REVIEW ARTICLE

Biocompatible Hydrogel for Various Tissue Engineering

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ABSTRACT

Objective: **To give an overview and evaluation of the biocompatibility of hydrogels that support tissue engineering.**

Materials and Methods: **A literature search was conducted in PubMed/Medline and Science. org databases. The keywords used were 'hydrogel' AND 'biocompatible' AND 'tissue engineering'. The search was limited to the studies carried out on humans or animals.**

Results and Discussions: **From the search of the related literature, we obtained seven studies that described hydrogel biocompatibility that was used in tissue engineering. The hydrogels were amorphous calcium phosphate and carboxymethyl chitosan (CMCh-ACP), collagen, polyaniline cryogel, bioink GLgel, nanocomposite hydrogel (nSi Gel), host-guest supramolecular hydrogel (HGSM-GelMA), and synthesized succinylated chitosan (SSC). Recent advances in the use of hydrogels in tissue engineering provide justification for further research and testing in clinical settings.**

Conclusion: **The hydrogels that were used in the seven examined studies did not trigger the immune system rejection, had no negative effect on surrounding tissues or cells, and were highly biocompatible with living tissue.**

KEY WORDS

hydrogel, tissue engineering, biocompatibility

INTRODUCTION

Tissue engineering can be used to support regeneration, when self-regeneration is impossible. It is considered a viable method for replacing certain damaged tissues, parts, or whole organs and accordingly, plays a role in tissue repair, which allows injured tissues to function again[1)]. The success of tissue engineering is largely affected by three factors, which have been proposed by many previous researchers. These factors are scaffolds, cells, and signaling molecules. The main method of tissue engineering is cell culture, which is grown in properly designed scaffolds before being transplanted into organisms. Due to their pluripotency or multipotency, and unlimited proliferation, stem cells are the cells, which are most commonly used in tissue engineering[2)].

Scaffolds in the form of biomaterial or synthetic scaffolds provide a place and environment for cells to grow, mature, and differentiate to reconstruct target tissues. Scaffolds must have a design that is suitable in terms of porosity, profile, self-degradation time, and the capacity to contain signaling molecules to be used by cells, either within-scaffold or in the surrounding of the scaffold, and should be suitable for cell growth[2)].

Hydrogel (HG) is a biopolymer with physical and chemical cross-links and is one of the most widely used scaffolds. Hydrogels have a three-dimensional (3D) structure with a relatively simple design, are porous, and consist of long polymer chains, which form a complex matrix with water molecules filling the spaces between the polymer chains. They are widely used in the medical field because of their water absorption capability and capacity to load other substrates, allowing them to carry or deliver bioactive molecules. Hydrogel scaffolds are made of materials, which are very similar to and have a structure similar to the extracellular matrix of the human body. They are ideal for tissue engineering as they allow the three components, i.e. scaffolds, cells, and signaling molecules to cooperate and their target is to repair a damaged tissue[2)].

Alginate, chitosan, collagen, fibrin, gelatin, HA, and synthetic polymers (PLA (Polylactide), PEG (Polyethylene glycol) derivatives, and PVA (Polyvinyl alcohol) are examples of polymers that produce hydrogels. The chemical properties of the polymers and the degree of cross-linking determine the quality of the hydrogel as matrix[2)].

The capacity of hydrogels to be used in the clinic without causing adverse effects on neighboring tissues or cells is the most important attribute for biological and pharmaceutical applications. Either the sub-

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stance or its degradation products should not be harmful to the patient, and must be proven before it can be categorized as biocompatible. As such, the biocompatibility of hydrogels that have been used in tissue engineering needs to be elaborated. The aim of this review was to give an overview and evaluation of the biocompatibility of hydrogels, which support tissue engineering, by discussing the physicochemical properties of hydrogels: their porosity, swelling properties, and biocompatibility, and to discuss recent various hydrogel used in various conditions.

MATERIALS AND METHOD

A literature search was conducted on November 23, 2021 in the PubMed/Medline and Science. org databases. The keywords used were 'hydrogel' AND 'biocompatible' AND 'tissue engineering'. The search was limited to the studies carried out on humans or animals in the last five years. The search results were checked manually. All studies that were related to hydrogel biocompatibility were included. This literature search was focused on articles on the biocompatibile hydrogels that support tissue engineering. Several physicochemical features of hydrogels including swelling ratio, porosity, and biocompatibility were highlighted, but only biocompatibility was discussed further in this review.

RESULTS AND DISCUSSION

From the search of the related literature, we obtained seven studies that described hydrogel biocompatibility that was used in tissue engineering (Table 1)[3-9].

HYDROGEL PHYSICOCHEMISTRY

The physicochemical properties of the hydrogel significantly influence the final material characteristics and biological compatibility. Several parameters/material properties, which should be considered in tissue engineering because of their significance for medical applications are swelling ratio, porosity, and biocompatibility[2].

Swelling Ratio

Swelling is a physical phenomenon in which a substance absorbs fluid and, by doing so, increases its volume and mass. The original shape of the material under study is maintained as the size of the substance increases. One of the most basic features of most hydrogels is swelling. Gels may absorb much more water than their dry mass, and on dried polymer matrix water is anchored and deployed to form the gels[10].

The hydroxyl and carboxylate groups are the first to hydrate in the presence of water, which cause solvent molecules infiltrate the gel matrix and stay there until an equilibrium condition is achieved. The hydrogel structure is stabilized by the hydrogen bonds that are produced between the water molecules and the functional groups of the polymer chains[11].

The swelling ratio is defined as the increase in the weight of hydrogels as a result of water absorption and is expressed as[12].

$$\text{Swelling Ratio} = \frac{\text{Water Mass}}{\text{Dry Mass}}$$

$$= \frac{\text{Swollen Mass - Dry Mass}}{\text{Dry Mass}}$$

The swelling process opens up the possibility of using hydrogels as medicinal material carriers. The use of this gel structure enables antibacterial, including antibiotics, to be administered. These gel structures can also act as promising scaffolds for both cell and protein delivery systems in tissue engineering. When the non-swelling gel is immersed in a solution of a drug, the solvent and drug molecules can enter the polymer network[12].

Porosity

Porosity is an important factor that support cells'ability to migrate and grow in a certain structure, which is giving biomechanical stimuli, as well as affecting the microenvironment (e.g. biofactor releases or efficient nutrient exchanges). In addition, porosity impacts vascularity and advocates mechanical interlocking between the scaffold and the surrounding tissues[13].

Porosity constitutes the ratio of the volume of voids in the tested material to the total volume of the material. The porosity of a material is calculated using the following equation[2]: Porosity = void volume/total volume

The size of the pores is also important. The International Union of Pure and Applied Chemistry, classifies the pore diameter as in Table 2.

Different pore divisions have been proposed in this regard, by considering both diameters and applications. Elbert *et al*. as cited by Chyzy and Plonska-Brzezinska[2]. offered the following classifications, as in Table 3. In addition, pore size can be distinguished in terms of the various uses of hydrogels as can be seen in Table 4.

Biocompatibility

Biocompatibility is considered an important feature of hydrogel materials for biological and pharmaceutical applications. By the simplest definition, biocompatibility is the capacity of a medical substance that does not cause negative effects in the body. This material should neither activate the immune system nor harm nearby tissues or cells and hence, will not be harmful. On grounds of their structure and consistency, as well as high water content, most hydrogels have high biocompatibility with living tissues. Furthermore, removing several unreacted substrates, monomers, initiators, and other compounds that are used in the hydrogel manufacturing process, which potentially may have negative effects in the body, is necessary to improve the biocompatibility of the hydrogel[14].

The biocompatible hydrogels are developed primarily for applications in both tissue engineering and regenerative medicine. They are mostly employed to heal wounds, for drug administration, and to create an environment for cell proliferation and growth[15,16]. An object design and production that is based on living organisms patterns is known as biomimetics (also known as bionics or biomimicry). Biomimetics is a concept that is closely associated with biocompatible materials, especially hydrogels. Biomimetics is concerned in producing drug/substance carrier scaffolds that are most similar to the components of the human body. It is very important to understand all the activities that occur in the human body, notably the immunological reactions, which is related to the main determinants of hydrogels'potential use[2].

In the case of hydrogels, biomimetics requires adjustment of the hydrogel structure so that it is not recognized as foreign by the human immune system, while allowing drug delivery to the target site. Apart from wound care, another important application of biocompatible hydrogels are for tissue engineering and regenerative medicine, where they should be mimicking extracellular matrix. In natural condition, extracellular matrix is made by cells, and serves as a living habitat covering the gaps between cells, including collagen, glycosaminoglycans (e.g., hyaluronic acid), and growth factors. Further, hydrogels can also aid in cell division, adhesion, and other processes[17].

Hydrogel substance or its degradation products, both should do no harm the body that should be proven before it can be categorized as biocompatible. For that purpose, several tests need to be conducted, which consists of three stages, e.g., primary, secondary, and preclinical test. The first two test are fully in vitro and in vivo, but the preclinical one requires the material to be administered to humans. An in vitro assay requires us to determine the cytotoxicity of hydrogels using a variety of cell lines, which cover human and animal cell lines. Fibroblasts, keratinocytes, macrophages, and lymphocytes are examples of cells that can be used. In vivo testing requires delivering a hydrogel to an animal model subcutaneously, intramuscularly, or epidermally and explaining its positive or negative effects. To determine the effect of hydrogels on the human body in clinical conditions, these three-stage tests are used. Commercial use can be given to the hydrogel that has passed these three stages of testing[2].

Various Tissue Engineering Supporting Hydrogels

Various tissue engineering supporting hydrogels have been used (Figure 1). They are amorphous calcium phosphate and carboxymethyl chitosan (CMCh-ACP), collagen, polyaniline cryogel, bioink GLgel, nanocomposite hydrogel (nSi Gel), host-guest supramolecular hydrogel (HGSM-GelMA), and synthesized succinylated chitosan (SSC).

Table 1: Hydrogel biocompatibility in tissue engineering[3-9)]

No.	Ref.	Hydrogel	Specification of Hydrogel	Purpose	Biocompatibility Test Results
1.	[3, 13, 19)]	CMCh and ACP	CMCh-ACP hydrogels were made by mixing GDL into an aqueous dispersion or using pH-triggered self-assembly. and to rehydrate freeze-dried acid nanoparticles	To regenerate bone tissue using osteoprogenitor cells	The biocompatibility of the CMCh-ACP hydrogel was proven effective to facilitate MSC growth and cell adhesion
2.	[4)]	Collagen	1 mL of 2 N acetic acid was used to dissolve rat tail type I collagen. At pH 7.4, this solution was combined with 10 PBS and sodium hydroxide (1N)	To treat clinical bone problems	The presence of elongated OE-MSC that adhered properly to the hydrogel indicated biocompatibility
3.	[5, 20)]	Polyaniline cryogel	Polyaniline cryogel is a novel type of polyaniline that combines the electrical conductivity of polyaniline with the qualities of a hydrogel material. Aniline was polymerized in a frozen polyvinyl alcohol solution to produce this product. Cryogel consisted of 2% polyaniline by weight, 5% polyvinyl alcohol by weight, and 93% water by weight.	For electrically excitable tissue regenerative medicine and tissue engineering	The low amount of low molecular weight contaminants in the polyaniline cryogel indicated biocompatibility. The surface energy, elasticity, and porosity of the cryogel were designed to simulate tissue ECM quality
4.	[6)]	Bioink GLgel	Electrostatic interactions between LA nanosheets and GAGNPs created GLgel bioinks	For regenerative medicine	W-20-17 BMSCs attached to a 3D bioprinted framework, promoting cell proliferation and osteogenic differentiation. In bioprinted structures, dispersion and cell adhesion are important. GLgel bioinks have high printability, shear-thinning characteristics, convenience and speed of crosslinking, cytocompatibility, and hydrogel bioactivity
5.	[7, 21, 22)]	nSi Gel	As a secretome delivery vehicle (nSi Gel+), gelatin and Laponite® were mixed to create nSi Gel	To treat myocardial infarction	When compared with the untreated control group, levels of inflammatory genes, microRNA, or cell marker expression demonstrated no significant difference. Treatment with nSi Gel+ resulted in significantly increased capillary density, scar tissue reduction, and improvement of cardiac functions
6.	[8)]	HGSM-GelMA	An aqueous solution that contains a mixture of A-TEG-Ad and GelMA	For the integration of host-guest non-covalent cross-linking and covalent cross-linking in a single-network hydrogel, enabling biocompatible 3D-printed hydrogel development with increased self-healing and mechanical strengths	HGSM-GelMA scaffold was fully integrated with naked mouse autologous tissue, and new blood vessels and subcutaneous muscle tissue grew in its pores, but no immunological reaction was observed
7.	[9, 23, 24)]	SSC-based hydrogel	SSC-L, SSC-M, and SSC-H	Bioactive Hydrogel	There were no problems of cytotoxicity, cell adhesion, or proliferation

CMCh = Carboxymethyl chitosan, ACP = amorphous calcium phosphate, GDL = glucono-lactone, MSC = mesenchymal stem cell, PBS = phosphate buffered saline, OE-MSC = olfactory ectomesenchymal stem cell, ECM = extra cellular matrix, LA = synthetic smectic silicate (laponite), GAGNPs = glycosaminoglycan nanoparticles, BMSCs = bone marrow stromal cells, nSi Gel = Nanocomposite Hydrogel, HGSM = host guest Supramolecular hydrogel, GelMA = gelatin methacryloyl, A-TEG = acryloylated tetra-ethylene glycol, CD = cyclodextrin, AOI2 = 2-Isocyanatoethyl acrylate, CD-AOI2 = CD conjugated with two AOI2, A-TEG-Ad = adamantane modified with A-TEG and CD-AOI2, SSC = Synthesized succinylated chitosan, SSC-L = Low succinylated chitosan, SSC-M = moderately succinylated chitosan, SSC-H = highly succinylated chitosan

Amorphous Calcium Phosphate and Carboxymethyl Chitosan (CMCh-ACP)

Bone tissue engineering and regenerative medicine, in combination with mesenchymal stem cell (MSC) might be a potent approach to address skeletal and bone reconstructions[18]. For bone tissue engineering, osteoprogenitor cells, scaffolds and osteoinductive factors are needed. Osteoprogenitors are usually the derivation of MSCs, which exists in many tissues, such as in the bone marrow stroma or adipose tissue. Bone morphogenetic protein (BMP) is considered an effective osteoinductive factor either in vitro or in vivo. Among the 14 types of BMP, BMP9 is considered the most powerful bone-building factor. BMP9 transduced MSCs, together with an ideal scaffold, will significantly augment bone regeneration to repair bone defects as well as non-union fracture[3].

Development and characterization of CMCh-ACP hybrid nanoparticle gel, which is sensitive to pH, was done using amorphous calcium phosphate (ACP) and carboxymethyl chitosan (CMCh) scaffolds. This hydrogel scaffold is ideal for successful progenitor-based bone repair. The biocompatibility of the CMCh-ACP hydrogel is excellent, and it promotes MSC growth and cell adhesion. In vitro, this hydrogel was shown to be osteoinductive, induced bone markers and osteogenic regulators. In a long-term model of ectopic osteogenesis, CMCh-ACP hydrogel scaffolds dramatically increased the maturity and efficiency of BMP9-initiated bone regeneration and reduced the resorption of bone[19].

In vitro, the biocompatibility and cytotoxicity of CMCh-ACP hybrid hydrogel were investigated on immortalized multipotent adipose-derived (iMAD) MSCs. The nuclei of Hoechst 33258 positive cells increased in a time-dependent manner after iMAD MSCs were combined with CMCh-ACP hydrogels and grown on cell culture plates, then stained with Hoechst 33258 and examined with at various time points. Even on day 5, most of the cells that were stained with Hoechst 33258 had healthy nuclei and showed no obvious apoptotic core characteristics such as pyknosis or cell shrinkage. The cell proliferation rate of iMAD MSCs that were combined with CMCh-ACP hydrogels showed no significant difference from those which were directly grown in a 2D monolayer culture in the same growth condition ($p > 0.05$). This result demonstrated that there was no negative effect of CMCh-ACP hydrogel on iMAD MSCs[3].

On the CMCh-ACP hybrid hydrogel's surface as well as its interior, a field emission scanning electron microscopy (FE-SEM) examination was carried out to analyze cell shape and mineralization. iMAD MSCs, which were infected with recombinant adenovirus AdBMP9, adhered effectively to CMCh-ACP hydrogel's outer surface. The internal surface of the hydrogel and mineralized nodules were detected easily, which indicated that the CMCh-ACP hybrid gel was a biocompatible and environmentally friendly, and suitable as a scaffold for bone tissue engineering[3].

Chitosan is easily carboxymethylated to yield CMCh, which elevates its solubility in an aqueous medium while maintaining its biocompatibility and biodegradability. CMCH shows a significant increase in solubility. Due to its porous structure, gelling properties, easy chemical modification, and high affinity for macromolecules in vivo, CMCh-based material is suggested for many applications in biomedical nanodevice development, formulations of the controlled-release drug, and a wide range of tissue engineering of blood vessels, bone, cartilage, nerves, liver, and skin[13].

Collagen

Collagen is a significant extracellular matrix (ECM) structural proteins. It has suitable biodegradability, high biocompatibility, and low immunogenicity for cell encapsulation. Additionally, a growing literatures supported its wide use in medicine. Collagen hydrogels are good candidates for the encapsulation of stem cell with a flexibility that is similar to natural tissue properties for bone regeneration. Bone ECM consists of mineral deposits of hydroxyapatite in a type I collagen substrate. Furthermore, in the field of tissue engineering, many types of stem cells have been analyzed and can be extracted from both adult and embryonic organs. The key mechanisms for stem cells in tissue regeneration are the release of paracrine chemicals, immunomodulatory effects, and high angiogenic ability[4].

In a study, 1 mL of 2 N acetic acid was used to dissolve type I collagen that was isolated from rat tails. This solution was combined with phosphate-buffered saline (PBS) and sodium hydroxide (1N) at pH 7.4 and prepared in four final concentrations, and were stored on ice. The concentrations were 4 mg/mL, 5 mg/mL, 6 mg/mL, and 7 mg/mL, and were tested independently. In all groups, FE-SEM after human olfactory ectomesenchymal stem cell (OE-MSC) encapsulation in collagen hydrogels revealed that human OE-MSCs were viable and had elongated shape, with moderate adherence to the hydrogels. The results showed that all four concentrations of scaffolds were highly porous, with a porosity rate exceeding 90%. The percentage of porosity was proportional to the amount of collagen. The 4 mg/mL of collagen scaffold had greater porosity than other scaffold concentrations. In addition, there was no significant difference in OE-MSC between groups[4].

Table 2: Pore type and pore diameter size

No.	Pore Type	Diameter size
1.	Micro-porous	< 2 nm
2.	Meso-porous	2-50 nm
3.	Macro-porous	> 50 nm

Table 3: Pore type classification according to Elbert et al as cited by Chyzy and Plonska-Brzezinska[2]

No.	Pore Type	Diameter size
1.	Nano-porous	< 100 nm
2.	Micro-porous	100 nm-1 micro m
3.	Macro-porous	> 1 micro m

Table 4: Pore size classification according to various hydrogel usages

No.	Pore Size	Use
1.	About 5 micro m	Neovascularization
2.	About 20 micro m	Hepatocyte growth
3.	Between 5 and 15 micro m	Fibroblast growth
4.	Between 20 and 125 micro m	Adult mammal cell
5.	Between 40 and 100 micro m	Osteoid in growth
6.	Between 100 and 350 micro m	Bone tissue regeneration
7.	> 500 micro m	Development of fibrovascular tissue

Polyaniline Cryogel

Polyaniline cryogel constitutes a new polyaniline form, which combines the material characters of hydrogel that is prepared by aniline polymerization in a frozen polyvinyl alcohol solution and its intrinsic electrical conductivity. Polyaniline cryogel biocompatibility was showed by a cytotoxicity assay on mice's embryonic fibroblasts and by embryotoxicity assay, which was based on beating foci formation in spontaneously differentiated embryonic stem cells. Excellent biocompatibility was achieved by low content of low molecular weight impurities in the polyaniline cryogel, that was proven by liquid chromatography. Attachment and growth of embryonic stem cells, neural progenitors, cardiomyocytes, and embryoid bodies revealed the biocompatibility and potency of polyaniline cryogels as cell carriers in tissue engineering. Further, its elasticity, surface energy, and porosity are more or less similar with natural ECM of various tissue properties[20].

Polyaniline cryogel is a water-bubbled material, which contains both a conducting agent and a polymer as its constituents. Polymerization occurs in the frozen state and the prefix "cryo" thereby refers to the preparation method; hydrogel is formed after thawing. Furthermore, polyaniline constitutes the hydrogel's conducting portion, and different water-soluble polymers may act as carrying agents that impart its mechanical traits. Polyaniline is made by oxidating the respective monomers with ammonium peroxydisulfate. Before oxidation, 2.59 g of aniline hydrochloride is dissolved in 5% of aqueous solution by weight of polyvinyl alcohol[5]. Polyaniline cryogels have a very porous structure, and the mean pore size obtained is estimated at 159 µm, whereas the mass ratio of the expanded and dry cryogel reveals 95.5 volume %. Thus, polyaniline cryogels meet the scaffold criteria, namely porosity and biocompatibility[5].

Cytotoxicity is a basic biocompatibility characteristic that reflects the capacity of a cell to survive in the presence of a foreign substance. Polyaniline powder is recognized to have a high level of toxicity, as it contains aniline hydrochloride and ammonium peroxydisulfate as impu-

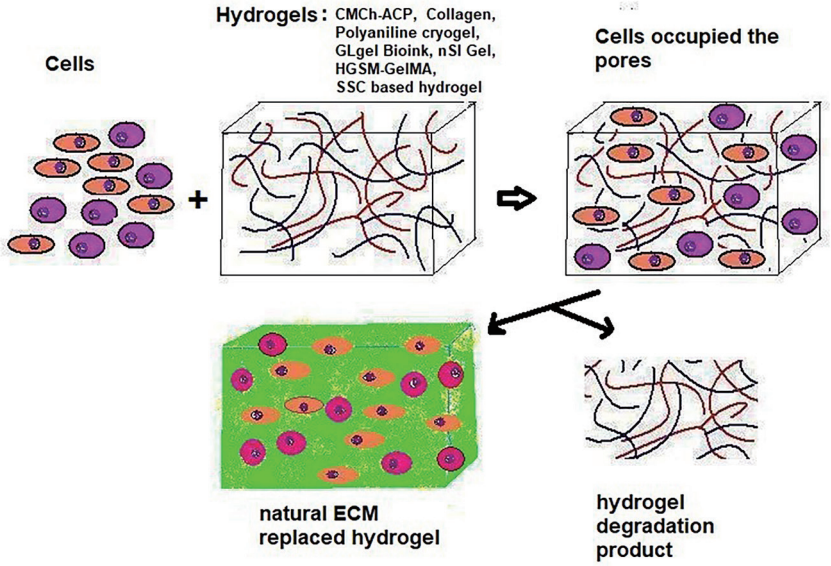

Figure 1: Schematic representation of various use of tissue engineering supporting hydrogels

rities. As a cryogel matrix contains 2% of polyaniline by weight, its toxicity should be lower than polyaniline powder. Cytotoxicity reduction was investigated after purification procedures were executed to remove impurities from the cryogel. The impurities of cryogel are related to residual precursors that were used in polymerization, and should be determined in the respective end product[5].

Mouse embryo fibroblasts (MEF) cytotoxicity assay should be performed not only on original polyaniline cryogels but also on pure polyaniline cryogel. The polyaniline cryogel extract should be tested for cytotoxicity by ISO 10993-5. According to ISO 10993-12, extraction is carried out by an aseptic approach in a closed chemically inert container at 37°C for 241 hours, where cryogel in solution was stirred. The parent extract (100%) is then diluted in culture media to produce dilutions at 50, 25, 10, 5, and 1% of concentrations. All extracts should be tested within 24 hours. Polyaniline extract at various dilutions serve as a substitute for the culture medium after the cells are grown in the course of 24 hours. Cells that are cultured in pure media are used as the reference of 100% cell growth. After one day of incubation of cells at 37°C, the MTT assay is used to determine the cytotoxic effect of various concentrations of polyaniline cryogel that is ascribed as viable MEF cell relative numbers, after the cells have been cultivated for 24 hours in the presence of the extracts. The viability limits by EN ISO 10993-5 are: viability of > 0.8, > 0.6 -0.8, > 0.4 - 0.6 and < 0.4 are indicative of no cytotoxicity, mild, moderate, and severe cytotoxicity, respectively[5].

The original and pure polyaniline cryogel showed similar cell viability at 5, 10, 25, 50, and 75% of the extracts, which did not produce cytotoxicity (the survivals of the cells were all above 80%, in comparison with the reference). However, the parent extract (100%) was shown to be cytotoxic (cell survival was 60-80%). As such, while purification reduced the low molecular weight contaminants in the polyaniline cryogel, leaching had no effect on cytotoxicity[5].

Bioink GLgel

The formation of nanocomposite glycosaminoglycan-laponite hydrogels (GLgel), which combines glycosaminoglycan nanoparticles (GAGNPs) with laponite (LA), is an auspicious strategy for producing cell-laden bioprint constructs. In a study, the effect of GAGNP was tested on the rheological characters, swelling, printability, and degradation characteristics of nano-engineered hydrogel. In vitro osteogenic characteristics and biocompatibility of the hydrogels were evaluated as well. The engineered shear-thinning hydrogel was exploited as a bioink to build a cell-laden construction with a high form accuracy. The shear-thinning hydrogels were used as osteoinductive bioinks to create a cell-containing bone tissue engineering construct[6].

In a study, LA solution was produced by solving LA powder in water and executing 20-minute vortexing. The GAGNP solution was rapidly mixed with LA solution and vortexed immediately for a minute to produce a homogeneous gel. Varying LA concentrations of 20, 25, 30, and 35 mg/mL at a fixed GAGNP/LA weight ratio (1:120) were exploited to make various GLgel concentrations, i.e. 20, 25, 30, and 35GLgel, respectively. GLgels were frozen in liquid nitrogen overnight. After that, the original weights of the samples were measured. Each sample was stored in a Transwell insert with a 1-µm pore[6].

For assessment of 30GLgel, NIH-3T3 fibroblasts and W-20-17 bone marrow stromal cells were used to assess cell viability, proliferation, and metabolic activity. A commercial on/off test and PrestoBlue kit were used to assess the metabolic activity and viability of 3T3 cells that were implanted on the gel surface (2D cell seeding). Cells seeded on GLgel for seven days of incubation had high viability (> 85%). Furthermore, the metabolic activities of 3T3 cells that were seeded on 30GLgels increased continuously for seven days of culture, indicating GLgels in vitro cytocompatibility. For the subcutaneous implantation trial, 30GLgel was chosen. All animals successfully passed the experiment without the development of cancer, abscess, or infection at the injection site. There was no necrosis or muscle degeneration in the tissues surrounding the gel, although there were mild inflammation and fibrosis. At 7 and 14 days after injection, H&E staining revealed the intact skin structure, with no dermal and epidermal changes, and inflammatory infiltrates[6].

Nanocomposite Hydrogel (nSi Gel)

In a study, an injectable and biocompatible hydrogel was used to provide a therapeutic mixture of biomolecules (secretome), which were secreted by human adipose-derived stem cells (huASCs), to the peri-infarct myocardium. Laponite® and gelatin were mixed to make a shear-thinning nanocomposite hydrogel (nSi Gel) as a secretome delivery vehicle (nSi Gel+)[21].

To make the nSi Gel, a solution of 10% gelatin (w/v) is made by dissolving it in PBS (pH = 7.4) at 37°C. After that, Laponite® is dissolved in ultrapure water at room temperature to make 1, 2, 3, and 4% (w/v) suspensions. Then, the gelatin solution is mixed with at an equivalent volume of the corresponding laponite suspensions to get a nanocomposite nSi Gel with gelatin final concentration of 5% (w/v) and laponite of 0.5, 1, 1.5, and 2% (w/v). Biocompatibility test can be carried out on human umbilical vein endothelial cells (HUVECs). A number of 8 x 10^4 HUVECs can be seeded in chamber slides followed by testing of various nanocomposite nSi Gel. Testing is done by substituting 10% of culture media volume with various gelatine-laponite mixtures. Medium only is used as positive control group (Cntrl+), while 50 M camptothecin containing medium is used as a negative control (Cntrl −). After 24 hours, cells are fixed in paraformaldehyde (4%) at 37°C for five minutes. Phalloidin-AlexaFluor488 and diamidino-2-phenylindole dilactate (DAPI) can be used to counterstain actin and nuclei, respectively. Then, immunofluorescence is conducted to study the effect of hydrogel addition on cell morphology[7].

In a study, Cntrl+ and treatment groups were followed for one, three, and five days, then the cells were washed using a fresh medium and MTS assays were performed. To calculate HUVECs' amount, each well absorbance was measured at 490 nm. The cell number was calibrated to a curve between 5 x 10^3 to 2 x 10^5 cells. The results of 2%

Laponite® containing nanocomposite hydrogel showed no toxicity; moreover, immunofluorescent F-actin labeling revealed no morphological changes[7].

Therefore, 2% w/v Laponite® containing nanocomposite hydrogel was selected for further in vivo study. A preliminary test of biocompatibility on immunocompetent mice (n = 5) was carried out to ensure that the nanocomposite hydrogel did not cause a substantial immunological reaction. Mouse models of myocardial infarction were anesthetized, followed by tracheal intubation and ventilation. The animal body temperature was maintained at 37°C during the operation, and nSi Gel+ was administered through intramyocardial injection at the peri-infarct region 14 days after infarction. Therapeutic adverse effect was analyzed by immunohistochemical examination of the myocardium. The result showed no significant inflammation or cardiomyocyte damages. There were no significant differences in proinflammatory gene (TNF, miR-146, or miR-155) or apoptotic marker (Cyclin D1, miR-145, or Rb1) and miRNA expressions between control and hydrogel-injected animals, according to qPCR data. Finally, no disturbance of cardiac activity is detected after injection of the nanocomposite hydrogel[22].

A Host-Guest Supramolecular (HGSM) GelMA Hydrogel

Natural polymer hydrogels are good biomaterials for soft tissue repair due to their low immune rejection, biodegradability, and biocompatibility. However, natural polymer hydrogels are devoid of desired functionality and mechanical strength when compared to ECM of natural tissues. To provide a new hydrogel with suitable characteristics, first a host-guest supramolecule (HGSM) is developed. This novel structure has three arms, which are covalently cross-linked with a natural polymer. The three-armed HGSM is made through an efficient non-covalent host-guest link between acryloylated tetra-ethylene glycol-modified adamantane (A-TEG-Ad) and isocyanatoethyl acrylate-modified β-cyclodextrin (β-CD-AOI2). Further, a HGSM-GELMA hydrogel is acquired through copolymerization between gelatin methacryloyl (GelMA) and HGSM arms to form a covalently crosslinked network. This HGSM-GELMA hydrogel allows the hydrogel to have better mechanical characteristics, easy to be 3D bio-printed, and have self-healing capacity[8].

To generate HGSM-GelMA hydrogel, HGSM and GelMA at various concentrations are initially dissolved in a PBS solution at a temperature of 37°C. This temperature is critical to prevent HGSM release. Subsequently, the mixture should be subjected to UV to initiate free radical copolymerization, to cause a strong HGSM-GelMA hydrogel formation. HGSM-GelMA hydrogel has both covalent and non-covalent cross-links. The polymerization between GelMA and HGSM occurs via covalent, while the host-guest interaction between cyclodextrin (CD) and adamantane (Ad) occurs via a non-covalent cross-linking. Strong and permanent covalent cross-links maintain the overall shape of the hydrogel, while weak host-guest interactions provide some mechanical functions simultaneously. Unlike pure GelMA hydrogels, the pore size decreases, and the pore density increases when the HGSM concentration in HGSM-GelMA increases. These changes in pore size and density might be used as indication of successful HGSM cross-linking with GelMA[8].

HGSM-GelMA hydrogel is easy to form into various forms that are more suitable for the environment of damaged tissue, and therefore may minimize mechanical stimulation. HGSM-GelMA can retain its initial shape and moisture content after the drying-swelling cycle. This might be due to the strengthened crosslinking network of the hydrogel because of the cross-linking between HGSM and GelMA, and the reversible HG interaction in HGSM. A majority of natural biopolymer hydrogels have poor mechanical qualities (for example, they are unstable and brittle), because of their high water content, especially those with non-covalent cross-link. HGSM-GelMA exhibits much better mechanical characteristics, even at a humidity level of 400%. Compared with pure GelMA hydrogels, HGSM-GelMA has excellent compression properties. The compression modulus and mechanical strength of HGSM-GelMA hydrogel increases dramatically when the HGSM concentration is increased. HGSM-GelMA compression modulus may reach 0.63 MPa when the concentration of HGSM increases to 15% (w/v), which is 5.25 times greater relative to that of pure GelMA hydrogels (0.11 MPa). A study used three hydrogels with HGSM concentration of 15% (w/v) to support the weight of 1 kg and analysed the mechanical strength of the hydrogels. None of the HGSM-GelMA hydrogels were damaged in testing, and they remained in their original state. On the other hand, the pure GelMA group was completely disintegrated even when subjected to a pressure of 500 g[8].

Biocompatibility constitutes a crucial evaluation criterion for novel biomaterials. TestCell Counting Kit-8 (CCK-8) might be used to evaluate cell density after cells are cultured in a 3D HGSM-GelMA scaffold. A study showed that cells in HGSM-GelMA had the same proliferation rate as in the pure GelMA scaffold. The on/off staining assay showed that most of the cells were viable (stained green). Moreover, HGSM-GelMA hydrogel showed a clearly visible cell filament and pseudopodia that corresponded with good cytocompatibility and cell proliferation ability when cultured on HGSM-GelMA hydrogel[8].

Synthesized Succinylated Chitosan (SSC) Composite Hydrogels

Chitosan is the material of choice for bone tissue engineering because of its biological and physicochemical properties. It may increase cell proliferation, adhesion, mineralization, and osteogenic differentiation; and its porous structure may play a role in osteoconduction. In addition, its intrinsic cationic properties allow growth factor linkage. However, it has weak mechanical properties, and modifications are needed to enhance it. A modification to synthesized succinylated chitosan (SSC) composite hydrogels showed an advantage in mechanical strength compared to natural chitosan[23,24].

SSC composite hydrogels can be made by combining glucose-6-phosphate (G6P), which serves as a crosslinker, with SSC at various levels of chitosan succinylation, and bone graft materials (BGM) equivalent to yield various SSC composite hydrogels. The various levels of chitosan succinylation are high-SSC (SSC-H), medium-SSC (SSC-M), and low-SSC (SSC-L), when are mixed with G6P and BGM are defined as SSC-HB (SSC-H with BGM), SSC-MB (SSC-M with BGM), and SSC-LB (SSC-L with BGM), respectively. These SSC-based hydrogels provide a mechanism for controlled delivery of biomolecules with potential pH and temperature sensitivity[23].

A study showed that SSC-H, SSC-M and SSC-L had empty pores, while the pores of SSC-HB, SSC-MB, and SSC-LB were filled with a proportion of BGM that were determined by the pore sizes. The lower the levels of chitosan succinylation, the smaller were the pores, compared to those with higher succinylation levels. This fact might be due to the large number of remaining amine groups that might play a role in increasing ionic bonds. These SSC-BGM hydrogels has a sponge like appearance with a strong mechanical property that could maintain their shape intact[9].

A study tested SSC hydrogels for their osteogenic differentiation ability on human adipose tissue-derived mesenchymal stem cells (huAT-MSCs) by performing alkaline phosphatase (ALP) test, which showed early osteogenic differentiation. In comparison to the control group, significant increases in ALP activity occurred in SSC-L after 5 and 10-day cultures. In bone formation, the activity of ALP usually increases and reaches a peak around 10-14 days and then declines. SSC-BGM scaffold has a sufficient bone formation ability and stable mechanical property. In addition, it has chitosan innate antibacterial activities, which prevent infections that may cause an implant failure. Combination of SSC with BGM leads to protection of chitosan polymer against enzymatic degradation, as well as neutralization of chitosan acidic degradation products. Therefore, SSC-BGM scaffold might last longer to yield better bone growth and bone tissue formation[9].

Assessment of SSC-BGM cytotoxicity on direct cell-to-material contact was done by seeding huAT-MSC on the surfaces of SSC-HB, SSC-MB, SSC-LB, and SSC-L. On/off staining was used to determine biocompatibility. The majority of huAT-MSCs were viable. After 24 and 48 hours, the viability of huAT-MSC in all groups was greater than 99%. After 48 hours, SSC-LB had the highest cell viability, which was 99.95%, relative to SSC-HB (99.91%), SSC-MB (99.94%), and SSC-L (99.94%). Therefore, during baseline time, there were no cytotoxicity or proliferation and cell adhesion problems in any of the experimental groups. Furthermore, proliferation rates were compared among different succinylation level. During cell proliferation studies, no cytotoxicity was seen in all groups. Over time, the three hydrogels (SSC-L, SSC-MB, and SSC-HB) promoted cell growth. However, at all-time points, SSC-L, SSC-MB, and SSC-HB had lower proliferation rates than SSC-LB. Therefore, an appropriate succinylation level choice will guarantee proper cellular compatibility[9].

CONCLUSION

Recent advances in the use of hydrogels in tissue engineering provide justification for further research and testing in clinical settings. The

hydrogels that were used in the seven examined studies did not trigger the immune system rejection, had no negative effect on surrounding tissues or cells, and were highly biocompatible with living tissue.

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