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Mecanisms of the chemical crosslinking to obtain the hydrogels: Synthesis, conditions of crosslinking and biopharmaceutical applications

Mecanismo de reticulação química para a obtenção de hidrogéis: Síntese, condições de reticulação e aplicações biofarmacêuticas

Mecanismo químico de entrecruzamiento para la obtención de hidrogeles: Síntesis, condiciones de entrecruzamiento y aplicaciones biofarmacêuticas

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Abstract
Hydrogels are three-dimensional polymer matrices with recognized biomedical applications. Chemically crosslinked hydrogels offer greater mechanical stability than physically crosslinked hydrogels due to the covalent bonds between their polymeric chains. The preparation of hydrogels by chemical crosslinking involves three basic components: monomer, initiator and crosslinking agent, which must be present in proportions that do not alter the integrity of the hydrogel. The chemical crosslinking mechanism can be designed from reactions between complementary functional groups, ultraviolet light reactions, radical polymerization, high energy irradiation, among others. In this review, we revisit the chemical crosslinking mechanisms involving synthetic or natural polymers. Finally, biomedical applications of hydrogels are discussed, such as drug delivery, cell culture, tissue engineering, cancer therapy, among others.
Keywords: Hydrogels; Crosslinking of hydrogels; Chemical crosslinking; Biomedical applications.

Resumo
Os hidrogéis são matrizes poliméricas tridimensionais com aplicações biomédicas reconhecidas. Hidrogéis reticulados quimicamente oferecem maior estabilidade mecânica que os hidrogéis reticulados fisicamente devido às ligações covalentes entre as suas cadeias poliméricas. A preparação de hidrogéis por reticulação química envolve três componentes básicos: monômero, iniciador e agente de reticulação, que devem estar presentes em proporções que não alterem a integridade do hidrogel. O mecanismo de reticulação química pode ser projetado a partir de reações entre grupos funcionais complementares, reações por luz ultravioleta, polimerização radicalar, irradiação de alta energia, entre outras. Nesta revisão, revisitamos os mecanismos de reticulação química envolvendo polímeros sintéticos ou naturais. Finalmente, são discutidas as aplicações biomédicas dos hidrogéis como na liberação de drogas, cultura de células, engenharia de tecidos, terapia contra o câncer, entre outros.
Palavras-chave: Hidrogéis; Reticulação de hidrogéis; Reticulação química; Aplicações biomédicas.

Resumen
Los hidrogeles son matrices poliméricas tridimensionales con aplicaciones biomédicas reconocidas. Los hidrogeles entrecruzados químicamente ofrecen una mayor estabilidad mecánica que los hidrogeles entrecruzados físicamente debido a los enlaces covalentes entre sus cadenas poliméricas. La preparación de hidrogeles por entrecruzamiento químico involucra tres componentes básicos: monómero, iniciador y agente de entrecruzamiento, los cuales deben estar presentes en proporciones que no alteren la integridad del hidrogel. El mecanismo de entrecruzamiento químico puede diseñarse a partir de reacciones entre grupos funcionales complementarios, reacciones por luz ultravioleta, polimerización radicalaria, irradiación de alta energía, entre otras. En esta revisión, revisamos los mecanismos de reticulación química que involucran polímeros sintéticos o naturales. Finalmente, se discuten las aplicaciones biomédicas de los hidrogeles, como la administración de fármacos, el cultivo celular, la ingeniería de tejidos, la terapia contra el cáncer, entre otras.
Palabras clave: Hidrogeles; Reticulación de hidrogel; Reticulación química; Aplicaciones biomédicas.

1. Introduction

Hydrogels consist of three-dimensional matrices formed by natural or synthetic polymers with a high capacity to absorb water or biological fluids. Due to their intrinsic properties, such as renewability, biodegradability, and biocompatibility, hydrogels have been applied mainly in healthcare (Alavarse, et al., 2021). Currently, the design and assembly of these materials have been delineated for specific biomedical and pharmaceutical interests, including cell recruitment, wound healing, tissue engineering, and drug delivery (Afzal, et al., 2018; Bernhard & Tibbitt, 2021).

Since their introduction by Wichterle and Lim, hydrogels have evolved to acquire versatile and adjustable properties and stimulus-responsive behaviors (Bernhard & Tibbitt, 2021). To achieve the adjustable characteristics, several preparation approaches have been reported (Sharma & Tiwari, 2018; Madduma-Bandarage & Madihally, 2021), all of which rely on two basic crosslinking mechanisms: physical crosslinking and chemical crosslinking. Crosslinking is a process of chemical stabilization of polymers, which leads to multidimensional extension of the polymer chain, resulting in a network structure (Alavarse, et al., 2021). In physical crosslinking, hydrogels have temporary but sufficient interactions to make the hydrogels insoluble in aqueous media (Liu, et al., 2018); while in chemical crosslinking, hydrogels are made up of higher-energy permanent junctions (covalent bonds) (Ahsan, et al., 2021).

As mentioned above, chemically crosslinked polymer hydrogels (CCPHs) are formed by covalent bonds, which occur between the polymer chains due to the presence of specific functional groups (-COOH, -OH, -NH_2, -CONH, -$CONH_2$, and -SO_3H). The chemical bonds between the polymer chains are mediated by a crosslinking agent, making these hydrogels more stable than physical hydrogels (Alavarse, et al., 2021). The preparation of CCPHs involves three basic components: (i) a monomer; (ii) an initiator; and (iii) a crosslinking agent. These three components must be present in a ratio that does not alter the consistency and integrity of the hydrogel. In the final step of forming the hydrogels, they are washed with water or ethanol to eliminate unreacted residues, initiator and crosslinker (Ahsan, et al., 2021).

The chemical crosslinking mechanism is a viable strategy that, by connecting one polymer chain to another, improves not only the chemical stability of the hydrogel, but also its mechanical stability (Alavarse, et al., 2021). In general, CCPHs are formed by the chemical reaction of a functionalized polymer or monomer with small molecule and/or polymer crosslinking agents. Examples of such chemical reactions include reactions between thiols and acrylate/vinyl sulfone groups or amines that react readily with aldehydes or activated ester groups. CCPHs of water-soluble monomers can be crosslinked using, among others, γ-radiation, X-rays, UV light, or heat. Both radiation and thermal crosslinking methods are inexpensive, safe, and allow the elimination of the purification step (González-Henríquez, et al., 2017).

In this review, chemical crosslinking methods for the preparation of hydrogels will be covered in detail. The properties and some possible applications of these hydrogels will also be discussed. The articles were retrieved from platforms

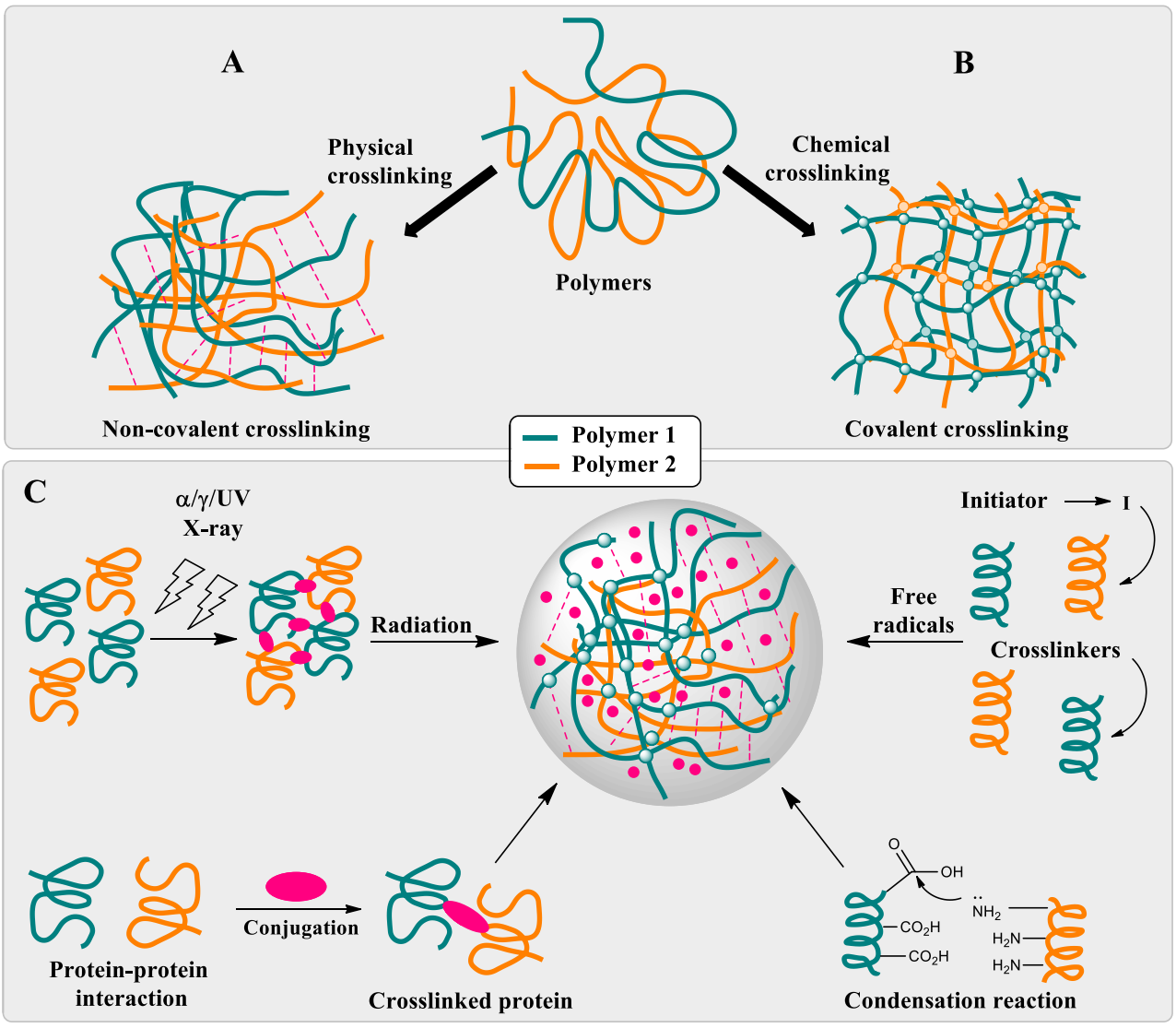
such as Science Direct, PubMed, Medline, Google Scholar, among others, in the period from 2022 to 2023, using the keywords hydrogels, crosslinking of hydrogels, and chemical crosslinking.

2. Preparation of Hydrogels by Chemical Crosslinking

Crosslinking of hydrogels is a stabilization process that leads to multidimensional extension of the polymer chains, resulting in network structures (Liu, et al., 2018). In addition, crosslinking is important to prevent crushing of the hydrogel during the swelling process (Ahsan, et al., 2021), providing these materials with greater physical integrity and mechanical strength (Singhal, et al., 2020).

Chemical crosslinking methods are divided into categories and include reactions between complementary functional groups (Schiff base, Michael addition, condensation, etc.), reaction by ultraviolet light, radical polymerization, and high-energy irradiation, among others (Figure 1) (Pita-López, et al., 2021).

Figure 1 - Schematic representation of the crosslinking methods: physical crosslinking (A) and chemical crosslinking (B). In (C): the schematic representation of the hydrogels synthesized by the mechanism of the chemical crosslinking.



Source: Adapted from Singhal, et al. (2020).

The next sections will discuss the main chemical crosslinking methods for the formation of hydrogels.

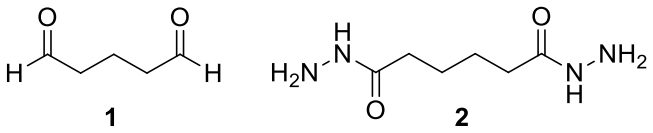
2.1 Crosslinking by complementary functional groups

Water-soluble polymers have hydrophilic functional groups that can be used for the synthesis of hydrogels. The junctions between polymer chains can be established by the reaction of specific functional groups of complementary reactivity, such as an aminocarboxylic acid, isocyanate-OH/NH2 reaction, or by Schiff base formation, which result in covalent bonds between polymer chains (Ullah, et al., 2015; Akhtar, et al., 2016).

a) Crosslinking with Schiff base formation

Schiff base formation occurs by the reaction between amino and aldehyde groups to generate an imine bond (Figure 2).

Figure 2 - Molecular structure of the glutaraldehyde (**1**) and the dihydrazide (**2**).



Source: Drawn using ChemDraw software.

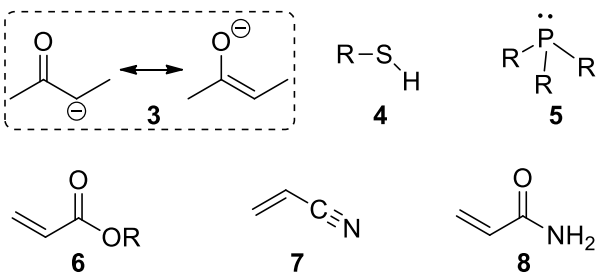
The most frequently used crosslinkers are aldehyde (glutaraldehyde, **1**) and dihydrazide (adipic acid dihydrazide, **2**). To establish this crosslinking, stringent conditions such as low pH, high temperature and addition of methanol as an inhibitor are applied (Akhtar, et al., 2016; Saini, 2016; Singh, et al., 2017).

Generally, proteins such as gelatin, albumin, and amino group endowed polysaccharides are used to obtain hydrogels (Saini, 2016). For example, polyvinyl alcohol (PVA) hydrogels have been crosslinked using glutaraldehyde (Akhtar, et al., 2016). Hyaluronic acid hydrogels were obtained by derivatization with **2**, followed by chemical crosslinking (Hennink & van Nostrum, 2012; Singhal, et al., 2020). The hydrogels were degraded by hyaluronidase and showed a potential to act as a carrier matrix for sustained drug release.

b) Crosslinking by Michael addition

Michael addition is a reaction that occurs between a nucleophile (enolates, amines (**3**), thiols (**4**), phosphines (**5**)) and an electrophile (acrylate esters (**6**), acrylonitrile (**7**), acrylamides (**8**)) (Figure 3) to prepare injectable hydrogels in tissue engineering.

Figure 3 - The examples of the nucleophiles and the electrophiles used in the Michael addition reaction.



Source: Drawn using ChemDraw software.

Natural polymers such as hyaluronic acid, dextran, and chitosan have been conjugated with these groups to prepare hydrogels by Michael reactions (Sui, 2010). The advantages of Michael addition include mild reaction conditions, regioselectivity, and efficient, favorable reaction rate (Hu, et al., 2019). Hydrogels based on chitosan, gelatin, and heparin were

prepared with poly(ethylene glycol) diacrylate (PEGDA) by Michael addition (Hu, et al., 2019). Among the obtained hydrogels, those of chitosan showed high mechanical strength.

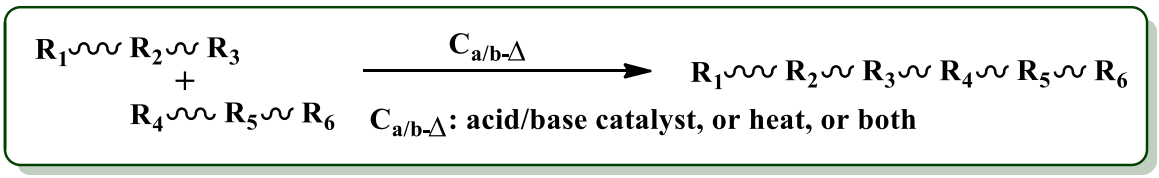
Pupkaite, et al. (2019) prepared crosslinked collagen hydrogels by Michael addition. To do so, collagen was modified to introduce thiol groups, and the hydrogels were prepared by crosslinking with polyethylene glycol-maleimide. The hydrogels showed minimal swelling (6%) for a period of 1 month in an aqueous buffer solution. Furthermore, they were suitable for delivery of encapsulated mesenchymal stromal cells (BMSCs) and endothelial cells (HUVECs). The viability of BMSCs and HUVECs *in vitro* was maintained at about 80 and 70%, respectively, for up to 4 days (Sui, 2010).

c) By condensation reactions

Hydrogel crosslinking by condensation reaction occurs from the joining of hydroxyl groups or amines with carboxylic acids or derivatives to form polyesters and polyamides. The condensation reaction for the formation of carbon-nitrogen double bond is best described because of its milder conditions and because it generates only water as a byproduct of the reaction (Singhal et al., 2020). One of the most efficient crosslinking agents used in this reaction is *N*,*N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide (EDC) (Akhtar, et al., 2015; Singhal, et al., 2020).

The simplified scheme for the synthesis of a crosslinked polymer by condensation reaction is depicted in Figure 4:

Figure 4 - The synthesis of the chemically crosslinked polymers obtained by the polymerization by condensation.



Source: Drawn using ChemDraw software.

A variety of polysaccharide-based hydrogels have been developed using the condensation reaction (Singhal et al., 2020). Polysaccharide hydrogels can be synthesized using Passerini and Ugi condensation reactions. Passerini condensation generates hydrogels with ester-type crosslinks, which degrade at room temperature and pH 9.5, whereas those prepared by Ugi condensation contain amide bonds in their crosslinks, which are stable under these conditions (Hennink & Nostrum, 2012).

2.2 Crosslinking by ultraviolet light

The mechanism of ultraviolet (UV)-induced crosslinking to produce hydrogels has gained importance in recent years, especially because it generates highly patterned structures. In addition, the cytotoxicity of hydrogels obtained by UV is relatively low (Hu, et al., 2019). For example, a chitosan hydrogel was prepared by the UV crosslinking method aiming at cell incorporation and loading (Li, et al., 2015). This hydrogel was found to exhibit low cytotoxicity in acute inflammatory response assays.

Hydrogels synthesized from UV light crosslinking can be multi-responsive to various factors. Choi, et al. (2018) obtained a multi-responsive dual-network hydrogel from allyl functionalized alginate (Alg) crosslinked with *N*-isopropylacrylamide monomers (NIPAM). The Alg-NIPAM hydrogels exhibited different swelling behaviors dependent on NIPAM content, pH, temperature, and added metal ions. The responsiveness of this hydrogel showed potential for biomedical applications (Choi, et al., 2018).

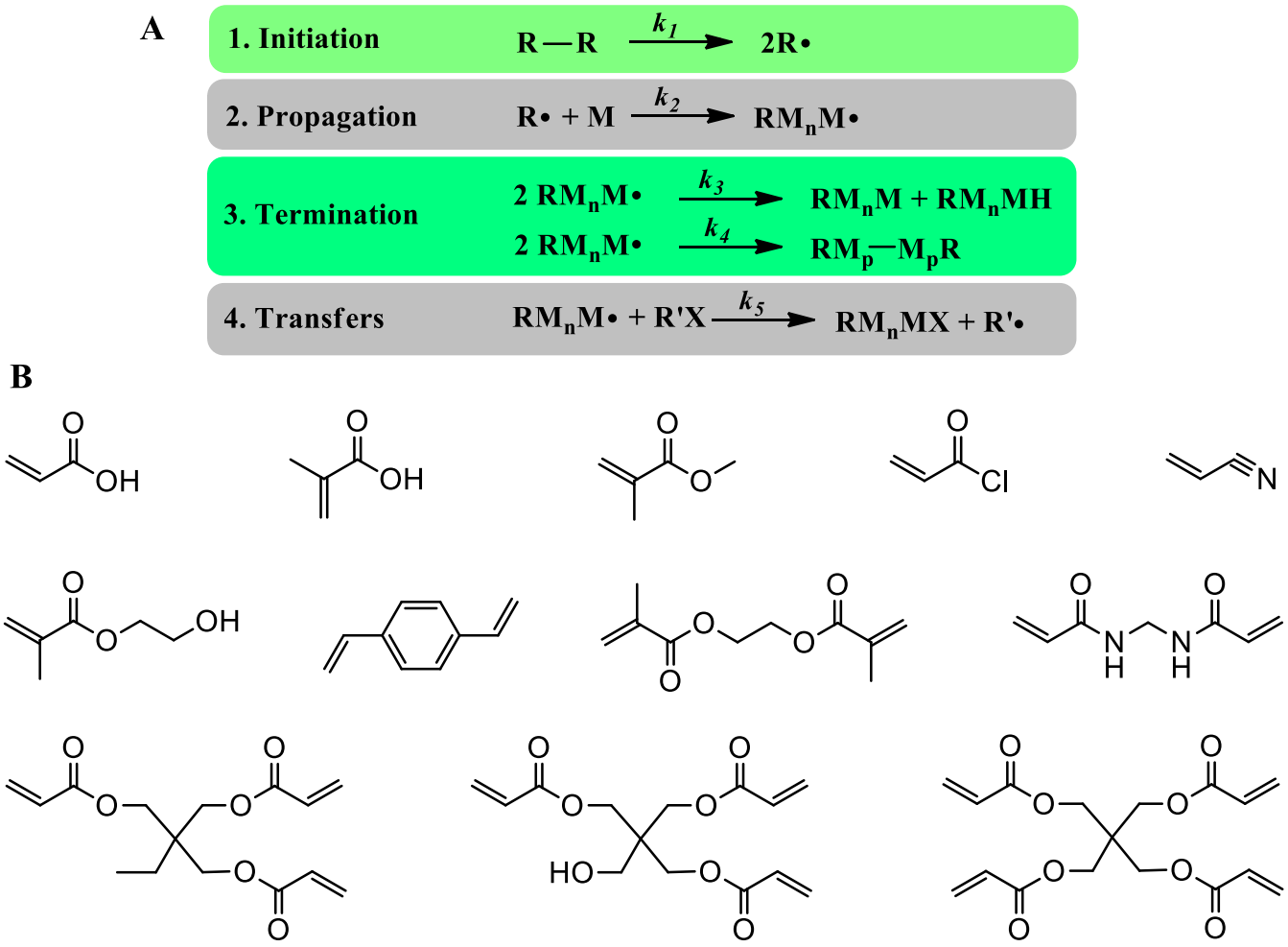
A methacrylated γ-PGA (mPGA) hydrogel was obtained from UV light crosslinking between γ-PGA and glycidyl methacrylate (GMA) (Hu et al., 2019). After synthesis, the hydrogel exhibited ionic properties and pH sensitivity, and low cytotoxicity to bovine chondrocyte cells.

Although the UV light-induced crosslinking method generates highly patterned structures, there are some concerns about the damage that UV radiation may cause to DNA (Hu, et al., 2019). In addition, the type of photo-initiator and solvent applied in hydrogel synthesis should be selected with care, as they may leak from the hydrogel once it is formed and cause damage to cells (Mahinroosta, et al., 2018).

2.3 Crosslinking through free-radical polymerization

This method consists of applying high-energy ionizing radiation (gamma rays and electron beams) to produce free radicals in the polymer chains. The radicals generated combine to form bonds between the polymer chains, creating a network (Sannino, et al., 2009). Natural and synthetic polymers can be used in high-energy radiation crosslinked hydrogels (Figure 5), with PVA, PEG, and PAA being the most common (Madduma-Bandarage & Madihally, 2020).

Figure 5 - The steps which constitute the radical polymerization (A) and the monomers and the crosslinking agents used during the polymerization by free radicals (B).



Source: Adapted from Madduma-Bandarage & Madihally (2020).

Tubular hydrogels have been prepared via radical polymerization (Ma, et al., 2018). In this process, an iron wire acted as a catalyst and mold for the formation of the PVA and poly(2-hydroxyethyl methacrylate) PVA/PHEMA hydrogel layers. The PVA/PHEMA hydrogels showed tube diameters ranging from a few hundred microns to a few millimeters, while the thickness was adjusted between tens to a few hundred microns. These hydrogels showed promise mainly for tissue engineering applications (Ma, et al., 2018).

Free radical crosslinking was used in the synthesis of polyvinyl/acrylic acid and gelatin-based hydrogels (g-NVP-AA) and gelatin nanocomposites (gNVP-AA/MMT) for cephalexin delivery (Hajikarimi & Sadeghi, 2020). The nanocomposite hydrogels showed an average size of 85 nm and porous morphology before the presence of the drug. The porosity directly affected the swelling, loading and drug release. The g-NVP-AA/MMT hydrogels containing cephalexin showed antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*.

2.4 Crosslinking through high-energy irradiation

High energy radiation such as gamma (γ) rays and electron beam can be used to polymerize unsaturated compounds without the use of a crosslinking agent (Akhtar, et al., 016; Singhal, et al., 2020). An advantage of this method is that it does not require any initiator and the swelling capacity of the hydrogel can be controlled only by adjusting the radiation dose. In addition, the hydrogels obtained by this approach are relatively pure, which makes them useful for various biomedical applications (Saini, 2016; Singh, et al., 2017).

Carboxymethyl hyaluronic acid (CMHA) hydrogels have been synthesized by gamma irradiation at concentrations of 10-60% (w/w) and doses of 20-120 kGy (Relleve, et al., 2021). At the 10 and 20% concentrations, the CMHA degraded and no gel content was measured in all dosage ranges. Whereas at the 40% and 60% concentrations, the gel content ranged from 15 to 68%. The CMHA showed swelling in water from 43 to 2,400 g H_2O/g dry gel. At doses of 60 and 120 kGy, the CMHA showed no cytotoxic effect by MTT assay (Relleve, et al., 2021).

Krommelbein, et al. (2021) studied the viscoelastic behavior, surface hydrophilicity and swelling behavior in agarose hydrogels in the sterilization range (0 kGy to 30 kGy). In this dosage range, the average number molecular weight between crosslinks increased by more than 6%. The mechanical properties (rheology measurements), on the other hand, decreased by more than 20% compared to the initial modulus. In addition, increasing the electron dose under high-pressure conditions reduced the formation of gas cavities in the hydrogels (Krommelbein, et al., 2021).

A ternary polyacrylamide-based hydrogel (PAAm) doped with boron nitride nanosheets modified with BNNS-TA tannic acid and Fe^{3+} ions (BNNS-TA/Fe^{3+}/PAAm) was described by Jiang et al. (2021). The BNNS-TA/Fe^{3+}/PAAm hydrogel exhibited excellent compressive strength, which was about four times that of the pure PAAm hydrogel. The BNNS-TA/Fe^{3+}/PAAm hydrogel can be applied as a sensor for radiation monitoring in the nuclear industry (Jiang, et al., 2021).

High-energy irradiation allows for micro/nano scale chemistry control without the need for the addition of initiators or crosslinking agents (Dispenza, et al., 2021; Kim, et al., 2021). Another advantage of this method is sterilization simultaneous to hydrogel synthesis, when appropriate doses are administered (Dispenza, et al., 2021). However, increasing the radiation dose may decrease the degree of hydrogel intumescence (Singhal, et al., 2020).

Finally, CCPHs generally require the addition of crosslinkers or an additional polymer pre-activation step. Despite the advantages of CCPHs in terms of better mechanical properties over physically assembled hydrogels, the residual chemicals and organic solvents employed for crosslinking can make them toxic to human cells (Francesko, et al., 2018).

2.5 Crosslinking by means of enzymatic reactions

Enzymatic crosslinking is one of the key biological processes for the development of supramolecular hydrogel networks with biomedical applications (Badali, et al., 2021). Enzymatically crosslinked hydrogels are highly biocompatible and have better pharmacokinetics. Their degradation produces no toxicity to the biological environment and can be monitored by varying the substrate and the concentration of the crosslinking agent (Singhal, et al., 2020).

Enzyme reactions that occur at neutral pH, in aqueous solutions, and at physiological temperatures are highly desired for biomedical applications (Badali, et al., 2021). Many enzyme systems have been investigated in hydrogel crosslinking. Among them, transglutaminase (TG), peroxidases, tyrosinase, phosphopantetheinyl transferase, lysyl oxidase, plasma amine oxidase, phosphatase/kinase, alkaline phosphatase (ALP), sortase A (SrtA), and horseradish peroxidase (HRP) stand out (Singhal, et al., 2020; Badali, et al., 2021).

Hydrogels obtained via enzymatic crosslinking are fully exploitable as devices for human cells and tissues (Francesko, et al., 2018). For example, supramolecular hydrogels of feruloyl-modified tripeptides and chitosan glycol (FerFFK/GC) were obtained via laccase-mediated crosslinking reaction (Wei, et al., 2019). The FerFFK/GC hydrogel showed higher compression modulus and storage. Feruloyl groups enabled the antioxidant effect of the gel by DPPH assays. In addition, FerFFK/GC hydrogel accelerated the skin wound healing process and was considered a promising material for dressings (Wei, et al., 2019).

Hydrogels composed of gelatin/cellulose nanocrystals (Gel-TG-CNCs) were prepared using microbial transglutaminase (mTG) as a crosslinking catalyst and cellulose nanocrystals (CNCs) as reinforcements (Dong, et al., 2021). The results showed that increasing concentrations of CNCs led to improved mechanical strength. The breaking strength of Gel-TG-CNCs with 2% CNCs could reach 1000 g, being 30 times higher than pure gelatin hydrogels. Gel-TG-CNCs hydrogels also demonstrated excellent biocompatibility by MTT method with Hela cells and can be used in wound repair and tissue engineering (Dong, et al., 2021).

Cao, et al. (2021) studied the effect of transglutaminase (TG) concentration on the physical and oxidative stabilities of filled hydrogel particles obtained by biopolymer phase separation. The filled hydrogels showed relatively smaller particle sizes, higher absolute zeta potentials, and greater interfacial layer thicknesses. In addition, the filled hydrogels exhibited lower degrees of lipid and protein oxidation during 10 days of storage when using TGase concentration of 10 U/g, which was mainly attributed to TGase crosslinking of protein molecules on the droplet surface. The authors suggest that the improved physical and oxidative stability is a result of protein crosslinking, which likely generates a thicker interface around the droplet surface (Cao, et al., 2021).

Hydrogels obtained by enzymatic crosslinking have biomedical applications that include everything from use in tissue engineering and wound healing to drug delivery (Badali, et al., 2021). Tyramine modified gel gum (Ty-GG) hydrogels were developed by crosslinking horseradish peroxidase (HRP) for encapsulation of betamethasone to increase its specificity and safety in treating patients with rheumatoid arthritis (RA) (Oliveira, et al., 2021). The Ty-GG hydrogels exhibited high mechanical strength and were shown to have a therapeutic effect for the controlled release of betamethasone over time. They also exhibited no cytotoxic effects and did not affect the metabolic activity and proliferation of primary chondrogenic cells (Oliveira, et al., 2021).

Hydrogels formed by enzyme mediation resemble extracellular matrices, exhibiting unique physicochemical properties and functionalities such as water-holding capacity, drug transport capacity, biodegradability, biocompatibility, biostability, self-healing capacity, and memory capacity (Badali, et al., 2021).

2.6 Hydrogels produced by hybrid double network crosslinking

Hybrid dual-network polymer hydrogels (HDNPH) consist of two crosslinked, interwoven networks. The first is generally rigid and strongly crosslinked through covalent bonds; the second is soft and weakly crosslinked through supramolecular interactions such as hydrogen bonds, ionic interactions, or coordination interactions (Figure 1.C) (Mondal, et al., 2020).

HDNPHs have emerged to improve the mechanical performance of synthetic hydrogels by combining high strength and toughness, which are tuned by inter/intramolecular interactions, using a wide range of monomers, crosslinkers, and synthesis strategies (Xu, et al., 2021). For example, Chen, et al. (2015) synthesized Agar/PAM based physicochemically crosslinked HDNPH and observed that it had a tensile stress of 3.3 MPa, higher than the control which was 2.8 Mpa. Furthermore, the Agar/PAM hydrogels exhibited excellent resistant properties for protein adsorption, cell adhesion and bacterial binding (Chen, et al., 2015).

Huang, et al. (2020) obtained HDNPH using chitin as surface repair material. The hydrogel was synthesized using hybrid regenerated chitin nanofibers (RCNs)-poly(ethylene glycol diglycidyl ether) (PEGDE) as the first network and polyacrylamide (PAAm) as the second network. The RCNs-PEGDE/PAAm hybrid hydrogel was strong and tough, possessing Young's modulus (elasticity) E 0.097 ± 0.020 MPa, fracture stress σ_f 0.449 ± 0.025 MPa and fracture work W_f 5.75 ± 0.35 $MJ \cdot m^{-3}$. Added to this, chitin endowed the hydrogel with good bacterial resistance and accelerated fibroblast proliferation, increasing the number of NIH3T3 cells by almost 5-fold in 3 days (Huang, et al., 2020).

Finally, Khodami, et al. (2022) proposed an HDNPH based on synthetic PVA and alginate. The ferrous ion was used to catalyze the decomposition of the initiator and subsequently, after its oxidation by the initiator to the ferric ion, it was coordinated by the carboxylic groups of the polymeric networks and acted as a physicochemical crosslinker. The composition of the hydrogel was optimized to obtain enhanced mechanical and electrical properties and self-healing ability (Khodami, et al., 2022).

2.7 Conductive hydrogels

Hydrogels obtained by hydration of conductive polymers have excellent electrical properties, being recognized as multifunctional materials that can perform intelligent functions in various devices, sensors and bioactuators, at the interface of robotics/molecular biology (Chen, et al., 2023). The absorption and release of water in these systems can perform work, generating complex and uniform stimuli, of great biological interest, enabling an acceptable performance in bioactuators, in particular the double-layer ones, which are efficient in bending and in asymmetrical actions in the matrix (Le, et al., 2019).

Yao, et al. (2016) and collaborators developed hydrogels of poly(*N*-isopropylacrylamide) nanocomposites in which changing the thickness of the two hydrogel layers with different clay contents, the thermoresponsive bending direction and the degree of hydrogel actuators can be adjusted.

Yu, et al. (2001) and collaborators presented a biomimetic hydrogel valve that can control the flow direction by activating the local pH in microfluidic channels. As the hydrogel valve is composed of a pH-sensitive hydrogel strip and a pH-insensitive hydrogel strip, the volume and shape of the hydrogel valve reversibly changes by changing the local pH. Under alkaline conditions, these devices can mimic anatomical venous valves, both functionally and structurally, due to the asymmetric swelling of the two strips, allowing fluid flow in one direction while preventing flow in the opposite direction.

3. Concluding Remarks

Throughout this review, the different chemical crosslinking approaches for the formation of hydrogels were discussed. The physicochemical properties and the different technologies currently adopted for the synthesis and modification of these

materials were also discussed. In addition, the innovative applications of hydrogels related to the biomedical area, such as in controlled drug release, cell culture, tissue engineering, imaging diagnostics, among others, were presented. Finally, it was discussed that chemical crosslinkers with cellular toxicity, such as glutaraldehyde, can result in cytocompatibility problems. In this sense, green chemistry routes represent a future strategy for the synthesis of hydrogels from natural or synthetic biopolymers, thus allowing the obtainment of biomaterials with desired characteristics.

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