style-type: none"> •Observed OA common features in the PBS group, no remarkable difference between the GelMA and PBS groups, and considerable enhancement in tidemark integrity, tissue cloning, as well as morphology change in the GelMA@DMA-MPC and GelMA@DMA-MPC@DS groups with the second group having the most intense positive staining •The best GAGs content and the least depth of lesion belonging to the GelMA@DMA-MPC@DS group, with a 50.5 % increase and 34.9 % decrease compared to the PBS group, respectively 	
<p>Rapamycin-loaded cationic liposome-incorporating AHAMA hydrogel microsphere</p>	<p>C-28/I2/Rat OA models</p>	<ul style="list-style-type: none"> -Confirmed cationic liposomes' targeting ability toward the negatively-charged GAGs chains within the cells -Exposed liposomes on the outer surface of hydrogel microspheres, leading to the generation of hydration layers with self-renewing capability in response to the frictional wear -Inferior drug encapsulation efficiency in the loaded hydrogel microspheres ($67.39 \pm 4.06 \%$), as compared to the liposomes ($87.55 \pm 6.49 \%$) owing to the drug leakage happening in the oil-removing step of the manufacture and exposed liposomes on the outer layer that diffused out during this step -More prolonged release of rapamycin from the liposome-loaded hydrogel microspheres than the sole liposomes (28 days versus 14 days) -Enhanced number of cells over time in each group (control, liposome-loaded microspheres, and rapamycin/liposome-loaded 	<p>[305]</p>

		<p>microspheres) without noticeable differences between them</p> <p>-Survival of approximately all of the cells</p> <p>-Considerably lower intercellular ROS content and apoptotic cells' number in the rapamycin/liposome-loaded microspheres in comparison with the other groups, indicating the ability of the proposed hydrogel microspheres in reducing ROS generation and inhibiting apoptosis via delivering rapamycin</p> <p>-Animal models' outcomes:</p> <ul style="list-style-type: none"> •Nearly 27.7 % greater osteophyte volume in the PBS group compared to the rapamycin/liposome-loaded microspheres, indicating the fabricated microspheres' ability to alleviate the joint injury •Significantly increased joint space width in the rapamycin/liposome-loaded microspheres in comparison with the other groups, illustrating the produced hydrogel microspheres' capability to act as a biolubricant •Disorganized chondrocytes and severe surface abrasion in the PBS group, relative integrity and regular AC structure in the liposome-loaded microspheres group compared to the PBS group, and no observable degenerative alterations in the rapamycin/liposome-loaded microspheres group 	
<p>GelMA hydrogel microsphere embedded with miR-140-loaded G5-AHP NPs</p>	<p>Primary mouse chondrocytes/Mice with the destabilized medial meniscus</p>	<p>-Survival of approximately all of the cells after five days of cultivation</p> <p>-Increased cell density after five days</p> <p>-Entire release of the miR-140 after five days and GelMA microspheres' disappearance because of their cargos' loss on the</p>	[313]

		seventh day	
		<p>-Osteophyte formation, wider articular space, excellent morphological integrity, improved tissue cloning, and diminished surface denudation at twelve weeks after surgery in the treated mice group compared to the PBS group possessing OA's common structural properties such as denudation erosion, deformities, and surface discontinuity</p> <p>-Reduced depth of cartilage lesions (48.2 % decrease) and higher collagen type II content in the treated mice group in comparison with the PBS group</p>	

6. Development of cartilage-related organ-on-chips

Apart from merely employing microfluidic technology in designing scaffolds, it can be utilized in creating leading-edge devices named OoCs.^[314,315] In essence, OoC refers to a single-channel or multi-channel 3D chip of a microfluidic cell culture simulating organ-level or tissue-level physiology, categorized into hydrogel-based OoCs (as one of the most promising microfluidic platforms) and those without embedding hydrogels.^[316–319] The difference between these devices and other traditional cell platforms is that they can be used for biochemical, mechanical, and electrical stimulations.^[320,321] Besides, OoCs can substitute animal models and markedly reduce experiments' time and cost.^[322] Other OoCs' benefits are the capability to recover exit fluids, real-time screening of drugs, live imaging, and disease modeling, to name but a few.^[323–325] Since the invention of OoC devices, tissues' models such as cartilage-related OoCs have been manufactured throughout the years. In this vein, the timeline of significant milestones in OoCs designed for cartilage TE is depicted in **Figure 17**.



Figure 17. Timeline of major milestones in the OoCs designed for cartilage bioengineering.

Importantly, a number of key GFs, namely bFGF and insulin-like GF-1 (IGF-1), exert major impacts on chondrocytes' biological behaviors. In this context, several studies have evaluated the effect of these GFs upon chondrocytes proliferation within a monolayer culture, not in a 3D

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culture.^[326,327] One of the first studies on employing microfluidic chips for cartilage TE was performed by Li et al.^[328], an innovative experiment in which an integrated cell-loaded microfluidic chip was devised in order to evaluate rabbit articular chondrocytes proliferation in the presence of bFGF, IGF-1, and their combination. Briefly, the PDMS device mainly consisted of downstream 3D cell culture chambers and an upstream concentration gradient generator (CGG), and the cells (1×10^5 cell mL⁻¹) loaded in the 1.5 % w v⁻¹ collagen type I gel in the cell culture chambers were cultivated for two weeks at the various GFs' concentrations created via the CGG. Indeed, PDMS was employed for two reasons: 1) it is biocompatible and very permeable to oxygen and carbon dioxide, thus ensuring these gases' rapid exchange between the medium within the culture chambers and the atmosphere surrounding the designed chip, and 2) being optically transparent, PDMS enabled direct counting of the proliferated cells' numbers using an inverted microscope. Furthermore, the collagen gel is an ideal biomaterial due to its ability to preserve the chondrocytes' phenotype in the time of cultivation. The round chondrocytes' survival was above 95 %, and the highest cellular proliferations were achieved in the concentrations of: 1) 57.14 ng mL⁻¹ IGF-1 (2.38-fold versus the control group), 2) 5.72 ng mL⁻¹ bFGF (3.85-fold versus the control group), and 3) 85.73 ng mL⁻¹ IGF-1/1.43 ng mL⁻¹ bFGF (4.76-fold versus the control group). In this regard, no further promotion of proliferation was observed in higher concentrations of IGF-1, mainly owing to the saturation of IGF-1 receptors in higher concentrations. At bFGF concentration superior to 5.72 ng mL⁻¹, the rate of proliferation decreased because of the alteration in extracellular signaling mediated via this GF. In the case of the GF combination, the mechanism underlying their interaction is still unknown, and more evaluations are required to define this interaction and specify their application in cells expansion *in vitro*. Hence, this device was the first of its kind that simultaneously enabled the production of detailed concentrations of two GFs and the evaluation of their impact on chondrocytes' proliferation in a 3D cell culture.

It is established that chondrocytes, compared to other cells, experience an acidic environment *in vivo*, affecting their metabolism and ability to synthesize the matrix.^[329] The following year was accompanied by the Huang et al. investigation^[330] on the impact of the extracellular pH upon activities of primary articular chondrocytes, which was conducted by utilizing a microfluidic cell

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culture chip. Within this experiment, cells (8×10^6 cell mL^{-1}) were immobilized in 1.5 % w v^{-1} agarose gel, and then the suspension was loaded in the cylindrical cavities on the polymethylmethacrylate plate, followed by injecting the culture medium with desired pH values (6.6, 7.0, 7.2, and 7.3) into microbioreactors on the PDMS layer. After a four-day culture, the biosynthetic and metabolic abilities of highly viable cells (95 ± 2 %) cultivated under different extracellular pH levels were assessed by measuring the GAGs and lactic acid contents in the medium, respectively. It was reported that their productions were affected by the pH, thus demonstrating that the biosynthetic and metabolic functions of primary articular chondrocytes were indeed sensitive to the environmental pH. The highest contents of GAGs and lactic acid were found in the pH value of 7.3, being 0.08 and 7 mg million cells $^{-1}$, respectively. Thus, the designed chip could be successfully used to examine chondrocytes' behaviors under various pH's. Moreover, by comparing the microfluidic cell culture with the conventional static, it was shown that the GAGs and lactic acid contents were different in the two cell culture formats due to the dissimilar biophysical or biochemical micro-environments. Therefore, the data extrapolation from a cell culture format to another one might result in bias error. Complementary investigations are required to perceive the mechanism causing these discrepancies.

The combination of the optically switched dielectrophoretic (ODEP) force-based approach and microfluidic platforms has produced an efficacious method for manipulating microparticles and biological cells.^[331] For the first time in 2012, a novel microfluidic cell culture chip was designed incorporating cell-encapsulating alginate microbeads and ODEP force for bottom-up cartilage TE. Within this experiment, primary steer articular chondrocytes were loaded at various cell densities (2.4×10^7 , 1.0×10^7 , and 7.7×10^6 cell mL^{-1}) into 1 % w v^{-1} alginate gel, followed by injecting cell-laden alginate gelled microbeads (created via a microfluidic-based micro-vibrator) into the photo-resist pillar array to mimic the cell distribution in the AC zones, and the ODEP force located at the central micro-chamber was then exerted to sort, separate, and pack the microbeads. Herein, the alginate hydrogel was employed owing to its biocompatibility and capability of maintaining the bovine articular chondrocytes' phenotype in the time of culture. It was concluded that the calculated manipulation force proportionally augmented with the increment of cells number in the microbeads,

and the prepared hydrogel-based microbeads possessed fantastic size uniformity. After four days of perfusion cell culture, the cellular viability remained as high as $96 \pm 2\%$, and the assembled 3D sheet-like construct resulted from the packed microbeads could successfully imitate the cell distribution in the AC's cross-section, with the reduction of cells per tissue unit with the increasing distance from the surface of cartilage. Notably, the patterned cell-loaded gel microbeads could be packed in a more compact way owing to the alginate hydrogel's elastic nature; in essence, the tight packing permitted the ECM produced by cells, to readily fuse in order to form a tissue construct having high integrity. As a result, not only the mentioned microfluidic chip was able to provide ODEP force for manipulating cell-loaded microparticles but was also capable of performing on-site perfusion tissue culture.^[332]

Fibrochondrogenic differentiation has cardinal importance in TE, particularly for the repair of damaged fibrocartilage, including the temporomandibular joint disc, intervertebral disc, and knee meniscus.^[333,334] Based upon the regulation of MSCs fibrochondrogenesis by mechanical and topographical cues^[335–337], a microfluidic perfusion platform simultaneously incorporating flow stimulus (oscillatory fluid flow) and nanotopography (aligned nanofibrous scaffolds) was engineered in March 2013. To be more specific, the developed microfluidic device with a PDMS layer allocated to the micro-channel networks had middle aligned PLGA nanofibers layer and contained rat MSCs-loaded (5×10^6 cell mL^{-1}) micro-chambers which enabled the oscillatory fluid flow (1 and $0.01 \text{ dynes cm}^{-2}$) created via a flow-driving system to form various angles with the fibers (0° , 45° , and 90°). The results were revealed as follows: 1) Collagen type I expression was higher in cells under flow stimulus compared to those in the static control, and in the angle of 0° with 1 dynes cm^{-2} shear stress, the gene expression enhanced more significantly than the other ones (**Figure 18.A**); 2) In the case of sulfated proteoglycans' secretion, the cells within 45° and 90° conditions had higher secretion (**Figure 18.B**); 3) Other fibrochondrogenic genes such as Sox9, Runx2, and collagen type II were significantly up-regulated under both parallel and perpendicular flow conditions (1 dynes cm^{-2}) compared to the static condition. Moreover, the MSCs cultivated under the perpendicular flow showed greatly higher expressions of collagen type II and Sox9 but a markedly lower Runx2 expression in comparison with

those that were cultured under the parallel flow (**Figure 18.C**); and 4) The fibrochondrogenic and fibroblastic phenotypes were observed resulted from the perpendicular and parallel conditions, respectively. Consequently, these findings suggested that the angle between the aligned nanofibers and perfusion flow could serve an important role in regulating MSCs' fibrochondrogenic differentiation. Nonetheless, the inability to produce large-scale tissue constructs should be solved in the future experiments.^[338] Although several experiments have employed the combination of two cell-loaded scaffolds for chondro- and osteo-differentiation of stem cells, these methodologies showed significant limitations. In particular, the constraint related to the concurrent induction of stem cells' differentiation and the absence of a generated interface between the formed chondrogenic and osteogenic zones need to be addressed.^[339,340] With respect to this issue, a group of researchers^[341] devised a gradient-generating microfluidic device that could simultaneously steer the stem cells' differentiation into osteoblasts and chondrocytes in an agarose hydrogel slab. Briefly, the platform consisted of two sections: 1) A PDMS pool incorporating human adipose-derived MSCs-loaded agarose gel (2×10^7 cells mL⁻¹ in 2.5 % w v⁻¹ gel) and 2) A polycarbonate membrane linked to a microfluidic chip with three channels for loading various culture media. In order to create a gradient phase between the chondrogenic and osteogenic zones, normal cell culture was injected into the middle channel to inhibit the combination of different differentiation media (chondrogenic and osteogenic media) on the chips' two sides. After twenty-five days of culture, the calcium content of cultured cells in the osteogenic zone was 45-fold higher than that of the cultivated cells in the chondrogenic zone, thus indicating the successful stem cells' osteogenic differentiation in the osteogenic region. Besides, the GAGs expression in the chondrogenic region was 8- and 3-fold higher than that observed for the cells in the osteogenic region and interface zone, respectively, illustrating the stem cells' chondrogenic differentiation in the chondrogenic zone. Furthermore, the expressions of the other cartilage-related markers such as aggrecan, collagen type II, and Sox9 were significantly increased from the osteogenic to the chondrogenic zones. At the final culture time point, the cells fully differentiated to osteoblasts, mineralized chondrocytes, and chondrocytes within the osteogenic, interface, and chondrogenic zones, respectively. These results demonstrated the ability of the proposed device to simultaneously steer the chondrogenic/osteogenic commitment of human adipose-

derived MSCs. Notably, fluid flow-induced shear stress is associated with the AC's metabolism regulation and its pathophysiologic processes; hence, various levels of shear stresses can impact cell signaling pathways within chondrocytes, thereby regulating the metabolism.^[342] Concerning this subject, it is of special importance to explore the effect of this stimulus upon the chondrocyte's metabolism. In 2013, Zhong et al.^[343] proposed an innovative microfluidic perfusion platform capable of exerting various magnitudes of fluid flow-induced shear stresses on chondrocytes in order to evaluate their metabolism responses. The microdevice contained four cell culture chambers loaded with rat articular chondrocytes (5×10^6 cells mL^{-1}) linked to four resistance channels and a peristaltic pump, which had the function of generating different values of fluid flow stress (0.007, 0.046, 0.189, 0.856, 1.2, 3.1, 7.5, and 15.4 dynes cm^{-2}) for twenty-four hours. These stresses were categorized as interstitial level (0.046 and 0.007 dynes cm^{-2}), low level (0.189-3.1 dynes cm^{-2}), moderate level (7.5 dynes cm^{-2}), and high level (15.4 dynes cm^{-2}). It was revealed that the expression of aggrecan was greatly up-regulated in the range of 0.007-1.2 dynes cm^{-2} . Conversely, its expression at moderate and high levels of stress was markedly down-regulated as compared to the static control. Importantly, the expression of interleukin-6 (IL-6), a proinflammatory cytokine detected in OA chondrocytes, was also assessed. No significant differences on its expression were observed at 1.2 and 3.1 dynes cm^{-2} in comparison with that of the control; nonetheless, at moderate and high levels of stress, the IL-6 expression was considerably enhanced by 4.5- and 9.6-fold, respectively, as compared with the static control. Even though this platform provided a robust tool to precisely screen cellular responses, future investigations should address some of its limitations, such as limited flow duration and cultivation of cells within a 2D culture. Yes-associated protein (YAP), a regulator serving role in the mechanical cues' nuclear transduction, can exert a notable impact on the cells fate. Nevertheless, its responses to the fluid flow shear stress and its relationship with the chondrocyte phenotype as well as MSCs lineage commitment have not been properly established.^[344] In this context, a group of scholars^[345] designed a microfluidic device with the ability of producing different magnitudes of shear stresses to evaluate the phenomena mentioned above. The platform comprised four cell culture chambers loaded with primary rat chondrocytes (1×10^5 cells mL^{-1}) and primary rat MSCs (0.5×10^5 cells mL^{-1}) linked to resistance channels and a peristaltic pump, which had the function of generating different values of

fluid flow stress (0.009, 0.055, 0.231, and 1.089 dynes cm^{-2}). Besides, MSCs were cultivated in co-induction adipogenic-osteogenic medium during the culture period. It was concluded that the MSCs' proliferation rate significantly augmented at 0.231 and 1.089 dynes cm^{-2} after twenty-four and forty-eight hours of perfusion in comparison with the 0.009 dynes cm^{-2} . In the case of chondrocytes, the proliferation rate considerably enhanced at 1.089 dynes cm^{-2} after twenty-four hours and at 0.231 and 1.089 dynes cm^{-2} after forty-eight hours. Following five days of perfusion for MSCs, the localization of YAP in the nucleus was not altered with the increment of stress, but its level increased in the nucleus. Furthermore, MSCs underwent adipogenic differentiation at low levels of stress, and osteogenic differentiation of cells was observed with augmenting levels of shear stress, which were in accord with the YAP distribution in the nucleus and cytoplasm. Following two days of perfusion for chondrocytes, YAP was mainly cytoplasmic at low stress levels, but its nuclear accumulation raised with increasing magnitude of stress. Additionally, when shear stress was enhanced, the expression of collagen type I and type II in chondrocytes increased and diminished, respectively. In essence, the chondrocytes dedifferentiation was simultaneous with the YAP nuclear accumulation, demonstrating the effect of fluid flow upon YAP expression and the resultant phenotype of chondrocytes. The nuclear YAP percentage within these two cells was drastically greater at 0.231 and 1.089 dynes cm^{-2} compared to 0.009 dynes cm^{-2} . In a nutshell, using a novel microfluidic platform, they deduced that the YAP expression in chondrocytes and MSCs was regulated via fluid flow shear stress and YAP could mediate the fate determination of chondrocytes and MSCs in answer to the fluid flow stimulus.

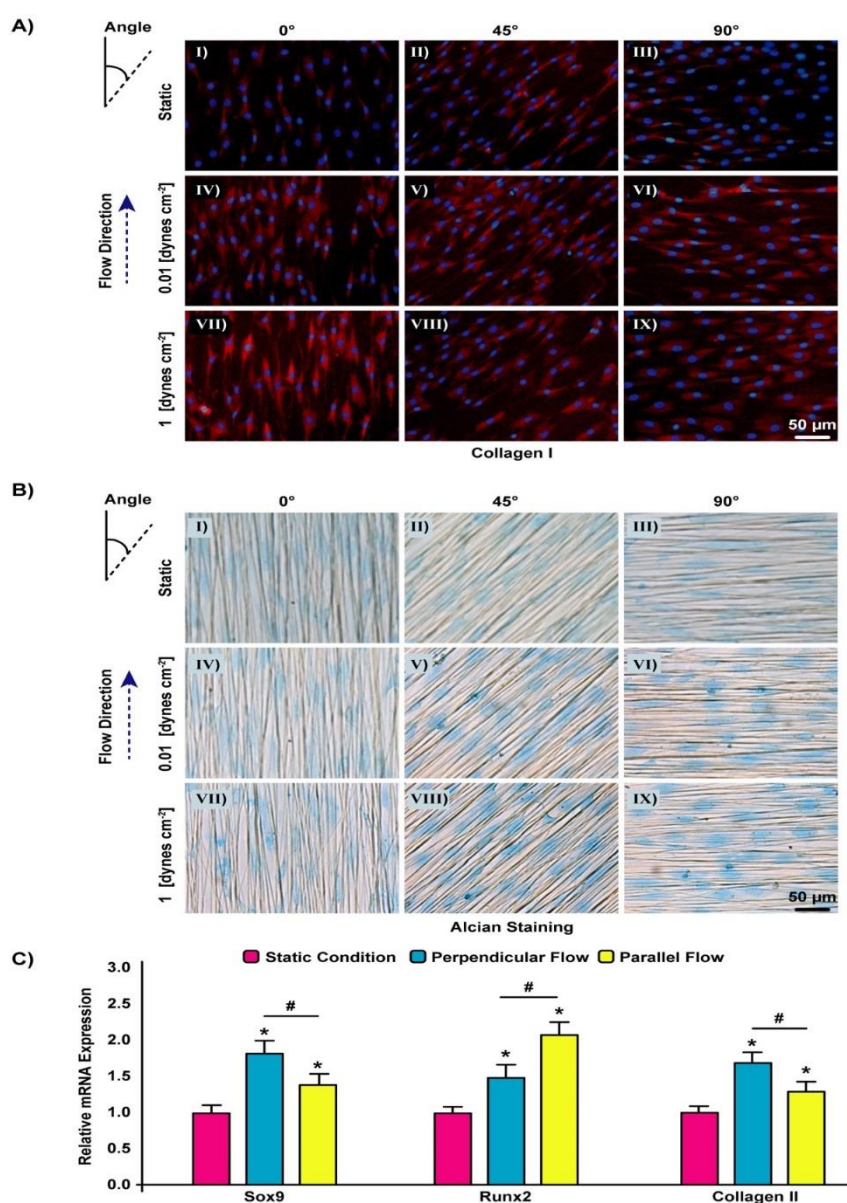


Figure 18. The fibrochondrogenic markers' expressions after four weeks of MSCs' induced differentiation at various angles with the nanofibers under the flow stimulus: A) Immunofluorescence staining for 4',6-diamidino-2-phenylindole (blue) and collagen type I (red). B) The sulfated proteoglycans' Alcian blue staining. C) The Sox9, Runx2, and collagen type II messenger ribonucleic acid expressions after four weeks of differentiation under perpendicular and parallel flows with 1 dynes cm⁻² shear stress as well as static condition (*p < 0.05, static versus flow condition and #p < 0.05, parallel versus perpendicular flow). Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[338] Copyright 2013, PLOS.

The articular chondrocytes within the cartilage tissue are constantly subjected to intricate physical events comprising tensile stress, shear stress, hydrostatic pressure, and compressive stress. Among these factors, compressive force serves an essential role in modulating the functions of articular chondrocytes.^[346,347] In 2014, an interesting study was performed to investigate the dynamic compressive loading's magnitude and frequency effect on articular chondrocytes' behaviors by employing a micro-cell perfusion culture system. Primary articular chondrocytes (2.4×10^7 cell mL⁻¹) were immobilized in 6 % w v⁻¹ alginate hydrogel. Then, the mixture was loaded into the cylindrical cavity within the microbioreactors, and a series of dynamic compressive loadings (frequency: 0.5, 1.0, and 2.0 Hz / strain: 20 % and 40 % / 3 cycles per day) were exerted on the cell-embedded constructs by the micro-cell device for five days. The cellular viability remained in the range of 95 % in all conditions (**Figure 19.A**), which was consistent with the former studies revealing that primary articular chondrocytes were not mechanically influenced by compressive loadings lower than 51.5 %. Although the cell proliferation did not change as the result of the compressions, the secretion of lactic acid, the indicator of metabolic activity as mentioned above, by cells cultivated under 40 % strain with 2 Hz frequency was nearly 18.77 % higher than that of cultured cells in the control group; however, it was not very different among the articular chondrocytes which experienced frequencies lower than 2 Hz (**Figure 19.B(I)**). Since the 40 % / 2 Hz compression was analogous to the condition cells experience during high intense exercise, it could be concluded that vigorous exercise with high frequency might up-regulate the articular chondrocytes' metabolism in the body. Of note, the compressive stimulation's frequency did not have a significant influence on cells when the strain level was 20 % (**Figure 19.B(I)**). Another finding was related to the GAGs synthesis, which was increased as the magnitude and frequency of loading raised. It is noteworthy to mention that the GAGs contents produced in 1 and 2 Hz (frequencies of walking and running states, respectively) were statistically higher than those generated in the control group, a phenomenon that did not occur among the articular chondrocytes undergoing the frequencies less than 0.5 Hz (**Figure 19.B(II)**). In brief, the proposed chip could provide invaluable insights both for cartilage rehabilitation and cartilage TE.^[348]

Chondrocytes can respond to bioelectrochemical and biomechanical stimuli via secreting proper ECM proteins, enabling the tissue to resist large forces it undergoes. Although ACs' biomechanical aspects are well described, its bioelectrochemical responses are not understood in depth.^[329] Regarding this matter, Stacey and co-workers^[349] developed an impedance microfluidic chip in which a suspension of primary human costal cartilage chondrocytes ($1000 \text{ cells mL}^{-1}$) was placed between two electrodes in order to investigate cells' bioelectrical characteristics. It was revealed that chondrocytes' dielectric features, such as membrane conductance, membrane capacitance, and membrane folding factor were analogous to those of rat cardiomyoblasts, a cell type with extensive ion channels. Furthermore, they employed a non-specific ion channel blocker, gadolinium, to decrease the cellular membrane conductance. Results demonstrated that ion channels were an essential component of the bioelectrochemical response and were an active process in preserving the cell homeostasis. There is rising evidence from clinical and *in vivo* studies that cartilage degeneration in the OA disease may happen subsequent to the occurrence of subchondral bone lesions, suggesting that OA is indeed an OC disease involving both cartilage and bone degenerative alterations.^[350,351] In order to assess the cartilage-bone interactions in this illness, a team of scientists^[352] developed a dual-chamber bioreactor that was fitted into a microfluidic base and contained hBMSCs-derived OC constructs. In brief, hBMSCs were suspended in the chondrogenic suspension (GelMA, HAMA, photo-initiator, and Hanks' Balanced Salt Solution) and osteogenic suspension (GelMA, hydroxyapatite, photo-initiator, and Hanks' Balanced Salt Solution) at a density of $20 \times 10^6 \text{ cells mL}^{-1}$. After loading them into separate chambers (first suspension in the top chamber and second suspension in the bottom chamber), cross-linking was performed to obtain cell-laden hydrogels in each chamber. The chondrogenic and osteogenic media were supplied through two conduits in the top and bottom chambers, respectively, for four weeks in the microtissue bioreactor. After the cultivation period, analyses illustrated the formation of biphasic OC constructs and a tidemark between osseous and chondral halves. In fact, cells within the chondral and osseous halves displayed increased expressions of chondrogenic genes (e.g., Sox9, aggrecan, and collagen type II) and enhanced expressions of osteogenic genes (e.g., osteocalcin, Runx2, and bone sialoprotein), respectively. Thereafter, the chambers were separately treated with IL-1 β (10 ng mL^{-1}) for one week so as to assess the possible communication between

components. Firstly, diminished expressions of chondrogenic genes were observed when separately treating the chondral and osseous constructs with IL-1 β , indicating the signaling between them. Interestingly, evidence of this interaction was even more obvious in the outcomes related to the catabolic genes expression; specifically, the expressions of MMPs in the chondral construct were significantly augmented in response to the osseous section's cytokine treatment. Furthermore, treatment of the chondral construct led to the reduced expressions of bone sialoprotein and osteocalcin, as well as enhancements in the production of MMPs in the osseous construct. Therefore, the proposed device enabled specifying the biochemically-relevant degenerative communication between bone and cartilage in the case of OA.

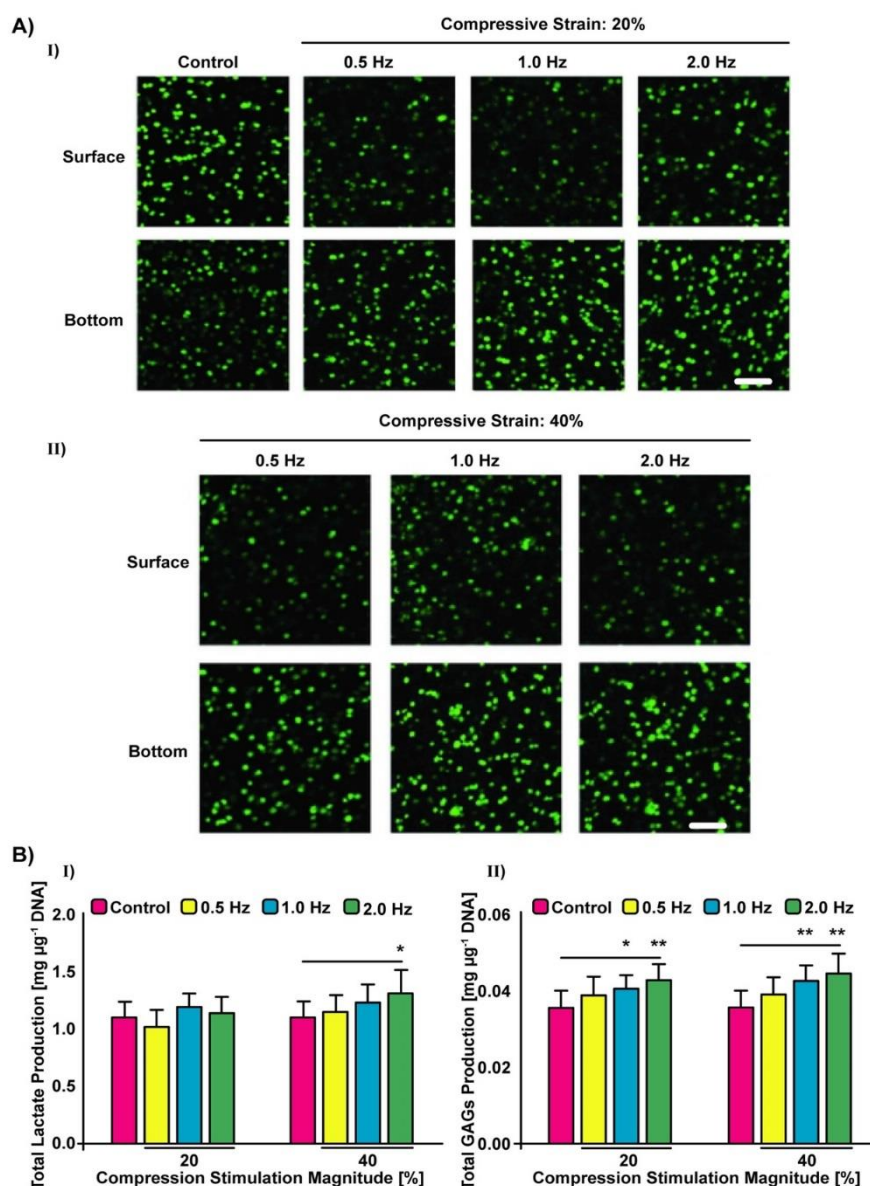


Figure 19. A) The cellular viability's microscopic observation after five days of cultivation under various compressive conditions (magnitudes of strain: I) 20 % and II) 40 % with frequencies of 0.5, 1.0, and 2.0 Hz) employing the live & dead assay. Red and green dots present dead and live cells, respectively. Furthermore, the upper and lower figures display the primary articular chondrocytes at the 3D culture constructs' surface and bottom, respectively. B) The investigation of: I) metabolic (total lactate production) and II) biosynthetic (total GAGs production) activities of the cells under various dynamic loading conditions, after five days of cultivation. Remarkable differences are presented as * $p < 0.05$ and ** $p < 0.01$. Reproduced under the terms of the Creative Commons

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As mentioned earlier, native AC possesses high mechanical strength provided by highly-organized collagen fibrils.^[353] Conversely, tissue-engineered cartilage constructs exhibit poor mechanical strength in comparison with the native AC because of lacking the abovementioned oriented micro-structure; in this regard, approaches to control the constructs' ECM structure have proved to be beneficial to manage this problem.^[354,355] Assessing the impacts of fluid flow-induced shear stress and biomolecular gradients on hMSCs' alignment and chondrogenesis in a microfluidic device having a PDMS gradient generator with the aim of controlling the construct structure was performed by a group of scholars^[227] in 2015. For the alignment experiments, the hMSCs were cultivated under various shear stresses in the microfluidic platform: 1) 0.1 dynes cm⁻² for four days at a confluent condition, and 2) 0.12, 0.2, and 0.4 dynes cm⁻² for eight days in non-confluent and confluent states. In the first condition, the hMSCs' long axis preferentially aligned in the direction of flow by the day four, while those cultured in the static condition (0 dynes cm⁻²) showed alignment in tiny localized regions that differed in alignment angle from each other and thus displayed no overall preferential alignment (**Figure 20.A**). The cells in the second states showed more alignment within the confluent groups (0-15°) than the non-confluent ones after eight days of culture, but in the shear stresses range, there was no significant difference in their impact on cell alignment degree for both groups varying in confluency (**Figure 20.B**). Therefore, the shear stress presence was adequate to induce cell alignment. For the chondrogenic investigations, cells were exposed to the flow-based TGF-β₁ gradients (0-10 ng mL⁻¹) for twenty-one days experiencing shear stress of 1.7 dynes cm⁻² in the microfluidic chip. Results indicated that more chondrogenic aggregates were formed as compared to the control group (with constant concentration) after the end of the culture period (14 versus 8 aggregates in the control group). Moreover, the aggregates were more aligned and elongated with a higher area (0.376 mm² versus 0.160 mm² in the control group) (**Figure 20.C**) and enhanced collagen type II deposition, suggesting that the TGF-β₁ gradients could control the chondrogenic aggregates' structure. In brief, by means of applying chondrogenic gradients and shear stress on hMSCs, organized cartilage constructs with superior mechanical features could be obtained.

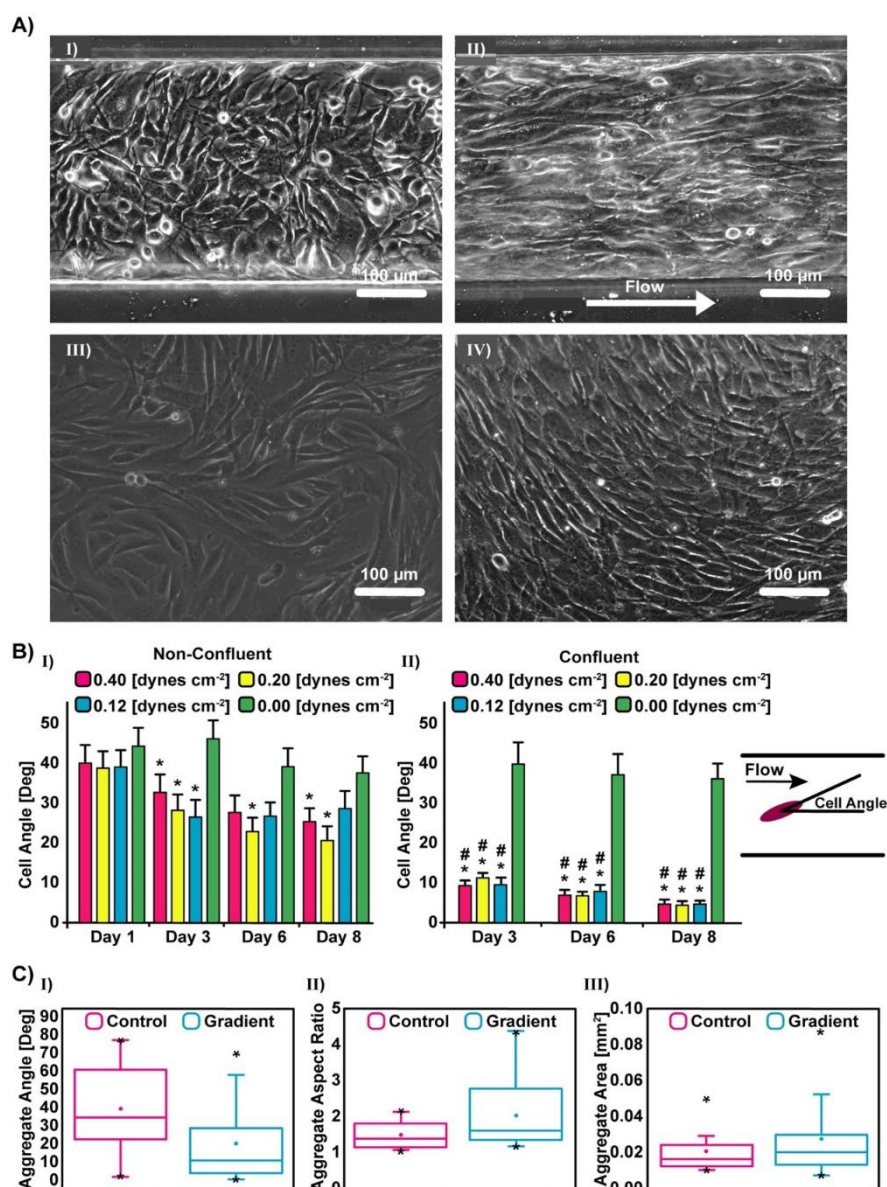


Figure 20. A) The impact of shear stress upon MSCs' alignment. The phase contrast photographs of cells that were cultivated in the microfluidic platform on I) day one (exactly subsequent to the attachment and prior to the flow initiation), and II) day four when the shear stress was 0.1 dynes cm⁻². The phase contrast photographs of cells that were cultivated in the static conditions (0 dynes cm⁻², polystyrene culture petri dish) on: III) day one; and IV) day four. B) The impact of shear stress upon MSCs' alignment (the average cell angle). The cell angle average was plotted at the various time points and values of shear stress for: I) non-confluent; and II) confluent areas. The angle measurements for day one were carried out after the MSCs' attachment and exactly before the flow. *

demonstrates a remarkable difference between the static control and the flow-based conditions.

Furthermore, # illustrates a remarkable difference between the non-confluent and confluent measurements of angle for a specific time point and shear stress state ($p < 0.001$). The seeding of MSCs was performed in a non-confluent condition; hence, on day one, no confluent areas were present. C) The impact of the TGF- β_1 gradients upon features of the MSCs aggregate. The box plots of: I) alignment angle of the aggregate ($p=0.04$); II) aspect ratio of the aggregate ($p=0.07$); and III) area of the aggregate ($p=0.24$). The measurements of alignment angle for the control and gradient devices showed a remarkable difference ($p < 0.05$) employing the Mann-Whitney procedure. $N=8$ and $N=14$ aggregates were utilized for the control and gradient devices, respectively. Reproduced under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.^[227] Copyright

2015, MDPI.

The expression of neuroleukin (NLK), a neurotrophic protein^[356], has been suggested to be associated with the cartilage development. Nevertheless, its direct effect upon chondrocytes proliferation remains to be thoroughly understood.^[357,358] In 2016, an innovative study was undertaken in which the exact role of NLK, on rat articular chondrocytes' proliferation was identified, and the NLK's working concentrations were optimized utilizing a microfluidic platform. Briefly, cells (0.8×10^5 cells well⁻¹) loaded into the downstream cell culture chamber were exposed to various gradients of NLK ($0-30$ ng mL⁻¹) produced via the upstream CGG in the microfluidic chip for six days, and the subsequent tests showed that among the eight generated concentrations (0, 4.28, 8.57, 12.85, 17.15, 21.42, 25.71 and 30 ng mL⁻¹), the NLK group with 12.85 ng mL⁻¹ concentration displayed a 3.96-fold increment in proliferation compared to the control group that was treated with a 2 % serum-containing culture medium; nonetheless, no further promotion was seen in concentrations higher than 12.85 ng mL⁻¹. Besides, the mentioned amount of NLK concentration could inhibit cultured chondrocytes' dedifferentiation by enhancing collagen type II production. Hence, these outcomes suggested that a specific concentration of NLK was capable of promoting cellular proliferation and collagen type II synthesis during the rat articular chondrocytes' in vitro propagation.

Future research projects may offer mechanistic insights into the NLK regulation of chondrocytes growth.^[359]

In an endeavor to design an OC unit-on-a-chip model including functional cartilage and bone tissue units, Goldman et al.^[159] developed a microfluidic chip incorporating bovine MSCs-derived OC constructs. In that work, bovine MSCs ($25 \text{ million cells mL}^{-1}$) were firstly encapsulated in a 2.5 % agarose gel, and followed the casting of the mixture against the PDMS micromolds. The top chondrogenic and bottom osteogenic regions were supplied with media supplemented with 100 ng mL^{-1} TGF- β_3 and 10 ng mL^{-1} bone morphogenetic protein-2, respectively, through two parallel microfluidic networks. After two weeks of culture, remarkable up-regulations of collagen type I-alpha I (29-fold) and Runx2 (86-fold) in the osteogenic region was observed. Moreover, significant up-regulations of aggrecan, Sox9, and collagen type 2-alpha I in the chondrogenic region relative to the osteogenic one were detected. Interestingly, the expression of collagen type X-alpha I enhanced from the chondrogenic region to the osteogenic area across the construct. Moreover, the content of GAGs in the chondrogenic and osteogenic regions were the highest and the lowest, respectively. As compared with conventional dual-chamber bioreactors, this system was capable of producing thicker constructs and optimizing culture conditions by microfluidic networks' incorporation within the constructs, suggesting its great potential for application in OC-related OoCs.

In the context of evaluating the impacts of mechanical forces on chondrocytes' responses, within a novel experiment, Yang et al.^[360] devised a microfluidic chip so as to analyze the effects of different fluid shear stresses on various primary rat chondrocytes' behaviors in 2017. The fabricated PDMS chip's chamber was loaded with primary rat chondrocytes ($5 \times 10^6 \text{ cell mL}^{-1}$) which were then cultivated under shear gradients (0.005 , 0.031 , 0.127 , and $0.576 \text{ dynes cm}^{-2}$) created via a fluid resistance network in the device. After twenty-four hours of shear stresses, the cellular viability remained high (above 90 %) in all conditions (**Figure 21.A**), illustrating that various fluid shear stresses did not affect the cell survival, and the expression of filamentous actin, an essential protein preserving cell morphology, showed an upward trend as the shear stress increased compared to the control group, with a significant enhancement after stimulating with $0.127 \text{ dynes cm}^{-2}$ shear stress

(**Figure 21.B**). Furthermore, with the shear stress increment, the cell area was increased. This result indicated that higher degrees of shear force promoted cellular adhesion. The expressions of collagen type I and type II were also up-regulated and showed an upward trend as the shear stress enhanced compared to the static condition, except for the $0.005 \text{ dynes cm}^{-2}$ shear stress effect on the collagen type II expression (**Figure 21.C**). As a result, the mentioned experiment provided novelty in investigating the cellular behaviors of chondrocytes in response to different fluid shear stresses. The investigations on the interactions between osteo-induced MSCs and other cells, including chondro-induced MSCs and chondrocytes, have been carried out under the static condition, not the dynamic one.^[361,362] In this regard, an intriguing study evaluated the combined effects of osteo-induced rat MSCs and fluidic shear stress on the phenotype of rat articular chondrocytes and chondro-induced rat MSCs by employing a two-layer microfluidic chip. First, osteo-induced primary rat MSCs ($1 \times 10^5 \text{ cell mL}^{-1}$) were injected in the chip's PDMS top layer containing a cell culture chamber, followed by the seeding of rat articular chondrocytes' and chondro-induced primary rat MSCs' ($1.5 \times 10^5 \text{ cell mL}^{-1}$) mixture on the PDMS bottom layer with three cell culture chambers capable of applying $0.05 \text{ dynes cm}^{-2}$ shear stress to the cells. The two layers were separated via a polycarbonate semipermeable membrane, which permitted the transfer of small molecular compounds from the top to the bottom layer, and concurrently inhibited the cells migration. The results revealed that cells were uniformly distributed, were mostly viable, and had more proliferation compared to the control group having no stimuli (nearly 1.5-fold enhancement). These cells displayed flattened morphology and enhanced adhering area per cell on the surface of substrate. Moreover, the aggrecan and collagen type II expressions were significantly superior than those of the control group, with a 1.75-fold increment in both cases. This investigation was the first one that showed synergetic, beneficial impacts of osteo-induced rat MSCs and fluidic shear stress on rat articular chondrocytes and chondro-induced rat MSCs in a fluidic environment. Despite the achieved favorable outcomes, one limitation was that the cultivation of cells on the bottom substrate was not done within a 3D matrix, meaning that merely the cells' upper surface was subjected to the fluidic shear stress. Therefore, future experiments should consider the design of a chip with an adoptable 3D cell culture platform on the bottom layer.^[363]

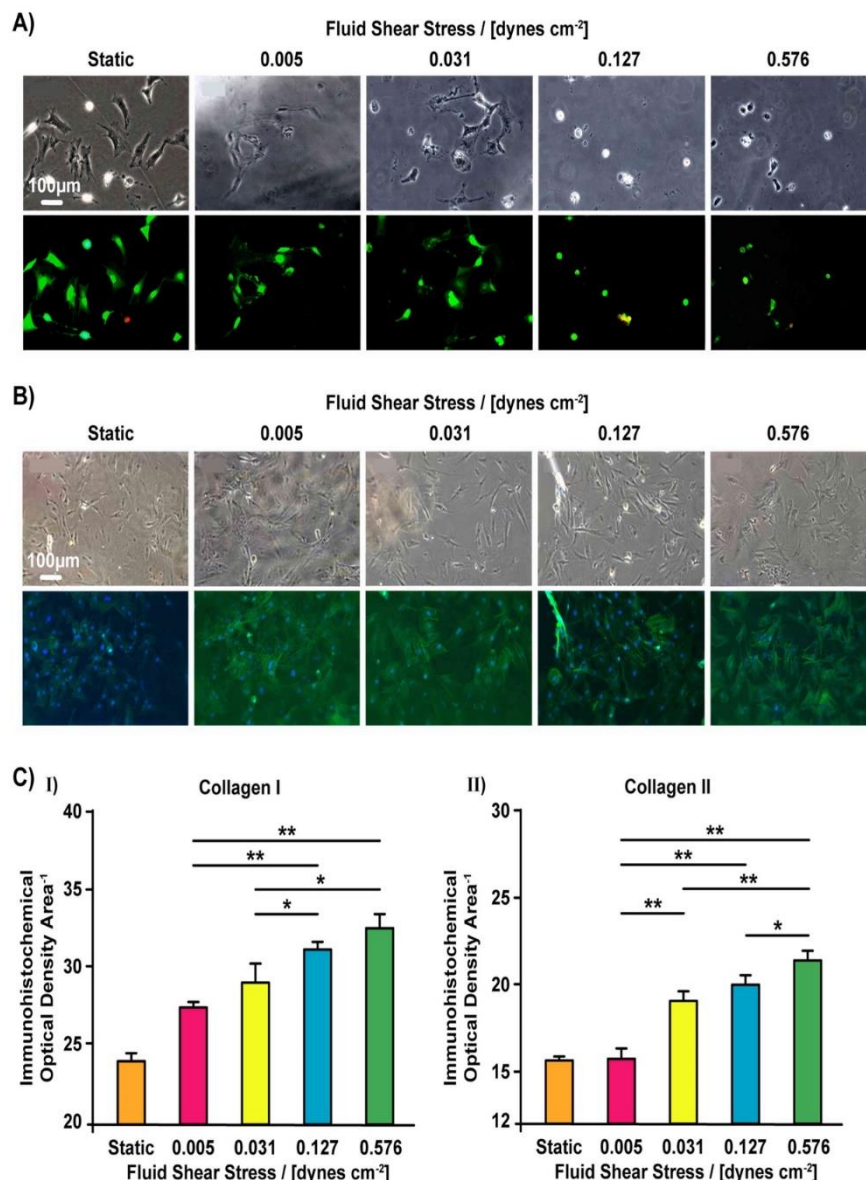


Figure 21. A) The cellular survival of primary rat chondrocytes under various fluid shear stress and static conditions. The colors green and red present the living and dead cells, respectively. **B)** The expression of filamentous actin protein in the cells under various fluid shear stress and static conditions. The colors green and blue present the filamentous actin and the nucleus, respectively. **C)** The expressions of: I) collagen type I and II) collagen type II under various fluid shear stress and static conditions (* $0.01 < p < 0.05$).

0.05 and $p < 0.01$). Reproduced under the terms of the Creative Commons Attribution

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Since conventional methods possess restricted productivity to assess chondrocytes' mechanobiological behaviors as a response to various magnitudes of compressive force^[364,365], a pioneering experiment tested the influence of static compression on the mouse chondrocytes' deformation using a microfluidic compression device. To delineate, a mixture of 1.5 % w v⁻¹ alginate gel and mouse chondrocytes (8×10^6 cell mL⁻¹) was loaded into a glass plate, followed by exerting various values of static compressive strain (14-34 %) for one hour on the constructs via PDMS balloons of different sizes. Alginate hydrogel has been considered as one of the fabulous biomaterials for the *in vitro* evaluation of chondrocytes' mechanobiology because chondrocytes within this gel culture behave in a manner analogous to those in native cartilage tissue constructs.^[366,367] It has been reported that cells strain was in the range of 7-19 % as a function of the PDMS balloon's diameter (1.2-2.0 mm), which can lead to the generation of 14-34 % compressive strain in the alginate gel. In fact, the cells strain enhanced ~ 2.9 % per 0.2 mm increase in the diameter of the PDMS balloon, thus illustrating that the chondrocytes displayed highly heterogeneous behaviors in response to the mechanical stimulation, and the cell compression amount could be readily manipulated via controlling the balloon size in the platform. One constraint of their experiment was the observed low viability of cells (58.6 %) after applying static compression as compared to that of cells under the dynamic compression (85.6 %), mainly due to the decreased transport of solute in the static condition that might be associated with the nutrient transport. Thus, future studies are in great need to help solving this type of issues. Nevertheless, the proposed device is interesting and enabled the cost- and time-efficient mechanobiological investigation of mouse chondrocytes.^[368]

Currently, DMOA drugs, that are employed to slow OA progression, are fairly scarce, primarily owing to the deficiencies in the relevant preclinical OA models; thus, *in vitro* load- and cytokine-based models that enable predicting DMOA drugs' efficiency are specifically required.^[234,369,370] In 2019,

Occhetta et al. ^[233] engineered a cartilage-on-a-chip disease model for evaluating the efficiency of these drugs. In the first step, HACs (5×10^4 cells μl^{-1}) loaded in MMP-degradable PEG-based hydrogel (12.5 % v v⁻¹) were injected in the top PDMS compartment of the micro-scale device, and after chondrogenic conditioning for fourteen days, a cartilage micro-construct was achieved. Subsequently, the established model experienced seven days of mechanical stimulation at various compression levels employing an electro-pneumatic actuation structure in the bottom PDMS compartment. PEG hydrogel was selected due to having higher standardization than naturally-derived biomaterials, and it was chemically modified in order to enable the MMP-mediated degradation, thereby allowing tissue remodeling by physiologically-relevant processes. Specifically, either hyper-physiological compression (30 %) or physiological compression (10 %) with the frequency of 1 Hz (duration: 2 hours of stimulation, 4 hours of rest, 2 hours of stimulation, and 16 hours of rest per day) was delivered to the model. By its turn, the 30 % compression condition led to a reduction in the expressions of aggrecan and collagen type II-alpha I collagen type I-alpha I messenger ribonucleic acid¹ and a higher expression of MMP-13 in comparison with those of the 10 % compression state. Furthermore, the up-regulation of collagen type X-alpha I and interleukin-8 (IL-8), an essential pro-inflammatory cytokine, expressions was revealed in the hyper-physiological condition, exhibiting the OA traits, namely HACs' hypertrophic differentiation to transient chondrocytes and inflammation, in the 30 % compression state. Thereafter, the effects of three commonly prescribed drugs named rapamycin, interleukin-1 (IL-1) receptor antagonist, and celecoxib were investigated in the model displaying OA-like features. The drugs diminished the expressions of MMP-13 (about 0.1 to 0.55-fold reduction versus the untreated control) and IL-8 (nearly 0.055 to 0.7-fold reduction versus the untreated control), with IL-1 receptor antagonist causing the maximum reduction in both of them. Consequently, hyper-physiologically-based cartilage-on-a-chip models can be designed by exerting specific compression forces similar to the mechanical cues involved in the OA pathogenesis. These models enable anti-OA drug candidates' screening, accelerate drug discovery procedures, and diminish demands for animal models. In the same year, a group of scholars ^[371] designed a microfluidic chondrocyte culture-on-a-chip for two purposes: 1) comparing the microfluidic model to the 2D monolayer culture and 2) as an in vitro inflammation-induced OA model. Primary equine

chondrocytes (3×10^6 cells mL^{-1}) were embedded in the fibrin hydrogel (20 mg mL^{-1}), and then the complex was loaded into PDMS semi-circular tissue chambers with uni-directional nutrient concentration gradients. The cells were re-differentiated due to the 3D gradient-based conditions, were highly viable after twenty-eight days of culture (**Figure 22.A**), exhibited spherical shapes (**Figure 22.B**), and had neither proliferation nor proteoglycans secretion (**Figure 22.C**). By comparing the microfluidic chondrocyte cultures ($3000 \text{ cell mm}^{-3}$) with monolayer control cultures ($2500 \text{ cell mm}^{-2}$), several results were revealed: 1) The increment of spherical cellular morphology from 84 % to 99 % of round cells (day one to day eight) in the 3D cultures was observed, which remained at this level during the rest of the 3D culture (**Figure 22.D(I)**), 2) The lowest metabolic activities related to the cells cultured in the 3D hydrogels on the chip were seen, whereas the highest metabolic rates were present within the 2D monolayer culture; indeed, the 7-fold reduction in the metabolic activity of cells cultivated in the 3D culture resembled the cells' low metabolic activity observed in vivo (**Figure 22.D(II)**), and 3) The up-regulation of all chondrogenic markers' expressions and down-regulation of collagen type I- α I expression in the 3D cultures after two weeks of cultivation were illustrated, thereby confirming the stable chondrogenic phenotype's establishment (**Figure 22.D(III)**). **Figure 22.D(IV)** illustrates the time-dependent changes in gene expressions during the culture periods, which supported the earlier chondrocytes' re-differentiation on the chip. Eventually, the micro-device was put under an inflammatory environment to simulate a biochemical injury associated with OA. Following twenty-four hours of biochemical stimulation employing 50 pg mL^{-1} IL-1 β and TNF- α , the expressions of all inflammatory mediators like ADAMTS5 were up-regulated, indicating the initiation of hypertrophic differentiation and thus supporting the beginning of an OA micro-environment. Furthermore, verification of this device as a disease model was conducted via simulating a common clinical OA therapy, corticosteroid triamcinolone's intra-articular injection. In this regard, $60 \text{ }\mu\text{g mL}^{-1}$ triamcinolone was used alongside the twenty-four hours of the stimulation mentioned earlier, and a significant decrease in the inflammatory markers' expression was demonstrated, thus illustrating the suitability of the proposed microfluidic culture for finding applications as an inflammation-induced OA model. However, the absence of biomechanical stimulation and lack of corresponding synovial fluid were the main

limitations of the present study. Following their studies on designing a microfluidic-based dual-chamber bioreactor for producing hBMSCs-derived OC constructs, Lin and a team of scholars^[372] decided to substitute hBMSCs with iPSCs-derived mesenchymal progenitor cells in order to develop an OC tissue chip derived from this population of cells. That work was established based on overcoming several challenges related to MSCs, such as finite capacity of expansion, decreased potential of differentiation with increasing the culture passage, and donor-to-donor variability, to name but a few, by means of using human iPSCs. This type of cells, with infinite proliferative capacity and chondrogenic/osteogenic potential, were firstly induced into an MSCs-like state (iPSCs-derived mesenchymal progenitor cells) utilizing an well-established protocol. Subsequently, the iPSCs-derived mesenchymal progenitor cells (20×10^6 cells mL^{-1}) were loaded into GelMA solution (15 % w v^{-1}) and cultivated in the dual-flow microbioreactor employed previously by this team under analogous circumstances. The optimized chondrogenic and osteogenic media were supplied through two conduits in the top and bottom compartments, respectively, for four weeks in the microtissue bioreactor. In the biphasic model, the chondral and osseous components of the OC model were abbreviated as OC-C and OC-B, respectively. Besides, chondral tissue-only constructs (CH) were fabricated via inserting the cell-laden suspension in the top compartment and filling the bottom chamber with cell-free GelMA solution, followed by the photo-cross-linking process. After four-weeks of culture, analyses demonstrated the successful formation of biphasic OC constructs. Indeed, the OC-C part showed higher expressions of collagen type II and aggrecan compared to the bottom section, and the OC-B displayed greater expressions of osteocalcin and bone sialoprotein. Additionally, the formation of a monophasic construct was verified owing to the observed elevated expressions of collagen type II and aggrecan in comparison with the undifferentiated tissue. Interestingly, the chondrosupportive behavior of OC-B was revealed due to the remarkably higher expressions of aggrecan, collagen type II, and increased deposition of GAGs as well as significantly lower expressions of Runx2 and MMP-13 displayed by the OC-C section than those of the CH construct. Thereafter, the OC-C was treated with IL-1 β (1 ng mL^{-1}) for one week so as to assess the communication between chambers. It was illustrated that the IL-1 β treatment led to several outcomes: 1) the diminished expressions of aggrecan and collagen type II in CH and OC-C constructs, 2) the

stronger impact of treatment on the collagen type II expression in OC-C as compared to that of the CH (20-and 5-fold decrease, respectively), and 3) considerably higher fold change of MMPs in OC-C than CH tissue, all of which indicated the aggravated degeneration of OC-C in the presence of OC-B under the treatment with IL-1 β . Ultimately, celecoxib with the concentration of 10 μ M was administered via introducing in both components or only OC-C section of the biphasic model (systemic and intra-articular routes, respectively). After one week, considerably suppressed expressions of inflammatory and catabolic factors were reported, with better achieved therapeutic effects of the drug when used for both components. Moreover, the osteoprotective impact of celecoxib was confirmed because of the partially rescued expression of osteocalcin and down-regulated expression of IL-1 β and IL-6. Multiple issues require evaluation in the near future: 1) the problem of not observing a tidemark-like structure in the chip, 2) the absence of other joint tissues, namely adipose and synovium tissues, in the model, and 3) the investigation of merely one drug in the OA-induced platform. Taking all the points into account, the designed model is of great value as a drug screening platform for potential DMOA drugs.

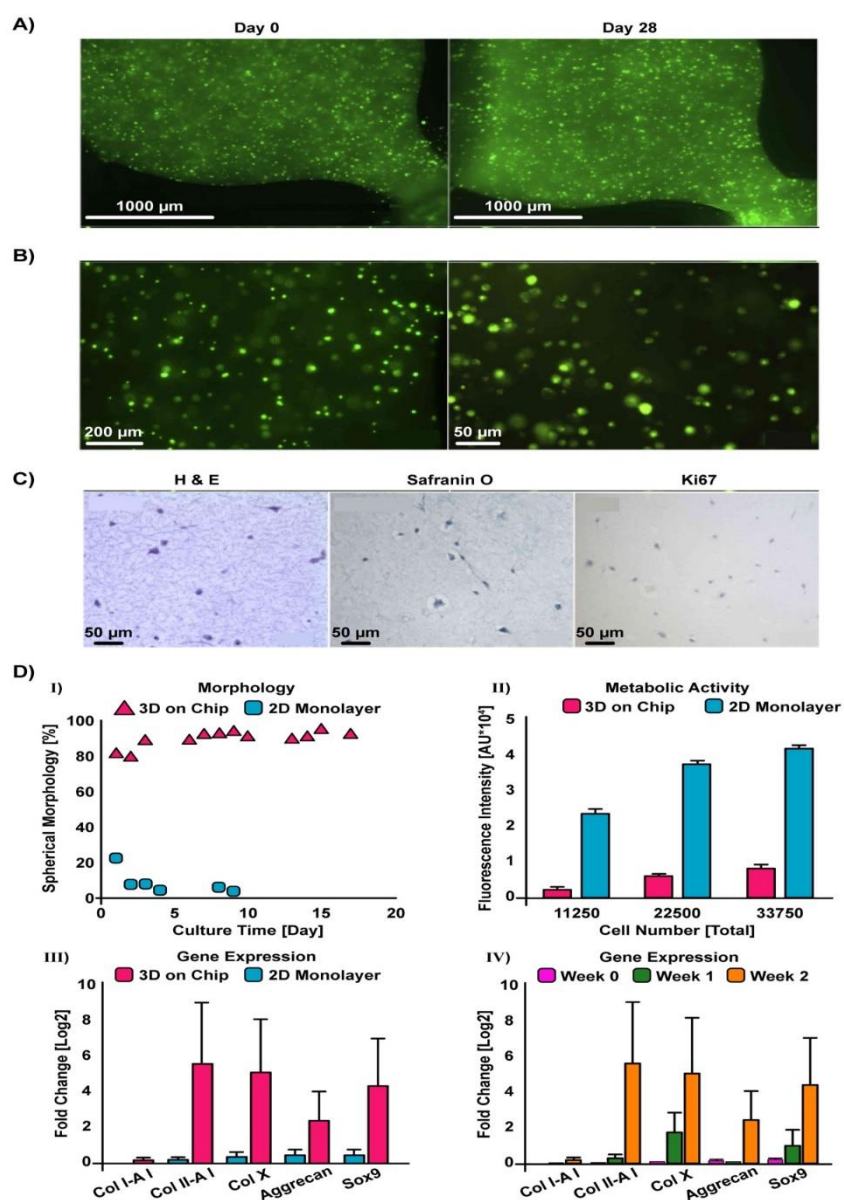


Figure 22. A) The cells' on-chip live staining after twenty-eight days of culture (right) and subsequent to loading at day zero (left), which confirmed high cellular viabilities within the cultivation period. B) The cells stained with the 5-chloromethylfluorescein diacetate dye in the culture compartment displaying round morphology. C) The chondrocyte-on-chip cultures' histological staining with Ki67 (right), safranin-O (middle), and hematoxylin and eosin (left). D) I) The cell morphologies' comparison between the 2D monolayer and the 3D cultures. II) The metabolic activities of 2D cultures compared to the 3D ones. III) The patterns of gene expression for 3D cell

cultures in comparison with the monolayer ones after two-weeks of culture, and IV) the alterations in the gene expression during culture. Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[371] Copyright 2019, Elsevier.

It is widely accepted that the chief reason behind the occurrence of RA and OA is synovial inflammation. Healthy synovium consists of a lining layer including condensed cells such as fibroblast-like synoviocytes and a loosely-organized sublining layer made up of scattered fibroblasts, mast cells, ECM, macrophages, blood vessels, nerves, and lymphatics.^[373] On the other hand, during the RA and OA diseases, synovial inflammation and hyperplasia, promoted by cytokines, can be observed, and synovium shows the lining layer thickening and cell condensation in the sublining layer.^[374,375] Nonetheless, since the mechanisms of these phenomena are not clearly understood, there is a dire need to produce reliable models for evaluating them.^[314] Concerning this subject, Rothbauer and co-workers^[376] decided to develop the first synovium-on-a-chip with non-invasive sensors in 2020 to better realize the initiation as well as the progression of inflammatory RA in response to stimulation with a proinflammatory cytokine, TNF- α in particular. The designed chip included time-resolved biosensing based upon light scatter measurements and micro-chambers containing synovium organoids formed by a mixture of non-induced or TNF- α -induced RA-derived human fibroblast-like synoviocytes (3000 cells μL^{-1}) and the Matrigel or commercial Tisseel fibrin hydrogel. Briefly, they incubated RA-derived human fibroblast-like synoviocytes without/with TNF- α (10 ng mL^{-1}) and then mixed the suspension with the Matrigel/commercial fibrin, followed by loading the mixture into the ring-shaped PDMS phase guides positioned within the culture chambers and the occurrence of gelation at 37 °C. The commercial Tisseel fibrin hydrogel was proved to be incompatible for the 3D biosensing approach owing to its turbidity, whereas Matrigel was compatible with their optical setup. The generation of a thin lining layer at the hydrogel-fluid interface and a loosely-connected sublining layer containing inter-connected synoviocyte network over a duration of three to four days were

confirmed by means of histological analysis of the 3D non-induced synovial organoid's cross-section. Furthermore, it was revealed that the collagen type I and type II fibers' deposition by fibroblast-like synoviocytes into the hydrogel matrix has occurred. Indeed, this deposition of reticular organotypic fibers at day four post-seeding demonstrated the formation of a healthy and functional 3D synovial organoids in the synovium-on-a-chip platform, which was chosen as the control group. Of note, no discernible loss of cellular viability in the organoids due to photo-toxicity was seen after eight days, which showed the light-scattering approach's suitability for biological applications. More importantly, signal gains of 16 % and 21 % in the presence of TNF- α on days three and four, respectively, were observed, indicating the synovial organoids' structural changes after stimulation. After four days of cultivation, further analyses revealed network remodeling, cellular hyperproliferation, thickened synovial lining layer, aggregated synovial networks, inflammatory tissue response, multi-layered network protrusions, over-expression of B-cell activating factor (1.6 ± 0.1 -fold increment), MMPs' activity increase (nearly 5 and 15-fold increment for matrix metalloproteinase-1 (MMP-1) and matrix metalloproteinase-3 (MMP-3), respectively), and enhanced cell migration for induced organoids compared to the non-induced control group, all of which illustrated inflammatory arthritis's onset and progress following the stimulation. Hence, the proposed device served as a rapid screening tool that could distinguish a diseased phenotype as early as possible (day 2-3 post-seeding versus 14 to 21 days post-seeding for conventional 3D microtiter-based synovial models). In an attempt to study the influence of different mechanical stimuli upon chondrocytes, a PDMS OoC device consisting of a mechanical actuation chamber that incorporated three sub-chambers, a human chondrocyte/agarose hydrogel chamber, and a perfusion chamber for providing nutrients to the construct, as well as arrays of pillars, was devised (**Figure 23.A**). Uniform shear and/or compressive strains (0-700 mbar) were exerted on the chondrocyte-embedded agarose hydrogel via actuation chambers, and the hydrogel was filled with 15 μ m microbeads in order to assess its deformation upon the stimulation. It was revealed that the higher pressure led to the higher strain, and mimicking both pathological (> 20 %) and physiologically healthy (5-20 %) strains were possible in this platform. Cellular viability was investigated at various mechanical stimulation conditions (0, 300, and 700 mbar applied at 1 Hz for 1 hour each day, initiating from day 1). Although cells cultured in the physiological situations remained

viable, those cultivated in the hyper-physiological conditions died after stimulation for three days, in the superficial zone in particular (**Figure 23.B**). In the next test, cells were cultured in three states, *i.e.*, static, compression, and compression/shear strains, to further determine the impact of mechanical stimulation on them. The formation of the pericellular matrix was enhanced due to the mechanical stimulation, and the combination of shear and compression strains significantly improved GAGs production (**Figure 23.C**). Therefore, this innovative microfluidic platform could be utilized to study the influence of different mechanical stimuli on chondrocytes. In addition, mimicking hyper-physiological and healthy stimulations was possible via this apparatus.^[377]

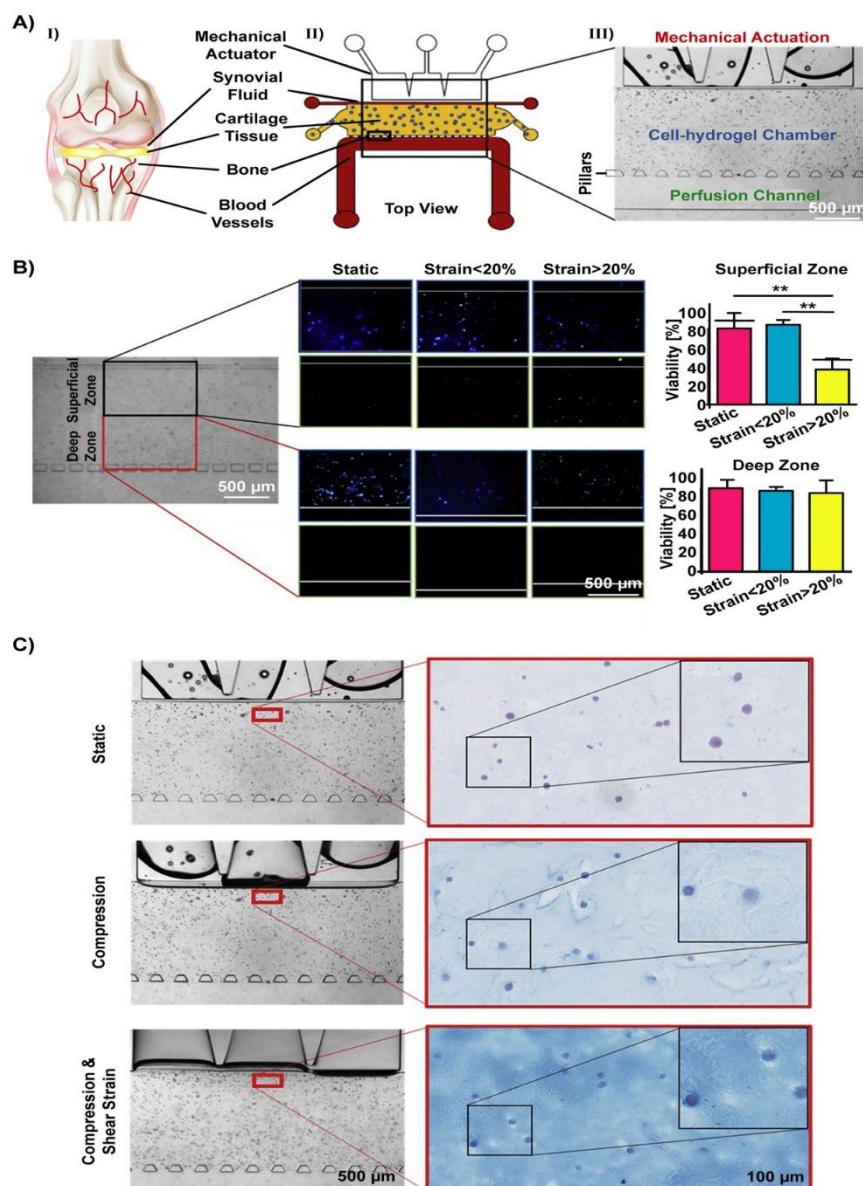


Figure 23. A) I) The knee joint's schematic presentation. II) Top view, and III) Schematic of the platform consisting of a mechanical actuation chamber, a cell/hydrogel compartment, arrays of pillars, and a perfusion channel. B) The left image depicting the system's top view that had a sub-division in two zones: the superficial zone (black frame) adjacent to the PDMS membrane and the deep zone (red frame) adjacent to the pillars. The middle figure depicting live & dead assay results after four days in two mentioned zones for the three conditions: static (no stimulation), strain < 20 % (healthy), and strain > 20 % (hyper-physiological). In this figure, the colors blue and green present living and dead cells, respectively. The right image depicting quantitative cell viability in the two zones (**p < 0.05). C) The system's transmitted image in the three conditions (left) and the hydrogel superficial part's

histology slide with cells in red/purple (given by nuclear fast red) and GAGs in blue (given by Alcian blue staining). The ring surrounding the cells presents the formed pericellular matrix after fifteen days of cultivation (stimulation for 14 days, 1 hour, 1 Hz, and 300 mbar). Reproduced under the terms of the Elsevier user license.^[377] Copyright 2020, Elsevier.

Anti-TNF therapies have been effectively employed in the RA treatment; nonetheless, it is still questionable whether therapeutic efficacy can be preserved over the time when they are linked to dendrimer NPs and encapsulated within a hydrogel.^[378,379] In this regard, Oliveira et al.'s investigation^[380] about the therapeutic impact of a novel NPs-loaded hydrogel on the modulation of inflammatory RA was conducted by utilizing an inflammatory cartilage-on-a-chip model. In this context, human chondrogenic primary cells (hCH) as target cells and human leukemia monocytic cell line (THP-1), both at a density of 2×10^6 cell mL⁻¹, were encapsulated in Matrigel, followed by injecting the mixture into the microfluidic platform's two lateral compartments and stimulating the THP-1 cells with lipopolysaccharide to create an inflamed environment. The central channel included the tyramine-modified GG hydrogel loaded with monoclonal anti-TNF- α antibody bound to CS-modified poly(amidoamine) dendrimer NPs, as the drug delivery vehicle. Within the inflamed condition, sole THP-1 cells induced the death of hCH cells; however, it was lower in the presence of the NPs-loaded hydrogel. After seven days of culture, although inflamed THP-1 cells negatively affected the hCH cells' metabolic activity (a 4-fold decrease in comparison with the hCH cells co-cultured with healthy THP-1), the hydrogel presence led to an increase in the metabolic activity (a nearly 2-fold increase compared to the previous condition). Furthermore, the collagen type II was highly expressed when the NPs-loaded hydrogel was used in the inflamed environment in comparison with the hydrogel-free environment having very low collagen type II expression. Since the process of inflammation induces the degradation of collagen type II, the obtained results suggested the possible therapeutic efficacy of the novel hydrogel-based drug delivery system. In spite of the favorable outcomes, these analyses were carried out in the normoxia condition, but hypoxia is the central characteristic of the inflamed cartilage. In essence, the enhanced consumption of oxygen by inflamed cells and the immune cells

infiltration plus an interrupted blood supply as a result of the vascular dysfunction cause tissue hypoxia in RA. Bearing this issue in mind, future investigations should focus on evaluating this model in a hypoxic condition so as to examine whether it would affect the results, and to precisely imitate the inflammatory environment of cartilage. In a nutshell, a preclinical model was developed in this experiment which enabled testing the therapeutic effect of an NPs-loaded hydrogel in an in vivo-like inflammatory RA-related condition. As mentioned earlier, synovial inflammation is an OA's active component, and OA synovium displays pathological alterations like macrophages' abnormal infiltration originating from circulating monocytes. Regarding this matter, in addition to DMOA, other OA treatments can be inflammation-targeting therapies that inhibit the progression of this disease.^[381] In order to better understand the monocytes' excessive recruitment to the synovium and target this process as a proper approach to hamper the negative effects related to macrophage accumulation, a group of scholars^[382] designed the first organotypic microfluidic model to particularly evaluate monocyte extravasation in the context of the OA. The study proposed a platform consisting of a channel for the injection of synovial fluid resembling an articular cavity, an endothelialized channel, an AC compartment, and two synovial compartments. Synovial fibroblasts (5×10^3 cell mL⁻¹) and HACs (2.5×10^6 cell mL⁻¹) embedded in the fibrin gel (10 mg mL⁻¹) were loaded into synovial and cartilage compartments, respectively, and human umbilical vein endothelial cells (6×10^6 cell mL⁻¹) and human primary monocytes (4×10^6 cell mL⁻¹) were loaded in the endothelialized channel to which fluid flow (30 μ L h⁻¹) and biochemical stimulation with 10 ng mL⁻¹ TNF- α , two factors of an inflamed environment as stated, were applied. As the final step, a known mixture of chemokines (C-C motif chemokine ligand 2 to 5) was injected into the synovial fluid chamber to further simulate the OA condition.

Fibrin gel is an excellent biomaterial due to several reasons, including providing cells with a biocompatible natural matrix, offering a physiological substrate that can be conducive to the endothelial assembly, possessing structural features reminding those of the collagenous matrix, and displaying intrinsic tendency to be gradually degraded and substituted by the ECM generated from fibrin-incorporated cells, thereby justifying its selection. Notably, monocyte extravasation happened

mostly toward the synovial fluid channel in which they added the chemokine mix, whereas a very few extravasated monocytes were identified in both of the synovial compartments within the control chips where the mix was not injected. It was also illustrated that the endothelial inflammatory preconditioning was essential to promote the extravasation process in response to the chemokines within the first hours. Furthermore, when anti-chemokine drugs, namely RS-504393 and cenicriviroc, were tested, the monocyte extravasation in reaction to synovial fluid was significantly diminished, with cenicriviroc leading to the most marked reduction, indicating their ability to inhibit monocyte extravasation within an organotypic setting. Multiple limitations existed in this assessment, two of which were the absence of a bone-mimicking compartment since adding osteoclasts and osteoblasts would have brought major complications regarding finding common culture conditions appropriate for all cell types and the unclarified effect of extravasated monocytes upon the cartilage compartment, thus suggesting the need for further investigations. Interestingly, this platform can be used to either investigate the impact of extravasated monocytes on OA conditions or to assess the capability of anti-chemokine drugs to antagonize the process of extravasation.

Several studies have employed the technique of cell shape engineering, *a.k.a.* cell imprinting, to induce the differentiation of stem cells.^[383–385] In this method, target cells are cultivated upon the cell culture plate's surface and fixed with a fixative solution. Thereafter, the cell membrane is patterned with PDMS and employed as a new substrate for the culture of stem cells. Employing cells as a template, upon which a mold made of silicon is cast, patterns are obtained, and the cells' topography can be imprinted on the cured substrates. It has been illustrated that the geometry of the imprinted substrates serves an essential role in the cultivated stem cells' cytoskeleton tension, which in turn exerts a significant impact on their differentiation.^[386–388] Concerning this matter, Yazdian Kashani and co-workers^[389] employed a microfluidic chip to generate a biomimetic cell-imprinted substrate for cultivation and subsequent differentiation of adipose-derived MSCs. In the first step, they placed a microfluidic chip upon a cell culture plate having channels side facing downward, followed by injecting a chondrocyte-containing solution (6×10^6 cell mL⁻¹) into the chip and incubating the set for seven hours. Then, the chip was removed from the plate, PDMS casting was performed for

transferring the cells' pattern to the PDMS, and finally attached cells were eliminated via thorough rinsing. The cell-imprinted PDMS substrate and another chip were bonded together at the final step. Adipose-derived MSCs (3×10^6 cells mL^{-1}) were inserted into the microfluidic device and cultured for two weeks. It was revealed that the spindle morphology of stem cells was changed into spherical morphology of chondrocytes fourteen days post-cultivation without utilizing any chemical GFs. Furthermore, enhanced expressions of collagen type II and collagen type II collagen type I⁻¹ ratio (2.3-fold increment) compared to those of cells cultured using traditional cell-imprinting approaches were observed, demonstrating the successful chondrogenic differentiation of stem cells. In brief, microfluidic-based imprinting method's is of great value for inducing stem cells' chondrogenesis. Following their previous investigation on developing a synovium-on-a-chip model ^[376], Rothbauer et al. ^[390] engineered a chip-based 3D chondro-synovial co-culture joint model so as to evaluate the reciprocal cross-talk between chondral and synovial organoids. In this regard, RA-derived human fibroblast-like synoviocytes (3000 cells μL^{-1}) were mixed with Matrigel, followed by loading the cell-embedded suspension into the chip's synovial chamber and the occurrence of gelation at 37 °C. Of note, HACs encapsulated in commercial Tisseel fibrin hydrogel have displayed good survival, morphology, and cartilage-specific functions independently of the cell culture approach in previous experiments. Thus, HACs (2000 cells μL^{-1}) were combined with Tisseel fibrin suspension, followed by injecting the mixture into the chip's opposite chondral chamber and the occurrence of polymerization at 37 °C. In this design, opposing organoid chambers permitted the tissue-level soluble communication by reciprocal signaling. The formation of stable synovial and chondral 3D organoids was confirmed after five weeks of culture. They compared the developed co-culture system with mono-culture synovial and chondral organoids formed by the same device except having a single cultivation chamber. Several results were obtained by comparing these systems: 1) Synovial organoids under the co-culture condition exhibited a roughly 4-fold decrease in the initial area of organoid during twenty-eight days of cultivation, which was analogous to that of mono-cultured organoids throughout twenty-one days of culturing, 2) The chondral organoids were more influenced by the tissue-tissue cross-talk and gained nearly 22 % organoid area when co-cultured (organoid area of $2.9 \pm 1.3 \text{ mm}^2$) compared to $0.65 \pm 0.33 \text{ mm}^2$ for mono-culture organoids at day twenty-one, 3)

After three weeks of culture, high cellular survival of 84 % and 91 % for synovial and chondral organoids, respectively, 4) Higher numbers of round chondrocytes for co-cultured chondral organoids relative to mono-cultured ones were observed, and 5) No significant alterations in secretion levels of MMP-13, IL-6, and IL-8 at day three were seen for the co-culture system. In a nutshell, the proposed chondro-synovial chip model could be successfully employed to investigate tissue cross-talks and can be further utilized to assess how these interactions stimulate the RA disease.

Within the 2022, in an attempt to develop a biomimetic in vitro OA model that could contain functional cartilage and bone tissue units, an OoC OA disease model without including a bioreactor was devised, incorporating cartilage and bone tissue units. The chip comprised a chondrogenic channel and an osteogenic channel separated via an electrospun PCL scaffold that consisted of a microfiber layer seeded with primary osteogenic cells (6.0×10^6 cells mL^{-1}) and a nanofiber layer seeded with primary articular chondrocytes (1.5×10^7 cells mL^{-1}). It was illustrated that the expressions of collagen type II, aggrecan, and osteopontin in the chip were high and analogous to the pellet cultures, thus suggesting that high-quality neo-cartilage and neo-bone matrices were deposited within the device after twenty-eight days. Furthermore, multiple cartilage and bone stainings revealed similar results, as follows: 1) The formation of a loose bone-like matrix beneath the dense cartilage-like matrix possessing a fairly high count of nuclei which lacked a calcified zone (**Figure 24.A(I)**), 2) Higher GAGs content in the chondrogenic zone than the osteogenic region (**Figure 24.A(II)**), and 3) Deposition of calcium in the osteogenic compartment but not within the neo-cartilage, with a relatively low rate of mineralization (**Figure 24.A(III)**). Subsequently, exposing the channels to the hypertrophy-inducing triiodothyronine hormone for five days was performed so as to simulate the OA condition in the dual-tissue model. The up-regulation of hypertrophy markers, namely integrin binding sialoprotein (5.04-fold change), ALP (2.83-fold change), and Runx2 (1.93-fold change), compared to the control groups in the chondrogenic compartment and the up-regulation of integrin binding sialoprotein (2.29-fold change) and ALP (2.57-fold change) as compared to the control groups in the osteogenic compartment were observed (**Figure 24(B)**). This result indicated that the designed dual-tissue chip can be used as a hypertrophy-induced OA model to simultaneously allow

evaluating the alterations in cartilage and bone. Nevertheless, several issues should be considered in the coming experiments: 1) Owing to the absence of a calcified zone and the relatively low rate of mineralization, the bone-like matrix was not mature yet. Thus, it can be optimized by means of homogenizing the osteogenic cells' distribution over the matrix or prolonging the cultivation period, 2) Mechanical stress, an essential trigger to the OA onset as stated, was not applied to the presented model. Hence, it would be of real value to add an actuation chamber to the system for exerting stress, 3) Not all the cell types were incorporated into the model. Consequently, implementation of other cells, such as immune cells, adipocytes, and synoviocytes will be desirable, 4) The cells were cultured under the normoxic condition (20 % oxygen), but *in vivo*, chondrocytes and cells within subchondral bone reside under hypoxic conditions (0-5 % and 5-10 % oxygen, respectively). Therefore, embedding of an oxygen gradient in the chip may be beneficial, and 5) Utilizing finite cell sources, scholars were unable to evaluate high-impact mutations in the interacting joint tissues. As a result, using another cell source like iPSCs for this model can be favorable.^[265]

In one of the most recent studies, Li and a team of scientists^[391] addressed the limitation associated with the absence of some joint tissues, namely adipose and synovium tissues, in the OA models by designing an OoC platform, referred to as miniJoint, that incorporated synovial-like fibrous tissue, OC complex, and adipose tissue. In brief, hBMSCs (20×10^6 cells mL⁻¹ for all except fibroblasts) were suspended in 15 % w v⁻¹ GelMA and the resultant suspensions were pipetted in the microfluidic-based bioreactor's inserts, followed by photo-cross-linking with UV light. Firstly, inserts containing cell-laden hydrogels were assembled in an OC dual-flow chip, and then perfusion of chondrogenic and osteogenic media through the bottom and top streams, respectively, was performed for twenty-eight days. Secondly, inserts incorporating cell-loaded hydrogels were cultivated within the adipogenic medium for the same amount of culture time. Thirdly, stem cells (2×10^3 cells cm⁻²) were cultured in fibrogenic medium in flasks for twenty-one days to achieve fibroblast-like cells, which were then suspended in 15 % w v⁻¹ GelMA, injected in the inserts, and photo-cross-linked. They integrated all microtissues into the chamber of miniJoint, where relevant media flowed on corresponding microtissues' tops and a commonly shared medium, simulating the synovial fluid's

function, flowed in the bottom stream. After twenty-eight-days of differentiation, higher expressions of osteocalcin in OC-B component, aggrecan and collagen type II in OC-C part, primary adipogenic marker genes in the adipose section, and fibrogenic markers in the engineered synovial-like fibrous tissue were observed. Notably, individual microtissues exhibited well-preserved tissue-specific phenotypes. In order to challenge the synovial-like fibrous tissue, introduction of IL-1 β (10 ng mL⁻¹) into the fibrogenic medium for one week was performed. Indeed, other tissues were indirectly exposed to the IL-1 β by the synovial-like fibrous tissue's secretome diffused in the shared medium. In this regard, up-regulated expressions of main catabolic genes in synovial-like fibrous tissue's chamber, and diminished expressions of aggrecan and collagen type II in OC-C section were observed. Furthermore, altered gene expressions related to the pathologic changes in OC-O compartment, and enhanced expressions of lipoprotein lipase and adiponectin in the adipose part were revealed. They also reported the up-regulated expression of nerve GF, an essential mediator of joint pain sensation, after treatment compared to the control groups. After the treatment with IL-1 β for three days, naproxen sodium, an anti-inflammatory nonsteroidal drug, with a concentration of 5 $\times$ 10⁻⁶ M was added to all streams and remained for a total of four days. Of note, the IL-1 β treatment continued for another four days (seven days in total as mentioned above). Moreover, four potential DMOA drugs comprising sclerostin protein (250 ng mL⁻¹), fibroblast GF-18 (100 ng mL⁻¹), IL-1 receptor antagonist (250 ng mL⁻¹), and adavivint (1 $\times$ 10⁻⁸ m) were introduced in the shared medium to imitate the intra-articular use and remained for a total of four days. Treatment with naproxen sodium resulted in the down-regulated expressions of MMP-3 and MMP-13 in synovial-like fibrous tissue's section, reduced expressions of nerve GF and osteopontin in OC-C compartment, diminished expressions of lipoprotein lipase and adiponectin in the adipose part, and suppressed expression of ALP in the OC-O section in comparison with the control groups. Regarding testing the other drugs, considerable up-regulation of chondrogenic genes and significant down-regulation of IL-6 in the model were reported. In the future, including a mechanically active mechanism in the miniJoint, exploring hydrogels with various microstructural and mechanical features, incorporating immune cells like macrophages in the model, and testing a wider range of drugs should be taken into account.

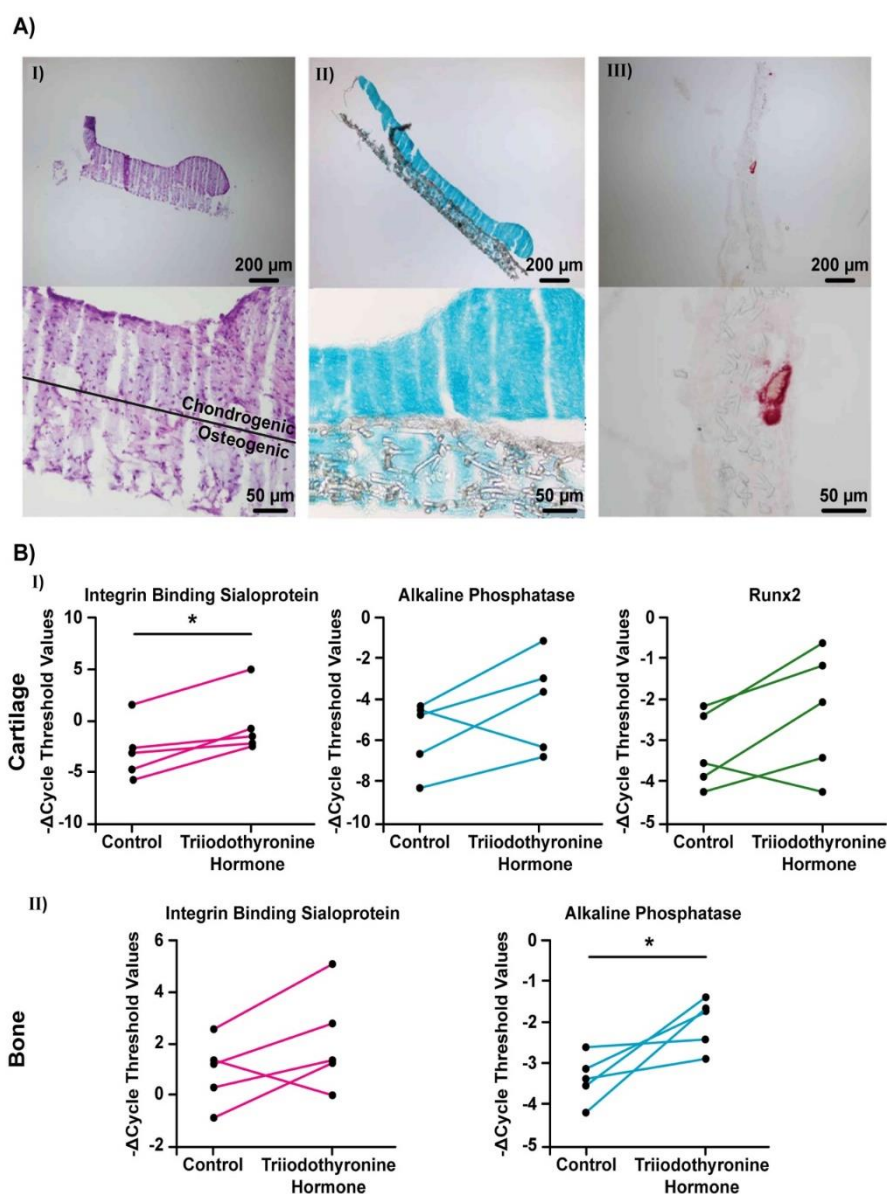


Figure 24. A) The immunohistochemistry figures on the OC unit-on-a-chip platform's cross-sections: I) Hematoxylin and eosin staining; II) Alcian blue staining; and III) Alizarin red staining. **B)** The hypertrophy markers' gene expressions in: I) the chondrogenic chamber, and II) the osteogenic chamber after the addition of triiodothyronine hormone (* $p < 0.05$). Reproduced under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.^[265] Copyright 2022, Wiley.

Table 6 is a summary of cartilage-related OoCs' experiments. The present gaps in translating the preclinical discoveries to clinical applications have been the primary motives for tissue engineers to develop innovative OoCs that are capable of closely mimicking the physiological and pathophysiological functions of the tissues and organs.^[392] Since the introduction of OoCs technology, diverse experiments have been conducted so as to explore their features as well as applications for tissues bioengineering such as cartilage TE, in particular. By surmounting the chief obstacles and taking into account the bigger picture, scientists will be able to enter the next phase of this intriguing field of cartilage-related OoCs.

Table 6. Summary of cartilage-related OoCs' experiments.

OoCs' main structure	Cell source	Results	Ref.
A PDMS microfluidic device consisting of downstream 3D cell culture chambers filled with cell-loaded collagen type I gel and an upstream CGG capable of creating various GFs' concentrations	Rabbit articular chondrocytes	<p>-High survival of round chondrocytes (above 95 %)</p> <p>-Highest cellular proliferations in the concentrations of: 1) 57.14 ng mL⁻¹ IGF-1 (2.38-fold versus the control group), 2) 5.72 ng mL⁻¹ bFGF (3.85-fold versus the control group), and 3) 85.73 ng mL⁻¹ IGF-1/1.43 ng mL⁻¹ bFGF (4.76-fold compared to the control group)</p> <p>-No further promotion of proliferation in higher concentrations of IGF-1, mainly owing to the saturation of IGF-1 receptors in higher concentrations</p> <p>-Decreased rate of proliferation in the bFGF concentration beyond 5.72 ng mL⁻¹, because of the alteration in extracellular signaling mediated via this GF</p> <p>-Unknown mechanism of GFs' interaction, requiring more evaluations to define this interaction and specifying their application in cells expansion in vitro</p>	[328]

<p>A chip comprising cylindrical cavities on the polymethylmethacrylate plate loaded with cell/agarose gel suspension and microbioreactors on the PDMS layer filled with culture medium having desired pH values</p>	<p>Primary articular chondrocytes</p>	<p>-Obtained highly viable cells ($95 \pm 2 \%$)</p> <p>-Highly affected production of GAGs and lactic acid by the pH after a four-day culture, demonstrating the sensitivity of the biosynthetic and metabolic functions of cells to the environmental pH</p> <p>-Highest contents of GAGs and lactic acid in the pH value of 7.3 (0.08 and $7 \text{ mg million cells}^{-1}$, respectively)</p> <p>-Observed different GAGs and lactic acid contents in two cell culture formats (microfluidic and static culture) due to the dissimilar biophysical or biochemical micro-environments</p> <p>-Probable bias error resulted from the data extrapolation from a cell culture format to another one, requiring complementary investigations to perceive the mechanism causing these discrepancies</p>	<p>[330]</p>
<p>A perfusion microfluidic culture system including a photo-resist pillar array filled with cell-laden alginate gelled microbeads and a central micro-chamber having ODEP force</p>	<p>Primary steer articular chondrocytes</p>	<p>-Proportional augmentation of the calculated manipulation force with the increment of cells number in the microbeads</p> <p>-Observation of cellular viability that remained as high as $96 \pm 2 \%$ after four days of perfusion cell culture</p> <p>-Imitated cellular distribution in the AC's cross-section, with the reduction of cells per tissue unit with the increasing distance from the surface of cartilage, by the assembled 3D sheet-like construct that resulted from the packed microbeads after a four-day perfusion cultivation</p> <p>-Tightly packed cell-loaded gel microbeads enabled by the alginate hydrogel's elastic nature, permitting the ECM, produced by cells, to readily fuse in order to form a tissue construct having high integrity</p>	<p>[332]</p>
<p>A microfluidic device with a PDMS layer</p>	<p>Rat MSCs</p>	<p>-Higher collagen type I expression in cells under flow stimulus compared to those in the static control with a</p>	<p>[338]</p>

<p>allocated to the micro-channel networks, a middle layer composed of aligned PLGA nanofibers, cell-embedded micro-chambers, and a flow-driving system for generating oscillatory fluid flows</p>		<p>more significantly enhanced gene expression in the angle of 0° with 1 dynes cm^{-2} shear stress in comparison with the other ones</p> <ul style="list-style-type: none"> -Higher secretion of sulfated proteoglycans in cells within 45° and 90° conditions -High up-regulation of Sox9, Runx2, and collagen type II expressions under both parallel and perpendicular flow conditions (1 dynes cm^{-2}) in comparison with the static condition, with MSCs cultivated under the perpendicular flow showing greatly higher expressions of collagen type II and Sox9 but a markedly lower Runx2 expression compared to those cultured under the parallel flow -Observation of fibrochondrogenic and fibroblastic phenotypes resulted from the perpendicular and parallel conditions, respectively -Inability to produce large-scale tissue constructs 	
<p>A microfluidic platform consisting of two sections: 1) A PDMS pool incorporating cell-loaded agarose gel and 2) A polycarbonate membrane linked to a microfluidic chip with three channels for loading various culture media (chondrogenic, normal, and osteogenic culture media)</p>	<p>Human adipose-derived MSCs</p>	<ul style="list-style-type: none"> -Higher calcium content of cultured cells in the osteogenic zone than that of the cultivated cells in the chondrogenic zone after twenty-five days of cultivation (45-fold change), indicating the successful stem cells' osteogenic differentiation within the osteogenic region -Higher GAGs expression in the chondrogenic region than that of the cells in the osteogenic region and interface zone (8-and 3-fold change, respectively), thus illustrating the stem cells' chondrogenic differentiation within the chondrogenic zone -Increased expressions of collagen type II, aggrecan, and Sox9 from the osteogenic to the chondrogenic zones -Observation of cells that fully differentiated to osteoblasts, mineralized chondrocytes, and chondrocytes 	<p>[341]</p>

		within the osteogenic, interface, and chondrogenic zones at the end of the culture period, respectively	
A microdevice containing four cell culture chambers loaded with cells linked to four resistance channels and a peristaltic pump, with the function of generating different values of fluid flow stress	Rat articular chondrocytes	<p>-Great up-regulation of aggrecan expression in the range of 0.007-1.2 dynes cm⁻² and markedly down-regulation of its expression at moderate and high levels of stress as compared to the static control</p> <p>-No observed significant differences on IL-6 expression at 1.2 and 3.1 dynes cm⁻² in comparison with that of the control</p> <p>-Considerably enhanced expression of IL-6 at moderate and high levels of stress by 4.5-fold and 9.6-fold, respectively, as compared with the static control</p>	[343]
A platform comprising four cell culture chambers loaded with cells linked to resistance channels and a peristaltic pump, with the function of generating various values of fluid flow stress	Primary rat chondrocytes and Primary rat MSCs	<p>-Significant augmentation of MSCs' proliferation rate at 0.231 and 1.089 dynes cm⁻² after twenty-four and forty-eight hours of perfusion in comparison with the 0.009 dynes cm⁻²</p> <p>-Considerable enhancement of chondrocytes' proliferation rate at 1.089 dynes cm⁻² after twenty-four hours and at 0.231 and 1.089 dynes cm⁻² after forty-eight hours</p> <p>-Unchanged localization of YAP in the nucleus of MSCs and its increased level in the nucleus with the increment of stress</p> <p>-Adipogenic differentiation of MSCs at low levels of stress and their osteogenic differentiation with augmenting levels of shear stress, being in accord with the YAP distribution in the nucleus and cytoplasm</p> <p>-Observed YAP that was mainly cytoplasmic in chondrocytes at low stress levels and its raised nuclear accumulation with the increasing magnitude of stress</p>	[345]

		<p>-Increased and diminished expressions of collagen type I and type II in chondrocytes, respectively, with enhancement of shear stress</p> <p>-Simultaneous chondrocytes' dedifferentiation with the YAP nuclear accumulation, demonstrating the effect of fluid flow upon YAP expression and the resultant phenotype of chondrocytes</p> <p>-Drastically greater nuclear YAP percentage within the two cells at 0.231 and 1.089 dynes cm⁻² as compared to 0.009 dynes cm⁻²</p>	
<p>A micro-cell culture system comprising microbioreactors with cell/alginate hydrogel-loaded cylindrical cavities capable of exerting dynamic compressive loadings</p>	<p>Primary articular chondrocytes</p>	<p>-Observation of cellular viability that remained as high as 95 % after five days of culture in all conditions</p> <p>-No change in cell proliferation as the result of the compressions</p> <p>-Nearly 18.77 % higher secretion of the lactic acid by cells cultivated under 40 % strain with 2 Hz frequency than that of cultured cells in the control group</p> <p>-No drastic difference in lactic acid secretion among the cells experiencing frequencies lower than 2 Hz</p> <p>-Probability of articular chondrocytes' metabolism up-regulation in the body by the vigorous exercise with high frequency due to the similarity with 40 % dynamic compression (2 Hz)</p> <p>-No significant influence on cells by the compressive stimulation's frequency with 20 % strain level</p> <p>-Increased GAGs synthesis with raising of the magnitude and frequency of loading, along with GAGs contents produced in 1 and 2 Hz (frequencies of walking and running states, respectively) being statistically higher than those generated in the control group (a phenomenon</p>	<p>[348]</p>

		not observed in the frequency less than 0.5 Hz)	
An impedance microfluidic chip with two electrodes with a suspension of cells between them	Primary human costal cartilage chondrocytes	<p>-Observed similar chondrocytes' dielectric features, such as membrane conductance, membrane capacitance, membrane folding factor, <i>etc.</i> to those of rat cardiomyoblasts, a cell type with extensive ion channels</p> <p>-Demonstration of ion channels as an essential component of the bioelectrochemical response and an active process in preserving the homeostasis of chondrocytes</p>	[349]
A dual-chamber bioreactor fitted into a microfluidic base containing MSCs-derived OC constructs	hBMSCs	<p>-Formation of biphasic OC constructs due to the increased expressions of chondrogenic genes (Sox9, aggrecan, and collagen type II) and enhanced expressions of osteogenic genes (osteocalcin, Runx2, and bone sialoprotein) of the cells within the chondral and osseous halves, respectively</p> <p>-Formation a tidemark between osseous and chondral halves after four weeks</p> <p>-Diminished expressions of chondrogenic genes when separately treating the chondral and osseous constructs with IL-1β, indicating the signaling between them</p> <p>-Significantly augmented expressions of MMPs in the chondral construct in response to the osseous section's cytokine treatment, demonstrating the obvious interactions between two components</p> <p>-Reduced expressions of bone sialoprotein and osteocalcin, as well as enhancements in the production of MMPs in the osseous construct after treatment of the chondral construct</p>	[352]
A cell-loaded microfluidic device with	hMSCs	-First condition's (0.1 dynes cm ⁻² shear stress for four days at a confluent state) outcome:	[227]

<p>a PDMS gradient generator capable of creating GF and fluid-flow induced shear stress gradients</p>		<ul style="list-style-type: none"> •Preferentially aligned hMSCs' long axis in the direction of flow compared to those cultured in the static condition (0 dynes cm^{-2}) showing alignment in tiny localized regions with differing alignment angles from each other and thus displaying no overall preferential alignment -Second conditions' ($0.12, 0.2$, and $0.4 \text{ dynes cm}^{-2}$ shear stresses for eight days at non-confluent and confluent states) outcome: •More alignment of cells within the confluent groups ($0-15^\circ$) than the non-confluent ones after eight days, and no observed significant difference in their impact on cell alignment degree for both groups varying in confluency in the shear stresses range, indicating the adequacy of shear stress presence for inducing cell alignment -Formation of more chondrogenic aggregates in the group exposed to the flow-based TGF-β_1 gradients compared to the control group with constant concentration (14 versus 8 aggregates in the control group), with the aggregates being more aligned and elongated with a higher area (0.376 mm^2 versus 0.160 mm^2 in the control group) and enhanced collagen type II deposition, showing the controlling effect of TGF-β_1 gradients on chondrogenic aggregates' structure 	
<p>A microfluidic platform including an upstream CGG capable of producing NLK gradients and cell-laden downstream cell culture chambers</p>	<p>Rat articular chondrocytes</p>	<ul style="list-style-type: none"> -Increment in proliferation within the NLK group with 12.85 ng mL^{-1} concentration compared to the control group treated with a 2 % serum-containing culture medium (3.96-fold increase) -No further promotion in concentrations higher than 12.85 ng mL^{-1} -Inhibited cultured cells' dedifferentiation by enhancing the collagen type II production in the NLK group with 	<p>[359]</p>

		12.85 ng mL ⁻¹ concentration	
An OC unit-on-a-chip model consisting of a chondrogenic region and an osteogenic region with two parallel microfluidic networks	Bovine MSCs	<p>-Remarkable up-regulations of collagen type I-α1 (29-fold) and Runx2 (86-fold) in the osteogenic region compared to the chondrogenic one after two weeks of cultivation</p> <p>-Significant up-regulations of aggrecan, Sox9, and collagen type 2-α1 in the chondrogenic region relative to the osteogenic one after two weeks of cultivation</p> <p>-Enhancement of collagen type X-α1 expression from the chondrogenic region to the osteogenic area across the construct</p> <p>-Highest and lowest content of GAGs in the chondrogenic and osteogenic regions, respectively</p> <p>-Production of thicker constructs compared to the conventional dual-chamber bioractors</p>	[159]
A PDMS chip consisting of cell culture chambers with a fluid resistance network able to generate shear stress gradients	Primary rat chondrocytes	<p>-Obtained high cellular viability (above 90 %) after twenty-four hours of various fluid shear stresses</p> <p>-Upward trend for the expression of filamentous actin as the fluid shear stress enhanced, with a significant enhancement after stimulating with 0.127 dynes cm⁻² shear stress</p> <p>-Increased cell area with the shear stress increment, indicating the promotion of the cellular adhesion by higher degrees of shear force</p> <p>-Up-regulation of collagen type I and type II expressions and their upward trend as the shear stress enhanced compared to the static condition, except for the 0.005 dynes cm⁻² shear stress effect on the collagen type II expression</p>	[360]
A microfluidic chip	Osteo-induced rat	-Obtained uniformly distributed, mostly viable cells with	[363]

comprising a PDMS top layer with a cell culture chamber and a PDMS bottom layer with three cell culture chambers capable of applying shear stress	MSCs, Rat articular chondrocytes, and Chondro-induced rat MSCs	more proliferation compared to the control group having no stimuli (nearly 1.5-fold enhancement) -Displayed flattened cell morphology and enhanced adhering area per cell on the surface of substrate -Significantly superior aggrecan and collagen type II expressions than those of the control group, with a 1.75-fold increment in both cases	
A microfluidic device comprising a cell/alginate gel constructs-loaded glass plate and PDMS balloons with different sizes capable of creating static compressive strains	Mouse chondrocytes	-Achieved cells' strain in the range of 7-19 % as a function of the PDMS balloon's diameter (1.2-2.0 mm), leading to the generation of 14-34 % compressive strain in the alginate gel (enhancement of the cells strain ~ 2.9 % per 0.2 mm increase in the diameter of the PDMS balloon) -Displayed highly heterogeneous behaviors of chondrocytes in response to the mechanical stimulation -Easy manipulation of cell compression amount via controlling the balloon size in the platform -Observed low viability of cells (58.6 %) after applying static compression compared to that of cells under the dynamic compression (85.6 %), mainly due to the decreased transport of solute in the static condition that might be associated with the nutrient transport	[368]
A micro-scale device including a top PDMS compartment that incorporated the cell-laden MMP-degradable PEG-based gel micro-construct and a bottom PDMS compartment able	HACs	-Reduction in the expressions of aggrecan and collagen type II-alpha I collagen type I-alpha I messenger ribonucleic acid ⁻¹ and increase in MMP-13 expression, resulted from a 30 % compression condition in comparison with those of the cells in a 10 % compression state -Up-regulation of collagen type X-alpha I and IL-8 expressions in the hyper-physiological condition,	[233]

to generate various values of compressions employing an electro-pneumatic actuation structure		<p>exhibiting the OA traits, namely HACs' hypertrophic differentiation to transient chondrocytes and inflammation</p> <p>-Diminished expressions of MMP-13 (about 0.1 to 0.55-fold reduction versus the untreated control) and IL-8 (nearly 0.055 to 0.7-fold reduction versus the untreated control), with IL-1 receptor antagonist causing the maximum reduction in both of them</p>	
<p>A microfluidic platform consisting of cell/fibrin gel-embedded PDMS semi-circular tissue chambers with uni-directional nutrient concentration gradients capable of response to the biochemical stimulation</p>	Primary equine chondrocytes	<p>-Re-differentiated cells owing to the 3D gradient-based conditions with spherical shapes</p> <p>-Highly viable cells after twenty-eight days of culture having neither proliferation nor proteoglycan secretion</p> <p>-Results obtained from comparing the microfluidic chondrocyte cultures with monolayer control cultures:</p> <ul style="list-style-type: none"> •Increment of spherical cellular morphology from 84 % to 99 % of round cells (day one to day eight) in the 3D cultures remaining at this level during the rest of the 3D culture •Lowest metabolic activities related to the cells cultured in the 3D hydrogels on the chip (7-fold reduction compared to the control cultures), resembling the cells' low metabolic activity observed in vivo and highest metabolic rates within the 2D monolayer culture •Up-regulation of all chondrogenic markers' expression and down-regulation of collagen type I-α1 expression in the 3D cultures after two weeks of cultivation, thereby confirming the stable chondrogenic phenotype's establishment <p>-Up-regulation of all inflammatory mediators' expression like ADAMTS5 after biochemical stimulation, indicating</p>	[371]

		<p>the initiation of hypertrophic differentiation and thus supporting the beginning of an OA micro-environment</p> <p>-Significant decrease in the inflammatory markers' expressions subsequent to corticosteroid triamcinolone's intra-articular injection and biochemical stimulation</p>	
<p>A dual-chamber bioreactor fitted into a microfluidic base containing iPSCs-derived OC and chondral tissue-only constructs</p>	<p>iPSCs-derived mesenchymal progenitor cells</p>	<p>-Formation of biphasic OC constructs after four weeks due to the higher expressions of collagen type II and aggrecan in the OC-C part compared to the bottom section, and the greater expressions of osteocalcin and bone sialoprotein in the OC-B part</p> <p>-Formation of a monophasic construct owing to the observed elevated expressions of collagen type II and aggrecan in comparison with the undifferentiated tissue</p> <p>-The chondrosupportive behavior of OC-B due to the remarkably higher expressions of aggrecan, collagen type II, and increased deposition of GAGs as well as significantly lower expressions of Runx2 and MMP-13 displayed by the OC-C section than those of the CH construct</p> <p>-Obtained several outcomes after treatment with IL-1β: 1) the diminished expressions of aggrecan and collagen type II in CH and OC-C constructs, 2) the stronger impact of treatment on the collagen type II expression in OC-C compared to that of the CH (20-fold and 5-fold decrease, respectively), and 3) considerably higher fold change of MMPs in OC-C than CH tissue, all of which indicated the aggravated degeneration of OC-C in the presence of OC-B under the treatment with IL-1β</p> <p>-Considerably suppressed expressions of inflammatory and catabolic factors, with better achieved therapeutic</p>	<p>[372]</p>

		<p>effects of the drug when used for both components after one week</p> <p>-Osteoprotective impact of celecoxib because of the partially rescued expression of osteocalcin and down-regulated expression of IL-1β and IL-6</p>	
<p>A microfluidic chip including time-resolved biosensors based upon light scatter measurements and micro-chambers containing synovium organoids formed by a mixture of cells and hydrogels</p>	<p>Non-induced and TNF-α-induced RA-derived human fibroblast-like synoviocytes</p>	<p>-Incompatibility of the commercial Tisseel fibrin hydrogel for the 3D biosensing approach owing to its turbidity and compatibility of Matrigel with the optical setup</p> <p>-Generation of a thin lining layer at the hydrogel-fluid interface and a loosely-connected sublining layer containing inter-connected synoviocyte network over a duration of three to four days employing the histology analysis of 3D non-induced synovial organoid's cross-section</p> <p>-Deposition of collagen type I and type II fibers by fibroblast-like synoviocytes into the hydrogel matrix</p> <p>-Demonstrated formation of a healthy and functional 3D synovial organoids in the synovium-on-a-chip platform owing to the deposition of reticular organotypic fibers at day four post-seeding</p> <p>-No discernible loss of cellular viability in the organoids due to photo-toxicity after eight days, showing the light-scattering approach's suitability for biological applications</p> <p>-Signal gains of 16 % and 21 % in the presence of TNF-α on days three and four, respectively, indicating the synovial organoids' structural changes after stimulation</p> <p>-Illustrated network remodeling, cellular hyperproliferation, thickened synovial lining layer, aggregated synovial networks, inflammatory tissue response, multi-layered network protrusions, over-</p>	<p>[376]</p>

		<p>expression of B-cell activating factor (1.6 ± 0.1-fold increment), MMPs' activity increase (nearly 5 and 15-fold increment for MMP-1 and MMP-3, respectively), and enhanced cell migration for induced organoids compared to the non-induced control group after a four-day cultivation, all of which illustrated inflammatory arthritis's onset and progress following the stimulation</p> <p>-Proposed device as a rapid screening tool distinguishing a diseased phenotype as early as possible (day 2-3 post-seeding versus 14 to 21 days post-seeding for conventional 3D microtiter-based synovial models)</p>	
<p>A PDMS OoC device consisting of a mechanical actuation chamber that incorporated three sub-chambers, a cell/agarose hydrogel chamber, arrays of pillars, and a perfusion chamber</p>	Human chondrocytes	<p>-Higher pressure leading to the higher strain</p> <p>-Ability to mimic both pathological (> 20 %) and physiologically healthy (5-20 %) strains via this platform</p> <p>-Observation of viable cells cultured in the physiological situations and dead cells cultivated in the hyper-physiological conditions after stimulation for three days</p> <p>-Enhanced formation of the pericellular matrix due to the mechanical stimulation</p> <p>-Significantly improved GAGs production owing to the compression/shear strains</p>	[377]
<p>A microfluidic platform comprising two lateral compartments filled with cell-laden Matrigel (as an inflamed environment) and one central compartment incorporating NPs-loaded modified GG</p>	THP-1 and hCH cells	<p>-Induced death of hCH cells within the inflamed condition by sole THP-1 cells, with a lower amount of death in the presence of the NPs-loaded hydrogel</p> <p>-Negatively affected hCH cells' metabolic activity (4-fold decrease in comparison with the hCH cells co-cultured with healthy THP-1) by inflamed THP-1 cells</p> <p>-Increase in the metabolic activity of hCH cells (a nearly 2-fold increase compared to the previous condition) due to the hydrogel presence after seven days of cultivation</p>	[380]

hydrogel drug delivery system		-High expression of collagen type II when using NPs-loaded hydrogel in the inflamed environment in comparison with the hydrogel-free environment having very low collagen type II expression, indicating the possible therapeutic efficacy of the novel hydrogel-based drug delivery system	
An organotypic OA-stimulated microfluidic model including a channel filled with synovial fluid, an endothelialized channel, an AC compartment, and two synovial compartments	Synovial fibroblasts, HACs, Human umbilical vein endothelial cells, and Human primary monocytes	<p>-Occurred monocyte extravasation mostly toward the synovial fluid channel with the chemokine mix</p> <p>-Presence of an extremely low number of extravasated monocytes in both of the synovial compartments within the control chips without the chemokine mix</p> <p>-Necessity of endothelial inflammatory pre-conditioning to promote the extravasation process in response to the chemokines within the first hours</p> <p>-Significant decrease in the monocyte extravasation in reaction to the synovial fluid when testing RS-504393 and cenicriviroc, with cenicriviroc leading to the most marked reduction, indicating their ability to inhibit monocyte extravasation within an organotypic setting</p>	[382]
A microfluidic device consisting of a cell-imprinted PDMS substrate and a chip loaded with stem cells	Adipose-derived MSCs	<p>-Modified morphology of stem cells from spindle morphology to spherical morphology of chondrocytes fourteen days post-cultivation without utilizing any chemical GFs</p> <p>-Enhanced expressions of collagen type II and collagen type II collagen type I⁻¹ ratio (2.3-fold increment) compared to those of cells cultured using traditional cell-imprinting approaches, demonstrating the successful chondrogenic differentiation of stem cells</p>	[389]
A chip-based 3D co-culture joint model	RA-derived human fibroblast-like	-Formation of stable synovial and chondral 3D organoids after five weeks of cultivation	[390]

containing two opposing synovial and chondral chambers	synoviocytes and HACs	<p>-A roughly 4-fold decrease in the initial area of organoid during twenty-eight days of cultivation under the co-culture condition, being analogous to that of mono-cultured organoids throughout twenty-one days of culturing</p> <p>-More influence exerted by the tissue–tissue cross-talk on the chondral organoids</p> <p>-Gained nearly 22 % organoid area when co-cultured (organoid area of $2.9 \pm 1.3 \text{ mm}^2$) compared to $0.65 \pm 0.33 \text{ mm}^2$ for mono-culture chondral organoids at day twenty-one</p> <p>-High cellular survival of 84 % and 91 % for synovial and chondral organoids, respectively, after three weeks of culture</p> <p>-Greater numbers of round chondrocytes for co-cultured chondral organoids relative to mono-cultured ones</p> <p>-No significant alterations in secretion levels of MMP-13, IL-6, and IL-8 at day three for the co-culture system</p>	
A microfluidic OA model consisting of a chondrogenic channel and an osteogenic channel separated via an electrospun PCL scaffold that incorporated a microfiber layer and a nanofiber layer, both seeded with cells	Primary osteogenic cells and Primary articular chondrocytes	<p>-High expressions of collagen type II, aggrecan, and osteopontin analogous to the pellet cultures, suggesting the deposition of high-quality neo-cartilage and neo-bone matrices within the device after twenty-eight days</p> <p>-Formation of a loose bone-like matrix beneath the dense cartilage-like one possessing a fairly high count of nuclei and lacking a calcified zone</p> <p>-Higher GAGs content in the chondrogenic zone than the osteogenic region</p> <p>-Deposition of calcium in the osteogenic compartment but not within the neo-cartilage, with a relatively low rate of mineralization</p>	[265]

		<p>-Up-regulation of integrin binding sialoprotein (5.04-fold change), ALP (2.83-fold change), and Runx2 (1.93-fold change) compared to the control groups in the chondrogenic compartment</p> <p>-Up-regulation of integrin binding sialoprotein (2.29-fold change) and ALP (2.57-fold change) compared to the control groups in the osteogenic compartment</p>	
<p>An OoC platform (miniJoint) incorporating synovial-like fibrous tissue, OC complex, and adipose tissue</p>	hBMSCs	<p>-Remarkably higher expressions of osteocalcin in OC-B component, aggrecan and collagen type II in OC-C part, primary adipogenic marker genes in the adipose section, and fibrogenic markers in the engineered synovial-like fibrous tissue after twenty-eight-day differentiation</p> <p>-Exhibited well-preserved tissue-specific phenotypes by individual microtissues</p> <p>-Up-regulated expressions of main catabolic genes in synovial-like fibrous tissue's chamber, and diminished expressions of aggrecan and collagen type II in OC-C section</p> <p>-Altered gene expressions related to the pathologic changes in OC-O compartment, and enhanced expressions of lipoprotein lipase and adiponectin in the adipose part</p> <p>-Up-regulation of nerve GF expression (essential mediator of joint pain sensation) after treatment with IL-1β in comparison with the control groups</p> <p>-Down-regulated expressions of MMP-3 and MMP-13 in synovial-like fibrous tissue's section, reduced expressions of nerve GF and osteopontin in OC-C compartment, diminished expressions of lipoprotein lipase</p>	[391]

		<p>and adiponectin in the adipose part, and suppressed expression of ALP in the OC-O section after treatment with naproxen sodium compared to the control groups</p> <p>-Considerable up-regulation of chondrogenic genes and significant down-regulation of IL-6 in the model after treatment with other DMOA drugs</p>	
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7. Challenges and outlooks

The AC anatomy, damages, and its different treatment strategies were overviewed in detail herein. Although cartilage treatment approaches have been significantly improved over the years, a grand challenge is still posed by the cartilage's limited self-healing capacity which hinders full recovery of the damaged tissue even after treatment. Among treatment techniques, a promising way to treat cartilage damages is the employment of hydrogel-based biomaterials capable of replicating the chondrocytes' ECM. In this regard, microfluidic hydrogel-based systems are designed to provide a suitable biological and chemical micro-environment for cartilage regeneration. In addition, the enhancement of mass transfer, injectability, controllable cell populations' interactions, low contamination, modular design, and adjustable porosity are features that make microfluidic hydrogel-based systems suitable for cartilage TE studies. Above all, microfluidic systems based on pneumatic pumps, valves, and channels supply the programmed circulation of a trim level of fluids in the chip, which scale down cell capturing, chemical analyses, immunoassays, and transfection. Several matters should be taken into account before utilizing microfluidic hydrogel-based systems for clinical applications. Firstly, the hydrogels' mechanical features should be enhanced to generate 3D microfluidic networks within cartilage platforms possessing biologically and clinically relevant sizes. Even though the applied technologies are proper for fabricating size-dependent microfluidic systems with simple channel architectures, currently developed methods are suggested to manufacture

qualified structures such as DN hydrogels for cartilage damages repair. Secondly, employing a single polymer may pose some significant challenges, particularly in drug-loaded constructs. To address this key issue, stratified and multi-material structures can be designed via using a mixture of technologies, even when components are not miscible. In addition, although external syringe, vacuum, or peristaltic pumps in microfluidic devices allow outstanding control of fluid dynamics, preventing bubble formation when the setup is adjusted requires a high level of expertise. It is also necessary to extend the hierarchically branched vascular networks and architectures that are intra-complex within the microfluidic hydrogel-based systems. Indeed, inter-connected channels in the perfusion system cannot fulfill the vascular systems' function. These critical issues increase the need for the functionalization of microfluidic hydrogel-based systems as well as the chemical and mechanical stimulation of cells for better vascularization and regeneration of the native tissue. By controlling culture parameters that can be readily achieved via keeping continuous re-circulation of small amounts of media within the culture of micro-tissues, crosstalk between cellular components can be stimulated physiologically. In addition, the clogging of channels by cells, intricate design, high expenses related to the fabrication process, and irreproducible gel-medium interference are other obstacles that should be overcome. Despite the abovementioned obstacles, micro-engineering technologies and novel hydrogels can open up excellent opportunities to develop microfluidic hydrogel-based systems for cartilage bioengineering.

Concerning the materials used in OoCs, PDMS is the first and most widely employed material owing to its ease of prototyping, low-cost, transparency, and gas permeability. Nonetheless, one of its major drawbacks is that a decreased absorbance of small, hydrophobic molecules influences solvent efficacy and toxicity. Hence, it is critical to find proper alternative materials, but this issue can be challenging since various cell types respond differently when moved from a material such as PDMS to another microfluidic culture with a different material (e.g. softer). As mentioned in the studies, diverse materials have been proposed so far, and advancements have been made in the recent years, but there is still a need to search for ideal materials. Natural polymers prevail over others because they are highly biocompatible, and therefore, particularly suitable for cell culture. Furthermore, bioinert

polymers such as PEG are promising for biosensors in OoCs to avoid unspecific protein bonds and biofouling. It is worthy to mention that the stimuli-responsive polymers such as the temperature-sensitive PNIPAAm hydrogel can potentially develop active elements within the chips. One essential challenge in this field is that the manufacturing process and experimental implementation are relatively expensive, hindering the widespread use of OoCs. Thus, inexpensive ingredients must be chosen, and more expensive elements should be reusable.

Although cartilage-related OoC technology has already obtained spectacular success, there is still plenty of room for future improvements. The inability to produce functional large-scale tissue constructs in the chips should be overcome. Other challenges, including the problems associated with the absorption of small, hydrophobic molecules into the bulk of chip's channel walls, the drug adsorption to the chip materials' surface, leaching of chip's uncross-linked materials oligomers into the cell culture media, improper vascularization, bubble formation in the channels, difficult inclusion of immune components, and high rates of water transmission leading to changes in osmotic concentrations must be tackled. Moreover, a deeper understanding of physiological and pathological mechanisms playing roles in the cartilage unit is needed to facilitate the design of the ideal platforms. Furthermore, when OoC is employed for disease modeling, multiple compartments should be included in the device to better mimic the illness conditions. For example, in joint-related diseases, the lack of a bone compartment, a synovium chamber, or an adipose section in some studies should be addressed, and when this essential issue is tackled, inter-organ scaling, providing suitable flow rates of various media, creating ideal oxygenation and nutrient levels for different organs, and the organs' interdependent functionality must be meticulously considered. In OoCs, media perfusion subjects cells to shear stress and changes effective cell to media volume ratios; issues that can have unintended impacts upon cells and should be explored. Furthermore, the neuroimmune expression profile must be considered in order to

understand the mechanism behind OA-related pain. It should be mentioned that the investigation of the interactions between neuronal cells and non-neuronal cell types, including bone cells, muscle cells, chondrocytes, adipocytes, *etc.*, can help in the better design of new treatment courses or pain relief methods. Despite the fact that OoCs are increasingly employed to examine the onset and development of diseases, the OoCs' culture times are just a few weeks, thereby making them inappropriate for evaluating the long-term impacts in which a disease may progress for years. Since gathering samples on the chip may interrupt its operation and alter the concentration of media, significant advances in some technological areas are needed, such as upgrading critical output sensors for better screening of biomarkers in the medium, establishing robust connections between chips to maintain sterility, prevent bubble formation, and avoid media leakage, as well as improving the automation of OoCs by employing machine learning and robotics. The advancement of technology can enhance the metrological properties of biosensors, such as sensitivity, specificity, detection threshold, and reduction of sample volume. It is also noteworthy to mention that for boosting the market of OoCs, raising the research funding all over the world in the biotechnology/pharmaceutical sector and investing in various clinical trials are certainly needed.

8. Author contributions

Hamidreza Tolabi and **Niyousha Davari**: Conceptualization, Methodology, Validation, Investigation, Data Curation, Visualization, and Writing Original Draft. **Mehran Khajehmohammadi**: Visualization, Methodology, Validation, Investigation, Writing, and Editing. **Haniyeh Malektaj**, **Katayoun Nazemi** and **Samaneh Vahedi**: Validation and Investigation. **Behafarid Ghalandari** and **Rui L. Reis**: Conceptualization, Writing, Review, Editing, and Project

Administration. **Farnaz Ghorbani** and **Joaquim Miguel Oliveira**: Conceptualization, Writing, Review, Editing, Supervision, Project Administration, and Funding Acquisition.

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10. Conflict of interest

The authors whose names are listed certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

11. Abbreviations

Articular cartilage	AC
Osteochondral	OC

Osteoarthritis	OA
Rheumatoid arthritis	RA
Tissue engineering	TE
Extracellular matrix	ECM
Mesenchymal stem cells	MSCs
Three-dimensional	3D
Growth factor	GF
Organ-on-chip	OoC
Hyaluronic acid	HA
International Cartilage Repair Society	ICRS
Poly(lactic-co-glycolic acid)	PLGA
Matrix-induced autologous chondrocyte implantation	MACI
Induced pluripotent stem cells	iPSCs
Methacrylated HA	HAMA
HA modified by aldehyde and methacrylate groups	AHAMA
ELRs	elastin-like recombinamers
Bone marrow MSCs	BMSCs
Human MSCs	hMSCs
Silanized-hydroxypropyl methylcellulose	Si-HPMC
Gellan gum	GG

Chondroitin sulfate	CS
Silk fibroin	SF
Nanoparticles	NPs
Glycosaminoglycans	GAGs
Transforming GF-β_1	TGF- β_1
Polyethylene glycol	PEG
Polyvinyl alcohol	PVA
Human-like collagen	HLC
Ultraviolet	UV
Poly(<i>N</i>-isopropyl acrylamide)	PNIPAAm
Polycaprolactone	PCL
Matrix metalloproteinase-7	MMP-7
Scrambled control	sc
PEG diacrylate	PEGDA
Semi-interpenetrating network	sIPN
Transforming GF-β_3	TGF- β_3
Polydimethylsiloxane	PDMS
Deoxyribonucleic acid	DNA
Two-photon lithography	TPL
Gelatin methacryloyl	GelMA

Amino ethyl methacrylate	AEMA
Human articular chondrocytes	HACs
Human BMSCs	hBMSCs
Two-dimensional	2D
Computer-aided design	CAD
Disease-modifying OA	DMOA
Human bone marrow endothelium cells	BMECs
Pure bacterial cellulose	pure BC
Particle-templated bacterial cellulose hydrogel microparticles	pBC-M
Chitosan/PEGDA hydrogel microspheres	CP-MS
Skeletal stem cells	SSCs
Micro ribonucleic acid-140-3p	miR-140-3p
Micro ribonucleic acid -146b	miR-146b
Kartogenin	KGN
Diclofenac sodium	DS
Phosphate buffer saline	PBS
Periodontal ligament stem cells	PDLSCs
Gingival mesenchymal stem cells	GMSCs
Basic fibroblast GF	bFGF
Stromal cell-derived factor-1	SDF-1

Tumor necrosis factor-α	TNF- α
β-tricalcium phosphate	β -TCP
Alkaline phosphatase	ALP
Double-network	DN
DS-loaded poly (dopamine methacrylamide-to-sulfobetaine methacrylate)-grafted microfluidic GelMA spheres	MGS@DMA-SBMA@DS
Thrombospondin type 1 motif 5	ADAMTS5
Matrix metalloproteinase-13	MMP-13
DS-loaded poly (dopamine methacrylamide-to-2-methacryloxyethyl phosphorylcholine)-coated microfluidic GelMA spheres	GelMA@DMA-MPC@DS
Poly (dopamine methacrylamide-to-2-methacryloxyethyl phosphorylcholine)-coated microfluidic GelMA spheres	GelMA@DMA-MPS
Interleukin-1β	IL-1 β
Reactive oxygen species	ROS
Micro ribonucleic acid	miRNA
Micro ribonucleic acid-140	miR-140
G5-polyamidoamine dendrimers modified with arginine, histidine, and phenylalanine-modified generation 5 polyamidoamine	G5-AHPs
Insulin-like GF-1	IGF-1

Concentration gradient generator	CGG
Optically switched dielectrophoretic	ODEP
Interleukin-6	IL-6
Yes-associated protein	YAP
Neuroleukin	NLK
Interleukin-8	IL-8
Interleukin-1	IL-1
Chondral component in the OC model	OC-C
Osseous component in the OC model	OC-B
Chondral tissue-only constructs	CH
Matrix metalloproteinase-1	MMP-1
Matrix metalloproteinase-3	MMP-3
Human chondrogenic primary cells	hCH
Human leukemia monocytic cell line	THP-1

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13. Authors' biographies

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Yazd Reproductive Sciences Institute. He manufactured two types of bioprinters and became one of the winners of the best innovative projects in 2021 at Iran's Science and Technology parks. His research interests focus on innovating medical engineering technologies to recreate functional biomimetic tissues employing bioprinting, biomaterials, and organs-on-chips.

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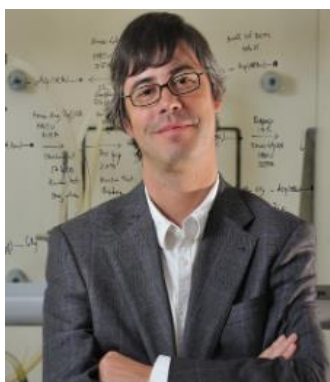
Samaneh Vahedi received her M.Sc. degree in materials science and engineering from Imam Khomeini International University, Iran in 2019. Her research interests focus on the development of high-performance electrochemical energy storage devices for renewable energy storage and delivery, understanding the fundamentals of electrochemical devices, production of high-quality carbon biosensors, and tissue engineering-related projects. Vahedi is keen to be part of international collaboration teams.

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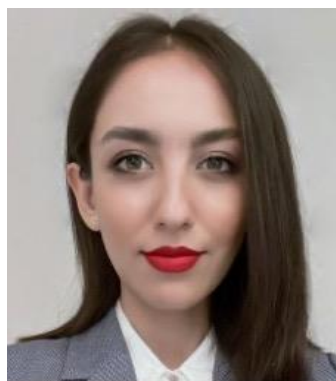
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Joaquim Miguel Oliveira is a principal investigator with Habilitation at the PT Government Associate Laboratory ICVS/3B's and vice president of I3Bs - Institute 3B's (University of Minho). Currently, he is a lecturer in doctoral program in tissue engineering, regenerative medicine, and stem cells at the University of Minho. Over the years, his research work has focused on the field of biomaterials for tissue engineering, nanomedicine, stem cells, and cell/drug delivery. He is also the Editor-in-chief of the *In vitro Models Journal* (Springer Nature). In 2021, he was listed in the World TOP 2 % most cited researchers.

Table of Content text

Schematic presenting various applications of microfluidic hydrogel-based scaffolds and microfluidic chips. Hydrogels are among the most promising options employed for cartilage repair. Subsequent to analyzing different types of hydrogels, microfluidic hydrogel-based scaffolds were selected due to displaying superior characteristics compared to their counterparts. The application of microfluidic chips, known as organ-on-chips, was also investigated in the cartilage tissue engineering.