Squaramide-based supramolecular materials for 3D cell culture applications
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CHAPTER 1

Introduction
1.1 Synthetic hydrogel materials

Hydrogels (either natural or synthetic) imbibe a large amount of aqueous solution throughout their entire 3D networks and as a result can be used as biomaterials for various biomedical applications.\textsuperscript{1-3} In particular, synthetic hydrogels are attractive to researchers due to their tunable properties that are enabled through simple structural modification and high reproducibility.\textsuperscript{1,2} More specifically, synthetic hydrogels can be formed either through the chemical or physical crosslinking of functional polymeric monomers or low molecular weight molecules (Figure 1.1).\textsuperscript{3}

![Diagram showing classes of synthetic hydrogel building blocks (polymeric and molecular) and crosslinking strategies (physical and chemical).](image)

**Figure 1.1** Classes of synthetic hydrogel building blocks (polymeric and molecular) and crosslinking strategies (physical and chemical). Image adapted from reference 3.

1.2 Supramolecular hydrogels

Supramolecular materials are typically formed through non-covalent interactions (e.g., ionic interactions, host-guest binding, hydrogen bonding, aromatic stacking, and/or hydrophobic effect), and can be divided into polymeric and low molecular weight hydrogels (Figure 1.2).\textsuperscript{3-6} These materials have unique properties, for example, modularity, tunability, responsiveness and potential for biomimicry, in comparison to their covalent counterparts. Consequently, they are being explored for use as drug/protein carriers, cell culture substrates, and in disease detection and therapy.\textsuperscript{4,5}
As shown in **Figure 1.2**, the mechanism to prepare supramolecular hydrogels can be further separated into one of two key general strategies, specifically, molecular recognition of polymeric precursors or through one-dimensional self-assembly by monomer stacking. For example, polymeric precursors or protein molecules (e.g., polypeptides) can be crosslinked into 3D networks through specific molecular recognition. Supramolecular assembly involving host-guest chemistry, protein-protein interactions, metal-ligand complexes and multiple hydrogen bonds have been used to drive molecular recognition (**top, Figure 1.2**). While in the one-dimensional self-assembly approach, based on other small synthetic molecules or peptides, two steps are used to achieve 3D hydrogel networks: i) the formation of nanofibers through self-assembly of the stacking units; ii) their further entanglement in 3D to form gel phase materials in water (**bottom, Figure 1.2**).
Among non-covalent interactions, hydrogen bonding is highly important in the construction of supramolecular materials. These interactions not only help to promote chain extension and crosslinking of polymers, but also trigger the 1D-directed stacking of small molecules. While the strength derived from a single hydrogen bond is relatively weak, using motifs with multivalent hydrogen bonds or their combination with other non-covalent interactions is necessary. In aqueous environments, the strength of hydrogen bonds can be drastically weakened. Therefore, to trigger the effective self-assembly of monomers with
hydrogen bond motifs in water, a hydrophobic segment needs to be used to protect them, while at the same time maintaining a balance with the hydrophilic segments to enable monomer self-assembly. These points need to be kept in mind in the design and preparation of such monomers. Over the past decade, numerous supramolecular polymers and hydrogels have been successfully engineered by applying various types of hydrogen bonding units.\textsuperscript{20-33} For example, in the Meijer and Lloyd laboratories, benzenetricarboxamide (BTA) motifs that contain 3 sets of hydrogen bonds between the amides, together with the π-π interactions from the benzene core, enable directional self-assembly in water and have been widely used as a platform for hydrogelators (Figure 1.3A).\textsuperscript{24-30} Meijer and co-workers have also prepared supramolecular hydrogels by decorating polyethylene glycol (PEG) polymers with two quadruple hydrogen-bonding ureidopyrimidinones (UPy) and a hydrophobic alkyl pocket to shield the hydrogen bonding moieties (Figure 1.3B).\textsuperscript{31-36}

\textbf{Figure 1.3} Chemical structure and cartoon for the self-assembly of multiple hydrogen bond motifs (A) benzenetricarboxamide (BTA) and (B) ureidopyrimidinone (UPy) based monomers. Image adapted from reference 24 and 32.
Squaramides,\textsuperscript{37-39} that are composed of N-H and C=O groups on a rigid cyclobutenedione ring, can act as multivalent hydrogen bonding motifs (Figure 1.4A). Based on a previous computational study, this four-membered ring shows the partial aromatic character (Hückel’s rule ($4n + 2$) $\pi$ electrons, $n = 0$),\textsuperscript{37} that can be further enhanced on hydrogen bond formation.\textsuperscript{38,39} Squaramide synthons have been widely probed in chemical synthesis, medicinal chemistry, and chemical biology.\textsuperscript{38,40} Recently, in our group, we explored the application of squaramide synthons for building supramolecular polymers in water. We successfully incorporated these units into bolaamphiphilic monomers that result in the formation of stiff and directional fibers in aqueous solution through the combination of the hydrogen bonding and aromaticity gain on oligomerization of the squaramide units when placed in a hydrophobic environment in the surrounding alkyl chains (Figure 1.4B).\textsuperscript{41} Systematic modulation of the hydrophilic or hydrophobic core side chains within these monomers resulted in a variation of the morphology from sphere to fibers in water (Figure 1.4B).\textsuperscript{42} Importantly, despite the use of higher monomer concentrations, these bolaamphiphiles did not form hydrogels effectively. In another vein, Costa and co-workers designed the amphiphilic squaramide-squaramate conjugates (Figure 1.4C) and aryl-squaramides (Figure 1.4D) as potential hydrogelators using an organic solvent (e.g., DMSO), or pH.\textsuperscript{43,44} However, a squaramide-based supramolecular material that can be made in a straightforward manner, easily modifiable with biomolecules and cytocompatible for a wide range of biomedical applications is still rare.
**1.3 Polymeric hydrogels**

To build synthetic polymeric hydrogels, chemical crosslinks are the most commonly used as they are stable and can precisely control the network properties. The approaches that have been examined in this area involve covalent and dynamic covalent chemistries.\(^1,2,45,46\)

**1.3.1 Dithiolane based adaptable hydrogel materials**

Among these chemistries, polymeric hydrogels with dynamic and reversible crosslinks are of interest because of their potential for spatiotemporal control and adaptable character. They can form-break-reform, which enables them with self-healing performance similar to supramolecular hydrogels as previously
So far, dynamic covalent linkages, e.g., hydrazone reactions between the aldehyde and hydrazine motifs, amine-aldehyde based Schiff base bonds, aldehyde-hydroxylamine based oxime reactions, disulfide crosslinks (thiol-disulfide exchange) and Diels-Alder reactions, have been explored to prepare adaptable hydrogels for a range of applications in the biomedical field, namely those involving 3D cell culture such as cell therapies, drug delivery and 3D bioprinting.

Disulfide exchange is one of the most attractive dynamic covalent chemistries, as it can respond to several external stimuli, such as light, temperature, pH, force or reagents such as free thiols. In contrast to linear disulfides, the increased ring tension of the cyclic disulfides, results in the improved S-S bond sensitivity with heat or light. Cyclic disulfides such as 1,2-dithianes and 1,2-dithiolanes, have been widely explored as intracellular catalysts, stabilizers of micelles, and in capping groups of nanoparticles. More recently, Waymouth’s group used 1,2-dithiolanes to build a new class of dynamic polymeric materials (Figure 1.5A). The formation of hydrogels with the Injectable and self-healing properties was achieved through the ring opening polymerization of 1,2-dithiolane triggered by a free thiol reagent. However, typically, reagents such as maleimides that are toxic are required to react with the existing free thiols to make the network stable for further applications. Therefore, gel phase materials formed through the nucleophilic addition-elimination together with their stabilization by small molecules, would be limited for certain biomaterials applications. More recently, according to the maximum absorption of the 1,2-dithiolane unit at around 330 nm, Li’s lab created a new type of stable and multi-responsive hydrogels that were formed by photo-crosslinking of 1,2-dithiolane-based F127 polymers (Figure 1.5B). Their mechanical properties could be adjusted with the polymer concentration and UV irradiation time. The obtained hydrogels also showed good self-healing ability as a result of dynamic disulfide exchange upon UV irradiation. This work offered a very promising strategy for engineering multifunctional hydrogels to a broad range of applications through these 1,2-dithiolane motifs.

To highlight here, the cyclic disulfides can also be used as a highly efficient thiol protecting unit. It is well-known that free thiols are easily oxidized to disulfides during storage which causes ineffective further reactions like conjugation. As an effective thiol protected strategy, the cyclic disulfide structures offered their unique properties over the linear disulfide, e.g., atom-
efficient nature and lack of by-products during the ring opening process. Moreover, the cyclic disulfide can also provide two reactive thiol moieties once the ring opening, which has the potential to act as a bifurcated crosslinker to build new hydrogel materials.

![Figure 1.5](image)

Figure 1.5 (A) The preparation of dynamic hydrogels through free thiol-induced ring opening of 1,2-dithiolanes and further stabilization of hydrogels through maleimide capping. Image adapted from reference 69. (B) Polymeric hydrogels preparation through photo-induced cross-linking of 1,2-dithiolanes and the proposed mechanism of gelation and its self-healing properties. Image adapted from reference 72.

1.3.2 Photo-responsive hydrogels

Responsive synthetic materials are especially required for some fields like controllable drug delivery, chemical sensors, and complex 3D cell culture scaffolds. Photo-responsive hydrogels are appealing due to their tunable properties through varying the parameters of the light source, for example, intensity, irradiation time, and wavelength. With this control light can be applied at a user-defined point in space and time to provide the hydrogel materials with spatiotemporal regulation of their properties. To date, several types of photoreactions were used to fabricate photo-responsive materials.
them, photoreactions such as cleavage, addition, exchange and isomerization have been frequently used to construct these photoinduced hydrogel materials to meet the required applications.\textsuperscript{75}

1.4 Hybrid hydrogels

Despite the use of supramolecular and/or polymeric-based synthetic hydrogels for a wide range of applications in the biomedical field, their relatively weak stiffness and strength limit them in mimicking the mechanical properties of load-bearing tissues.\textsuperscript{78-80} Numerous efforts have been tried to overcome these drawbacks, including increasing the monomer concentration, cross-linking density, and employing hybrid hydrogels consisting of interpenetrating networks (IPNs) or dual-crosslinked double network (DN).\textsuperscript{78}

Using approaches to interpenetrate or dual-crosslink two polymer networks by means of physical or chemical reactions, the generated materials not only take the merits of each independent system, but also can exhibit new attractive properties that are not found in the independent materials.\textsuperscript{81} As reported by Gong’s lab,\textsuperscript{82} DN hydrogels with high mechanical strength and toughness can be prepared if three design features are followed. Firstly, one network should be rigid and brittle, and the other one should be soft and ductile. Secondly, the monomer concentration (molar) for preparing the rigid network should be 20-30 times higher than the monomer concentration for creating the soft network. Thirdly, a high density of crosslinks was required for the rigid 3D network, while loose crosslinks for the soft 3D network. Recent studies indicate that incorporation of reversible physical cross-links (e.g., hydrogen bonding, host-guest complex, electrostatic interactions and hydrophobic interactions) can further help to promote energy dissipation of the DN network system.\textsuperscript{78, 83-86} Therefore, DN hydrogels with additional non-covalent crosslinks can exhibit some unique properties such as stress relaxation and self-healing.\textsuperscript{78} For example, Burdick’s lab developed a new type of DN hydrogel through orthogonal supramolecular (host-guest) and covalent (thiol-ene) crosslinks in one-pot.\textsuperscript{85} The obtained DN hydrogel showed tough stiffness and strength, self-recovering, compressive, stress relaxation, and also cytocompatibility in 3D cell culture. In Wang’s group, they mixed together peptide-based supramolecular polymers and polyacrylamides to form novel supramolecular/polymer-based DN hydrogels with UV irradiation.\textsuperscript{86} The outstanding mechanical properties (as mentioned above) of the achieved physical-chemical crosslinks of DN hydrogels demonstrated the
comparable characters for the articular cartilage *in vivo*. However, their performance as scaffolds for 3D culture scaffold is unexplored. To date, using the self-assembled low molecular weight gels to create supramolecular polymer-based DN hydrogels especially in the application of tissue engineering is rarely exploited. Hence, the preparation of new and fully synthetic supramolecular and covalent polymer-based DN hydrogels as artificial scaffolds with variable properties, functionalities, and also biocompatibility in a synthetically accessible manner is still largely needed.

1.5 Synthetic hydrogels for engineering cell microenvironment

Over the past decades, synthetic polymer hydrogels have been used as platforms to mimic the *in vivo* cell microenvironment because of their water-rich composition similar to native tissues. However, most common used synthetic hydrogel materials like dextran, PEG, and alginate, lack these bioactive sites for cells to interact. Therefore, full-length proteins or short peptides have been

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*Figure 1.6* Schematic diagram of the interactions between the cell and ECM in vivo (*left*). The main biofunctions (biophysical and biochemical cues) that are needed to be taken into consideration during the design of synthetic materials (*right*). Image adapted from reference 1.

1.5.1 Short peptides to provide cues for cell adhesion

*In vivo*, maintenance of regular cellular activities demands ligand-receptor recognition between cells and the ECM. However, most commonly used synthetic hydrogel materials like dextran, PEG, and alginate, lack these bioactive sites for cells to interact. Therefore, full-length proteins or short peptides have been
physically or chemically incorporated into materials. Short peptides (e.g., RGD, YIGSR, GFOGER) with cell-adhesive amino acid motifs are more appealing as they offer a facile means to understand the relationship between each independent ligand and their effect on cell behavior, and overcome the challenges involved in using full-length proteins. Thus, selecting the suitable peptide concentration, type, and distribution is necessary to prepare materials for different cell types and biological activities.

1.5.2 Control over hydrogel mechanical properties by tuning polymer features

Synthetic hydrogels have been classically used to give structural support to cells residing within them and prevent their sedimentation. When the cells are cultured in environments that are mechanically comparable to a particular tissue, they can show in vivo-like behaviors. The stiffness of various tissues in the body can range from hundreds of Pa to MPa depending on their anatomical location, for example, soft tissues include liver, brain, skin, and breast tumor, and the stiffer skeletal tissues include bone, cartilage, and tendon. Therefore, in order to access this wide stiffness range in synthetic hydrogels, chemical strategies such as varying the gelator concentration/crosslink density, chain length or molecular weight of the polymer, crosslink types (physical or chemical), or using a hybrid network method (IPN/DN) have been examined. More recent reports have shown synthetic materials can also facilitate mechanotransduction through providing biophysical signals and further influence cell behavior (e.g., morphology, proliferation, differentiation, and mobility). For example, the changes of the cell morphology and actin cytoskeleton were obtained in response to hydrogel stiffness through multiprotein complexes formed by the cell-matrix adhesions. Moreover, different mechano-responsive behaviors could be observed due to different cell types, mechanical loading modes, cell development stages, and pathological processes.

For covalent polymer hydrogels, a large body of work has been dedicated to the effect of hydrogel stiffness on cell behaviors. However, structurally covalent hydrogels are isotropic and can’t mimic the fibrillar and anisotropic properties of native ECM, and are unable to be remodeled by cells. Cell-mediated degradation sites (e.g., MMP cleavable peptides) are required in this class of materials. Alternatively, supramolecular hydrogels based on physical crosslinks that are reversible allow encapsulated cells to push or remodel their environment because of the nature of the interactions that hold them together.
materials are attractive for applications, such as tissue regeneration, cell delivery, and bioprinting. However, their relatively weak stiffness limits their applications in areas that require stiff and tough properties. Hence, bringing these classes of materials together in the formation of materials such as double networks that have tunable viscoelastic properties, and also improved stiffness and strength, can broaden their application range in the 3D cell culture of stiff and tough tissues.

1.5.3 Hydrogel materials with dynamic and complex properties

In vivo, many tissue processes like tissue growth, wound healing or illness progression, are dynamic instead of static. Therefore, complex hydrogels with tunable properties over time, using pH, temperature, or light, have been used to prepare hydrogel platforms with inhomogenous properties to study biological processes. Light-based chemistry with its potential for user-defined control over materials properties, has been proven to be a useful method to increase the complexity of the materials.

Many studies have been shown the potential to spatially and temporally incorporate bioactive cues in hydrogels using light-mediated reactions. For example, Shoichet and co-workers used photoreactions to create channels with an RGD peptide in an agarose gel matrix, and the patterned area could guide the migration of a cell cluster. In the Anseth’s group, ideal and cytocompatible hydrogels were built using click chemistry (Figure 1.7A). The obtained 3D cell-laden hydrogels could be further functionalized using a thiol containing RGD peptide by a light-activated thiol-ene reaction. Because a photomask was applied, NIH 3T3 cells showed a spread morphology where RGD was patterned. More recently, Zhu’s lab constructed thiol Michael-based HA-MMP synthetic hydrogels with biocompatible properties, to permit RGD peptide patterning using an o-nitrobenzyl and alcohol-based photoreaction to guide the migration of HDF cells from spheroids in a path as defined by the user (Figure 1.7B).
Figure 1.7 (A) The formation of hydrogel networks for 3D cell culture through click chemistry. Confocal image of NIH 3T3 cells in an RGD-patterned region as prepared by the photo-activated thiol-ene reaction. Image adapted from reference 121. (B) Schematic illustration of thiol-Michael addition-based hydrogel materials and light-induced protein conjugation throughout. Schematic representation and confocal image of cell migration in the RGD patterned region after 12 days of culture. Image adapted from reference 127.

Control over the mechanical functions of hydrogels at a user-defined time point in time and space has also been widely explored by researchers. Utilizing light, the stiffness of the material could either be dynamically increased or reduced through non-reversible and reversible strategies.\textsuperscript{130-142} For example, in Zhang’s group, they reported short peptide-based supramolecular hydrogels with suitable stiffness and cytocompatibility for 3D cell culture (Figure 1.8A).\textsuperscript{136} The hydrogels could be degraded using the photo-activated biaryl-substituted tetrazole moiety and this resulted in a change in the hMSCs cell morphology. Recently, Anseth’s group exploited a new strategy to create complex 3D scaffolds that can be dynamically stiffened through photo-induced polymerization of azadibenzocyclooctyne (DBCO) units (Figure 1.8B).\textsuperscript{141} The 3D C2C12 cell-laden PEG based hydrogels were constructed using covalent crosslinks (azide-alkyne
The C2C12 cell morphology and YAP protein localization varied with increased stiffness at user defined time points.

Figure 1.8 (A) Schematic representation of photo-induced degradation of self-assembled supramolecular hydrogels (left). Fluorescent images of the RGD gel after applying different amounts of UV light irradiation and representative hMSCs cell morphology after 36 h culture (right). Scale bar: 50 μm. Image adapted from reference 136. (B) Chemical structures used to build the complex PEG hydrogel matrix and storage moduli before and after light irradiation measured by oscillatory rheology. C2C12 myoblast cell morphology and their mechanotransduction when the hydrogels are dynamically stiffened either at day 1 or day 7. Image adapted from reference 141.
More recently, Aranzazu’s lab described an even more complex hydrogel scaffold with tunable properties using various wavelengths of light, and explored their applicability for 4D cell culture. For example, the mechanical property (stiffness) of the hydrogel could be stiffened when the visible light (400-500 nm) was applied. When the visible light was replaced by UV light (365 nm), dynamic presentation of the RGD peptide occurred (Figure 1.9). The migration of the L929 fibroblasts from spheroids was induced, guided or hindered through photo-activated dynamic stiffening or RGD peptide conjugation to the hydrogels.

![Figure 1.9](image.png)

**Figure 1.9** (A) Design and mechanism of dextran-based hydrogels to build 4D functions with orthogonal and wavelength-dependent biochemical and mechanical cues. (B) Migration study of L929 fibroblast cell within the 4D hydrogels with varied RGD ligand density and stiffness when applied different wavelength of light. Image adapted from reference 143.

### 1.6 3D bioprinting

3D bioprinting is an attractive biofabrication technology that can be used to build the complex and heterogeneous architectures found in the extracellular matrix in 3D. During the bioprinting process, bioinks that include biological hydrogel materials, cells, and other biochemicals are layer-by-layer positioned into well-defined objects with the aid of a bioprinter (e.g., extrusion, lithography, drop on demand, laser-based printer, etc.). The obtained 3D tissues and organs after bioprinting have potential applications for use in vitro for drug screening and disease modeling, or even in vivo for transplantation into patients.
specifically, extrusion-based bioprinting has been most commonly used due to its unique advantages, for example, commercial affordability, high cell printing densities, and the capacity to build complex and hollow 3D constructs with multiple cell types and hybrid materials. To date, the application of extrusion-based bioprinting is still in its initial stage as most of the published works focus on utilizing known materials or chemical crosslinking reactions to meet the required printing parameters. To further print and mimic more complex tissues (e.g., bone, brain, and vessels), the development of new types of hydrogel materials as bioinks with various properties, for example, printable, high printing resolution for complex structures, biocompatible, and also multifunctional, is largely required to be explored. \textsuperscript{149-154}

1.7 Aim and outline

The development and application of synthetic hydrogels in simulating the cell microenvironment to support cell survival and biological activities, has aroused much attention over the past decade. In this introductory chapter, I have shared examples of various synthetic hydrogels including supramolecular and polymeric hydrogels and also disclose the non-covalent and covalent interactions for the contribution of the formation of 3D networks. Supramolecular hydrogels as adaptive materials garnered even more attention for 3D cell culture due to their unique features such as easy preparation, responsiveness and self-healing. However, their relatively weak strength and stiffness restrict their application in some areas. Therefore, developing new types of self-assembled biomaterials with tunable properties (e.g., hydrogel stiffness) is necessary. More importantly, supramolecular hydrogels with spatial and temporal control over their properties are required to meet the dynamic microenvironment in some complex biological processes.

In this thesis, I aim to develop and study a new type of tripodal squaramide-based supramolecular hydrogels. The mechanical properties of hydrogel with a wide stiffness range that can be easily modulated and also special and temporal controlled either by decorating with the activated group to offer additional chemical crosslinks or using hybrid hydrogel by incorporating the second network through light irradiation. Moreover, the designed synthetic hydrogel systems are biocompatible with several cell lines and have the potential to use as a 3D culture substrate.
In chapter 2, I explore the potential for creating a novel type of self-recovering supramolecular hydrogels by incorporating squaramide-based hydrogen bond motifs into a flexible TREN core with varied hydrophobic and hydrophilic domains. The design and synthesis of a family of tripodal squaramide-based monomers are reported. Experimentally, the formation of a network composed of entangled fibrils is explored by cryo-TEM and SAXS measurements. At the molecular scale, spectroscopic measurements (UV-Vis, FL, and FTIR) provided insight into the self-assembly properties of the squaramide-based supramolecular polymers. Moreover, their mechanical and self-recovering properties were explored by rheology. Finally, I explore their cytocompatibility in 3D cell culture for a range of cell types, including NIH 3T3 cells of which several are considered sensitive such as hiPSCs and their differentiated derivatives.

In chapter 3, the tripodal squaramide based supramolecular hydrogel presented in chapter 2 was further decorated with the photoactive cyclic 1,2-dithiolane motif. This functional monomer can be co-assembled with the native monomer developed in chapter 2 and to result in the formation of multi-component hydrogels. Their tunable mechanical properties with a wide stiffness range under light irradiation were probed by rheology. The entangled fibrils of the hydrogel before and after light irradiation were explored by Cryo-EM and SAXS measurements. A dithiolane-based RGD peptide could be photo-patterned into the hydrogel in 3D either using a benchtop LED under a photomask or direct laser writing (DLW). Encapsulation of the cells, for example, NIH3T3, Hs578T and C2C12 within our hydrogel materials, demonstrated their cytocompatibility for future applications in 3D cell culture. Additionally, the cell morphology and migration with respect to spatial and temporal changes of the hydrogel mechanical properties are also explored.

In chapter 4, I aim to create a new type of branched macromolecular architecture and polymeric hydrogel material through UV induced crosslinking of cyclic 1,2-dithiolanes with norbornene on linear poly(ethylene glycol) polymers. Using rheology and confocal microscopy, we demonstrate the spatial and temporal control of the hydrogel mechanical properties and functionality. Moreover, a cytocompatible response of NIH 3T3 fibroblasts was also observed within these materials, which opens the door for use as a new type of synthetic matrix.

In chapter 5, a new type of double network hydrogel is created by the combination of the supramolecular hydrogels from chapter 3 and the polymeric
hydrogel from chapter 4. The obtained double network hydrogels were characterized by rheology and SEM to better understand the change of their mechanical properties and also their corresponding microstructure. Two-photon patterning was used to special and temporal incorporate the RGD peptide. 3D cell encapsulation and LIVE/DEAD staining study demonstrate that our double network hydrogels are biocompatible with NIH 3T3 and human primary articular chondrocytes (hPACs). Moreover, the ECM production by the encapsulated hPACs cells indicated the possibility for further exploiting our DN hydrogel in the biomedical field for applications involving cartilage.

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