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Recent advances in the development of the physically crosslinked hydrogels and their biomedical applications

Recentes avanços no desenvolvimento de hidrogéis fisicamente reticulados e suas aplicações biomédicas

Avances recientes en el desarrollo de hidrogeles físicamente reticulados y sus aplicaciones biomédicas

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Abstract
Hydrogels are three-dimensional networks formulated from natural or synthetic polymers with a high capacity to absorb and transport water in their structure. Hydrogels are prepared from the crosslinking of their polymeric chains, which involves two basic mechanisms: chemical crosslinking and physical crosslinking. In chemical crosslinking, hydrogels are held together by covalent bonds; while physically cross-linked hydrogels are produced by non-covalent interactions, such as hydrogen bonds, electrostatic interactions, and hydrophobic forces, among others. Physically cross-linked hydrogels are more similar to biological systems due to their assembly dynamics, so they have wide biomedical applications. The most used approaches in the preparation of hydrogels by physical crosslinking include freeze-thaw, formation of stereocomplexes, ionic interaction, hydrogen bonding, crystallization, and crosslinking by hydrophobic interactions. These approaches are briefly discussed in this review. Some biomedical applications of these hydrogels will also be discussed.
Keywords: Hydrogels; Physical crosslinking; Assembly dynamics; Biomedical applications.

Resumo
Os hidrogéis são redes tridimensionais formuladas a partir de polímeros naturais ou sintéticos com alta capacidade de absorver e transportar água em sua estrutura. Os hidrogéis são preparados a partir da reticulação de suas cadeias poliméricas, que envolve dois mecanismos básicos: a reticulação química e a reticulação física. Na reticulação química, os hidrogéis são unidos por ligações covalentes; enquanto que os hidrogéis fisicamente reticulados são produzidos por interações não covalente, como ligações de hidrogênio, interações eletrostáticas, forças hidrofóbicas entre outras. Os hidrogéis fisicamente reticulados se assemelhem mais aos sistemas biológicos devido à sua dinâmica da montagem, por isso têm ampla aplicação biomédica. As abordagens mais usadas na preparação de hidrogéis por reticulação física incluem congelamento-descongelamento, formação de estereocomplexos, interação iônica, ligações

de hidrogênio, cristalização, reticulação por interações hidrofóbicas. Estas abordagens são brevemente discutidas nesta revisão. Serão discutidas também algumas aplicações biomédicas desses hidrogéis.
Palavras-chave: Hidrogéis; Reticulação física; Dinâmica da montagem; Aplicações biomédicas.

Resumen
Los hidrogeles son redes tridimensionales formuladas a partir de polímeros naturales o sintéticos con una alta capacidad de absorción y transporte de agua en su estructura. Los hidrogeles se preparan a partir del entrecruzamiento de sus cadenas poliméricas, lo que implica dos mecanismos básicos: el entrecruzamiento químico y el entrecruzamiento físico. En la reticulación química, los hidrogeles se mantienen unidos por enlaces covalentes; mientras que los hidrogeles físicamente reticulados se producen por interacciones no covalentes, como enlaces de hidrógeno, interacciones electrostáticas, fuerzas hidrofóbicas, entre otras. Los hidrogeles físicamente reticulados son más similares a los sistemas biológicos debido a su dinámica de ensamblaje, por lo que tienen una amplia aplicación biomédica. Los enfoques más utilizados en la preparación de hidrogeles por entrecruzamiento físico incluyen congelación-descongelación, formación de estereocomplejos, interacción iónica, enlaces de hidrógeno, cristalización, entrecruzamiento por interacciones hidrofóbicas. Estos enfoques se discuten brevemente en esta revisión. También se discutirán algunas aplicaciones biomédicas de estos hidrogeles.
Palabras clave: Hidrogeles; Entrecruzamiento físico; Dinámica de ensamblaje; Aplicaciones biomédicas.

1. Introduction

Hydrogels are polymeric materials used in various scientific and industrial processes. They consist of a three-dimensional network formed from synthetic or natural polymers. They are classified into conventional hydrogels and smart hydrogels, the latter being able to interact with specific biological targets. Hydrogels have several physicochemical characteristics, including broad functionality, biocompatibility, and biodegradability. In addition, they have a high capacity to absorb water and biological fluids, being a material with characteristics similar to those observed in living tissues (González-Henríquez, et al., 2017).

The term hydrogel was already widely used in 1894; however, the first biological use was not reported until 1960 by Wichterle and Lim. Since then, hydrogels have attracted the interest of many scientists in various research fields (Ahsan, et al., 2021). Currently, they have received special attention in the biomedical field, being used in drug delivery, cell encapsulation, cell culture, tissue engineering (treatment or replacement of tissues and organs), coatings and dressings for chronic and traumatic wound healing, diagnostics, optics, imaging, among others (Ng, et al., 2014; Akhtar, et al., 2015; González-Henríquez, et al., 2017).

The methodologies used in the development of hydrogels have also received special attention in recent years (Ng, et al., 2014). These methodologies are based on the type of bond formed between the polymer chains, and are divided into two basic mechanisms: chemical crosslinking and physical crosslinking (Shantha & Harding, 2002; Sharma & Tiwari, 2020; Madduma-Bandarage & Madihally, 2021). In chemical crosslinking, hydrogels are joined by permanent (covalent) bonds, whereas in physically crosslinked hydrogels the interactions are of the non-covalent type, i.e., weak and transient bonds (Akhtar, et al., 2015; Liu, et al., 2018; Ahsan, et al., 2021).

Physically crosslinked hydrogels can be reversibly formed or disrupted due to non-covalent interactions. These interactions can be of different nature such as hydrophobic interactions, hydrogen bridges, electrostatic interactions, or even crystallized segments. The main advantage of physical crosslinking is that this method does not produce toxic residues; unlike chemically crosslinked hydrogels (Akhtar, et al., 2015). In addition, physically crosslinked hydrogels have particular structures, capable of forming larger aggregates compatible for the incorporation of biological molecules (González-Henríquez, et al., 2017).

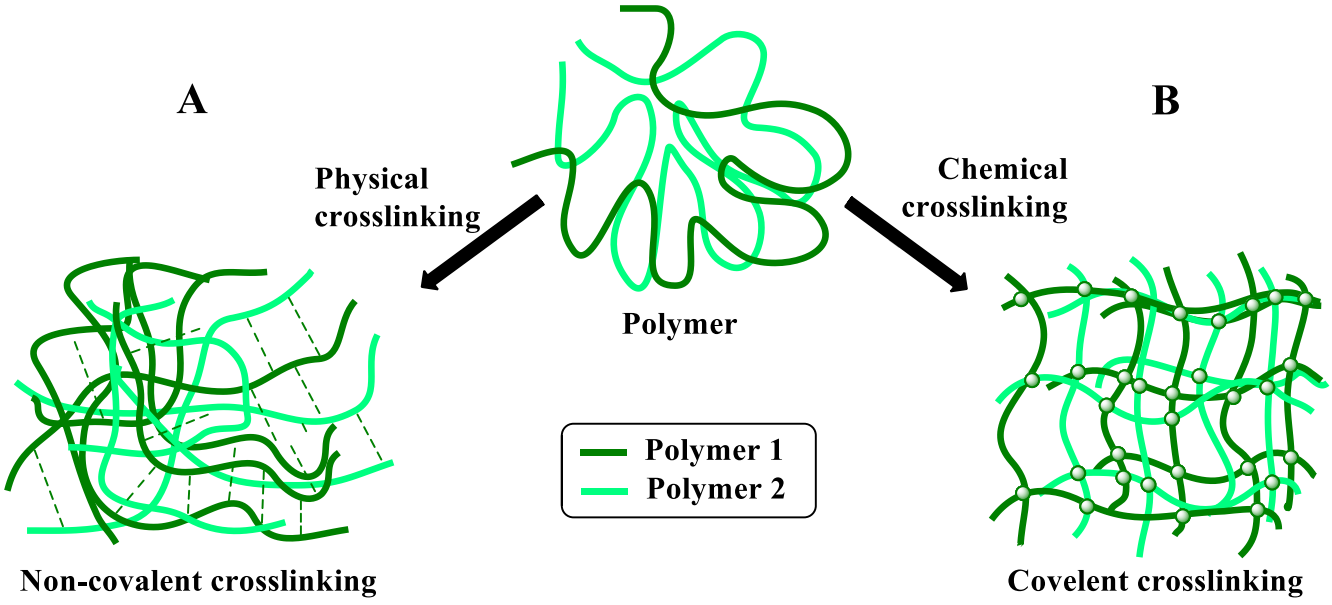
In this review, physical crosslinking methods for the preparation of hydrogels will be discussed in detail. The properties and some possible applications of these hydrogels will also be discussed. The articles were retrieved from platforms

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

2. Methods for Crosslinking Hydrogels

Natural and synthetic polymers contain various hydrophilic functional groups present in their structure, such as -COOH, -OH, -NH_2, -CONH, -$CONH_2$, and -SO_3H, which can interact physically (Figure 1 A) or chemically (Figure 1 B) to form three-dimensional networks (Ahsan, et al., 2021).

Figure 1 - Schematic representation of the methods of crosslinking: physical (A) and chemical (B).



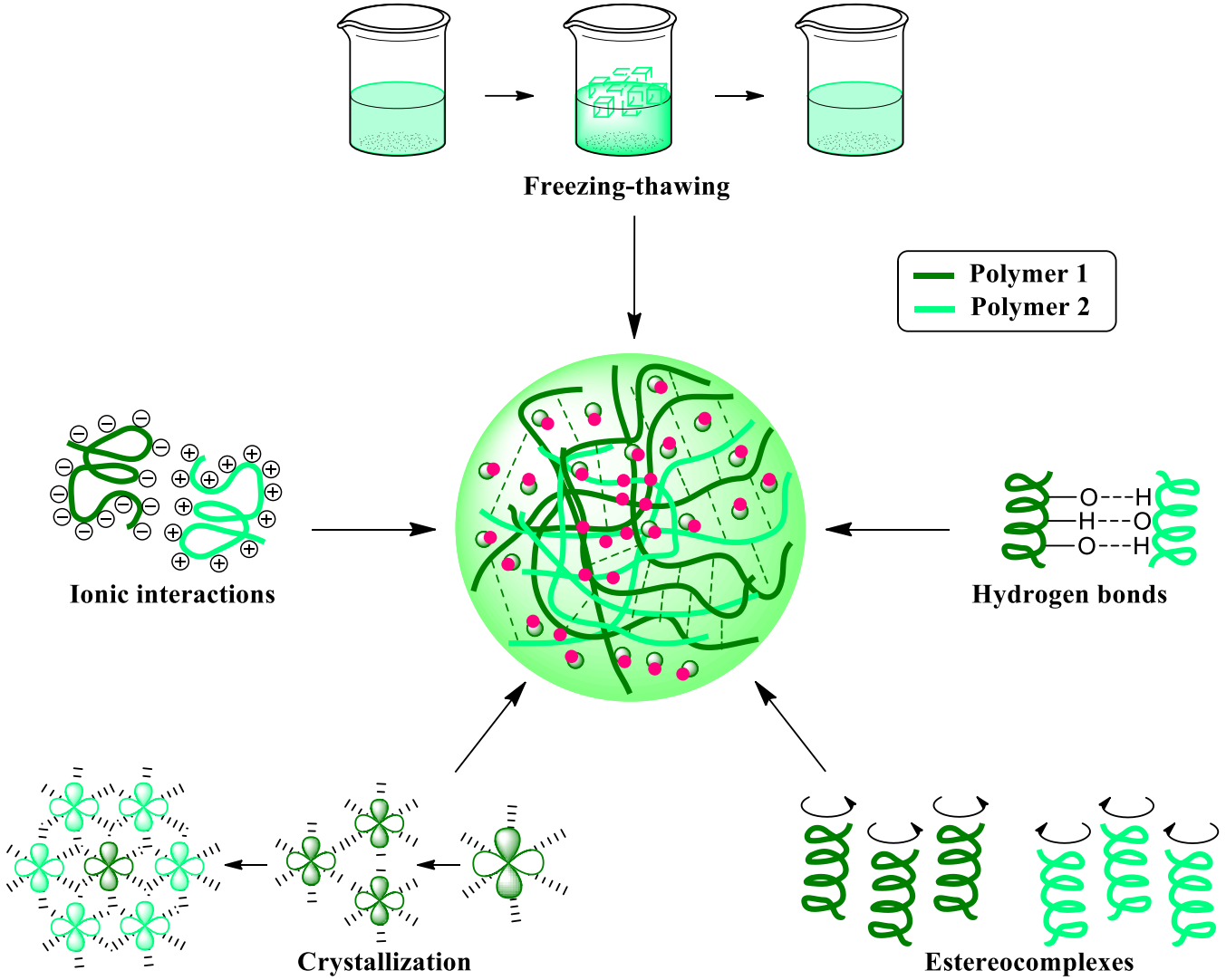
Source: Adapted from Singhal, et al. (2020).

As shown in Figure 1, the functional groups present in polymers are responsible for the hydrogel formation process, leading to a multidimensional extension of the polymer chains to form the three-dimensional network (Liu, et al., 2018). The crosslinking mechanism is very important to avoid crushing of the hydrogel during the swelling process (Ahsan, et al., 2021). It promotes an increase in the mechanical strength and physical integrity of the hydrogel. Additionally, it provides better resistance to dissolution of the material due to the cross interactions present between the polymer chains (Figure 1 A) (Liu, et al., 2018; Singhal, et l., 2020; Madduma-Bandarage & Madihally, 2021).

3. Development of Hydrogels by Physical Crosslinking

Physically crosslinked polymer hydrogels (PCPH) have received significant attention in recent years. PCPH do not require a bonding agent compared to those obtained by chemical crosslinking methods. Crosslinking agents for PCPHs not only affect the probity of the substances but can also produce some naturally toxic effects (Singhal, et al., 2020). Different methods involved in the synthesis of physically crosslinked hydrogels are shown in Figure 2, below:

Figure 2 - Formation of hydrogels by the physical crosslinking.



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

As shown in Figure 2, PCPHs are obtained from natural polysaccharides, poly(vinyl alcohol), poly(ethylene glycol), poly(N-isopropylacrylamide), poly(acrylic acid), poly(vinyl imidazole), and others (Mondal, et al., 2020). One of the advantages of PCPHs is that they avoid the potential toxicity produced by chemical crosslinkers (Liu, et al., 2018; Pita-López, 2021), so they are safer for applications *in vivo* assays, since chemical crosslinkers can leave toxic residues, resulting in damage to cells (Ahsan, et al., 2021).

The most commonly used methods to produce PCPH include freezing-thawing, stereocomplex formation, ionic interaction, hydrogen bonds, crystallization, and crosslinking by hydrophobic interactions (Figure 2), among others (Sharma & Tiwari, 2020).

3.1 Crosslinking by freezing-thawing

The mechanism of freeze-thaw physical crosslinking involves the formation of ice crystals in polymer-poor areas that pull the chains away from polymer-rich regions, resulting in the formation of crystals (from interchain hydrogen bonds) that act as crosslinking points in the hydrogel (Willcox, et al., 1999). The most common example of freeze-thaw physical crosslinking is the formation of poly(vinyl alcohol)-PVA hydrogels (Sharma & Tiwari, 2020). PVA is known for its

biocompatibility and hydrophilicity (Hong, 2017). The latter characteristic is due to the large number of hydroxyl groups that provides the formation of hydrogen bonds between the chains present in its structure (Willcox, et al., 1999).

Hassan, et al. (2000) investigated the incorporation of the model protein, bovine serum albumin (BSA), into PVA hydrogels by the freeze-thaw process and its subsequent release behavior. Hydrogels prepared with 3 or 5 cycles were found to have good stability over an intumescent period of 6 months. Although the release profile of BSA was not significantly different from the 3-cycle and 5-cycle hydrogels, the rate and total amount of PVA dissolution were considerably higher for the 3-cycle hydrogels (Hassan, et al., 2000).

Preparation of hydrogels by freezing-thawing can result in significant effects on their properties. Poly(vinyl alcohol)/polyacrylamide (PVA/PAM) double-network hydrogels were obtained by the freeze-thaw and annealing-intumescence methods (Ou, et al., 2017). Hydrogels made by freezing-thawing were found to have larger pore size and higher swelling capacity than those produced by annealing-intumescence (used as a control).

Hong (2017) developed an effective and potential dressing consisting of a hydrogel blend of PVA and tannic acid - TA (PVA/TA). TA can serve not only as a crosslinking agent, but also as a therapeutic agent. The PVA/TA hydrogel was prepared by three freezing cycles at -20 ± 3 °C for 18 h followed by thawing at 25 °C for 6 h. It was observed that the PVA/TA hydrogel showed an increase in mechanical strength and hardness and that the moisture content decreased as the concentration of TA was increased. Added to that, the PVA/TA hydrogel showed excellent antibacterial activity (>99.99%) and antioxidant capacity (>92%) (Hong, 2017).

PVA-chitosan hydrogels were also produced by the freeze-thaw method as a release system for Diflunisal, a nonsteroidal anti-inflammatory drug (Figueroa-Pizano, et al., 2020). For the preparation of the hydrogels, a polymer ratio of 50/50 (w/w %) was used, with variations of the freezing temperature (-4 °C, -20 °C, -80 °C) and the freeze-thaw cycles (4, 5, 6 cycles). It was observed that at lower temperatures the pore size decreased, while the porosity increased. The swelling percentage also increased at the lower freezing temperature. The drug release was maintained for 30 h and was regulated by a simple diffusion mechanism.

By the freeze-thaw physical crosslinking method, Hu, et al. (2023) developed a double-network hydrogel (DNCGel) consisting of PVA and xanthan gum (XG) chelated with ferric ion (Fe^{3+}). The DNGel3 hydrogels received the most attention in the study. DNCGel3 hydrogels were found to exhibit greater biocompatibility *in vitro*. Furthermore, DNCGel3 hydrogels were effective in wound healing *in vivo* (up to 97.8% healing rate) under electrical stimulation and real-time human motion monitoring.

3.2 Crosslinking by ionic interactions

Hydrogel synthesis via ionic interaction crosslinking can be achieved in two ways: (i) between a polymer and a small molecule with opposite charge as a ligand; and (ii) between two oppositely charged polymers (Singhal, et al., 2020).

Hydrogels prepared via ionic interaction are usually formed by ion-sensitive natural polysaccharides such as alginate (Ca^{2+} and other divalent ions), chitosan (Mg^{2+} or pH), and k-carrageenan (K^{+}). These hydrogels can be destabilized by removing cations using chelating agents or by modified ionic crosslinking through deprotonation by adjusting the pH (Sood, et al., 2016).

Crosslinking by ionic interaction is mechanically weak, resulting in rapid erosion or degradation (Francesko, et al., 2018), so some variables of this type of crosslinking are difficult to control, including gelation time, network pore size, and degradation time (Francesko, et al., 2018).

Calcium alginate (ALG-NG) and zinc pectin (PEC-NG) nanogels were synthesized by the reverse microemulsion method using ionic interaction crosslinking (Podgórna, et al., 2017). The ALG-NG and PEC-NG nanogels were 100 nm in size

and were stable for up to 2 months. In addition, they showed no toxicity to the human neuroblastoma cell line SH-SY5Y and were considered a suitable material for future use.

Zhou, et al. (2018) investigated the influence of different bivalent ions (zinc, copper, strontium, calcium) on the crosslinking of alginate/polyacrylamide hydrogels, as well as their antibacterial properties and biocompatibility *in vitro* and *in vivo*. The hydrogels crosslinked by copper ions showed higher breaking strength, while those crosslinked by strontium and zinc exhibited high mechanical strength. The *in vitro* and *in vivo* studies showed that the zinc crosslinked hydrogel exhibited a higher spectrum of antibacterial activities, cell viability, and wound regeneration capacity by stimulating fibroblast migration, vascularization, and granulation tissue formation (Zhou, et al., 2018).

Ionic crosslinking was used to produce hydrogels based on polyanionic and polycationic polymers *in situ* using quaternary ammonium salt of chitosan and sodium alginate (Geng, et al., 2021). The hydrogel formed possessed a homogeneous 3D network structure and exhibited reasonable mechanical properties. In addition, the hydrogels had excellent injectability and the compressive strength was up to 27.65 kPa. This strength was attributed to the Ca^{2+} forming secondary crosslinking. Cytotoxicity analysis showed that the hydrogel was nontoxic to NIH-3T3 cells. In summary, the hydrogel produced can be used as an ideal biomaterial for biomedical applications.

A system of oxidized alginate (di-aldehyde alginate, ADA) and gelatin (GEL) (ADA-GEL) was crosslinked via ionic (Ca^{2+}) interaction (Distler, et al., 2020). The crosslinking method allowed for adjusting the stiffness of the hydrogels over a wide range (from <5 kPa to 120 kPa), without changing their initial chemical composition. The degradation behavior of the hydrogels remained stable for up to 30 days of incubation.

Ionic crosslinking has also been used to prepare hydrogels based on oxidized nanocellulose and alginate, using Ca^{2+} as the crosslinking agent (Lin, et al., 2012; Mondal, et al., 2020). Carboxyl groups on the nanocellulose surface were induced by chemical oxidation via the 2,2,6,6-tetramethylpiperidin-1-oxyl radical (TEMPO). By the ion crosslinking process, hydrogel sponges were prepared and showed high porosity, promising water absorption and retention as well as compressive strength (Lin, et al., 2012, Mondal, et al., 2020).

3.3 Crosslinking by hydrogen bonding

Hydrogen bonding is a weak bond that results from an electrostatic attraction between a proton of one molecule and an electronegative atom of another (Lu, et al., 2018). Hydrogen bond crosslinking was created from the freeze-thaw method to result in a stronger and more elastic hydrogel, taking into consideration the high entanglement of its polymer chains (Gibas & Janik, 2010).

Hydrogels inspired by biological protein materials have been prepared by multiple hydrogen bonding from cellulose nanocrystals (CNCs) and PVA, using tannic acid (TA) as a molecular coupling bridge (TA-PVA/CNC) (Lin, et al., 2020). TA-PVA/CNC hydrogels showed ultra-high strength (up to 8.7 MPa), high elongation (up to 1107.6%), remarkable hardness (up to 58.2 MJ/m^3), and high compressive strength (up to 67.9 MPa at 90% strain). Due to the dynamic nature of the hydrogen bonds, the hydrogels were able to dissipate energy at the molecular scale and their self-recovery ability was much higher than that of most hydrogen bond-based hydrogels.

Hydrogen bond crosslinked hydrogels were obtained in the form of micelles in polymeric triblocks (LTL) from poly(acrylic acid) (PAA) and methacrylated ply(ether-thiourea) (MT) (PAA/LTL/MT) (Zhang, et al., 2020). As a result, the hydrogels exhibited remarkable mechanical performance, including ultra-extensibility and hardness. The maximum fracture strain, true fracture stress and hardness were 7800%, 4.80 MPa, and 1.73 MJ/m^3, respectively. In addition, the dense hydrogen bonds gave the PAA/LTL/MT hydrogel increased self-healing and good conductivity, making it a promising candidate for biosensors.

Liu, et al. (2023) synthesized a hydrogel based on *N*-[Tris (hydroxymethyl) methyl]-acrylamide (THMA), polyethylene glycol diacrylate (PEGDA), and sodium alginate (SA) as a wound repair material. The THMA/PEGDA/SA hydrogel exhibited strong adhesion to various surfaces and provided a mechanical microenvironment similar to normal skin tissue. In addition, the THMA/PEGDA/AS hydrogel was effective in wound closure, absorption of exudate, and keeping the wound moist and clean (Liu, et al., 2023).

Despite their wide application, hydrogen bond crosslinked hydrogels are generally not able to combine high elasticity and hardness, because water molecules can weaken the strength of hydrogen bonds (Zhang, et al., 2020).

3.4 Crosslinking by crystallization

Crystallization is the other method of forming physical crosslinking, and is a process associated with the partial alignment of polymer chains (Lu, et al., 2018). The crosslinking process for producing hydrogels occurs from cooling a polymer under certain conditions, such as heating or cooling a polymer solution, freeze-thaw cycles, changing pH, and mixing polyelectrolyte solution with an oppositely charged multivalent ion (Madduma-Bandarage & Madihally, 2021).

As mentioned earlier, some techniques involving freeze-thaw cycles in the presence of crystalline regions are applied to produce stable hydrogels such as PVA hydrogels (Lu, et al., 2018). Using 3D printing, Jiang et al. (2020) produced ultra-high strength hydrogels, obtained by building DPC crosslinking networks with PVA and chitosan (CS). The DPC hydrogel exhibited tensile strength of 12.7 MPa, strain of 302.3 %, Young's modulus of 14.0 MPa, and extension work of Wext fracture of 22.10 MJ/m^3 because of the dissipation energy of the rigid ionic network of CS.

A simple technique for preparing alginate hydrogels and the simultaneous crystallization of a drug, sildenafil, inside the beads has been reported (Cho, et al., 2020). The crystallization of sildenafil was accomplished by mixing water and DMSO droplets, containing alginate chains and a drug solution. The diffusion of solvents, alginate, drug, and Ca^{2+} ion resulted in *in situ* crystallization during crosslinking. The particle size of sildenafil crystals was significantly reduced to the submicron range while their crystallinity can be controlled by processing parameters (Cho, et al., 2020).

3.5 Crosslinking by stereocomplex formation

Another physical crosslinking method used in the production of hydrogels is based on stereocomplex formation (Lu et al., 2018). Generally, stereocomplex formation involves the enantiomers of polylactic acid (PLA), poly (D-lactic acid) (PDLA), and poly (L-lactic acid) (PLLA), which form stereocomplex crystals in a racemic mixture (Im et al., 2021). The ability of PLA to form stereocomplexes was first reported by Sharma, et al. (2020). The main advantage of stereocomplexed hydrogels is that they are easily formed by dissolving each component in water and mixing the solution. However, a significant limitation of stereocomplexation is the limited range of polymer compositions that can be used (Sharma, et al., 2020).

Injectable electroactive hydrogels (IEHs) were produced by condensation between carboxyl protected tetraaniline (CTA) and PLA blocks (PLA-PEG-PLA). The hydrogels were obtained as a mixture of enantiomeric copolymers CTAPLLA-PEG-PLLA-CTA and CTA-PDLA-PEG-PDLA-CTA (Cui, et al., 2013). Gelation was induced by stereocomplexation between PLLA and PDLA blocks and was dependent on time, temperature, and length of the PLA blocks. It was found that the hydrogels showed excellent cytocompatibility for L929 fibroblast cells when they were encapsulated with the hydrogels. Furthermore, when treated with pulsed electrical stimuli, the hydrogels were active, accelerating the proliferation of encapsulated fibroblasts, cardiomyocytes, and osteoblasts (Cui, et al., 2013).

A hybrid hydrogel made of PDLA/PLLA with an outer layer of gelatin/nano hydroxyapatite (PDLA/PLLA/Gel/nHA/Gen) was prepared for bone tissue engineering (Wang, et al., 2020). The PDLA/PLLA/Gel/nHA/Gen hydrogel exhibited optimal interconnectivity, vast porosity, and adequate size for nutrient delivery. *In vitro* tests with mouse

MC3T3-E1 pre-osteoblast cells showed that PDLA/PLLA/Gel/nHA/Gen hydrogels improved cell adhesion, cell proliferation, and calcium deposition (Wang, et al., 2020).

Physical crosslinking by stereocomplexes of PLLA and PDLA provides a robust approach and contributes to improving material properties such as stability and biocompatibility (Luo, et al., 2020). The main advantage of this process is the ease of obtaining the hydrogel, however the characteristic limitation has been the still restricted availability of polymer compositions (Lu, et al., 2018).

3.6 Thermogelation and hydrophobic interactions

Hydrophobic interactions are entropy driven, their strength depends on the temperature of the system, the molecular weight of the interaction blocks, and the concentration of the polymer (Bernhard & Tibbitt, 2021). For example, poly(ethylene glycol)-poly(propylene oxide) (PEG-PPO) forms hydrogels by hydrophobic interactions in aqueous media at high temperatures. These polymers are therefore thermogels and the increase in temperature will strengthen the hydrophobic interaction - entropic component of the interaction, which becomes more favorable and will induce polymer aggregation resulting in hydrogel formation. Other well-studied polymers are Pluronic and poly(*N*-isopropylacrylamide) - PNIPAm (Bernhard & Tibbitt, 2021).

Injectable and temperature- and pH-sensitive hydrogels have been prepared using carboxymethyl chitosan and poly(*N*-isopropyl acrylamide)-glycidyl methacrylate (CMCS-PNIPAm-GMA) (Zhang, et al., 2014). CMCS-PNIPAm-GMA hydrogels were used in the release assay of the drugs 5-fluorouracil (5-Fu) and diclofenac sodium (DCS) *in situ*. The release rate of DCS was 27% in solution at 37 °C and pH 2.1 for 24 h, while it was 89% in pH 7.4 solution at 37 °C for 24 h. This implies that the hydrogels could protect the drug in a low pH medium (such as the stomach) and release them substantially in higher pH condition (such as the intestine).

Jung, et al. (2017) prepared a type of thermosensitive hydrogel by simply physically mixing hyaluronic acid (HA) and Pluronic F-127 (HP) in aqueous solution. The addition of high molecular weight HA not only increased the mechanical strength of the hydrogel, but also caused a sustained release of the drug. *In vitro* and *in vivo* assays showed that the hydrogel not only exhibited superior mechanical strength, but also caused sustained drug release behavior. The authors believe that the hydrogel in question can be applied as a matrix for drug delivery in various disease treatments (Jung, et al., 2017).

While this class of hydrogels is attractive because of its ease of assembly and relatively simple design, the materials may be limited in their adjustability. Adjusting the mechanical properties requires an increase in polymer concentration or change in polymer structure. These modifications can affect the ability to encapsulate molecules and the amount of material that needs to be injected into the body (Bernhard & Tibbitt, 2021).

4. Outlook

The recent literature data show that physical crosslinking strategies to obtain the hydrogels have grown significantly. In this sense, the physical crosslinking mechanisms are reviewed in this work in order to provide an overview of the most appropriate strategies to obtain hydrogels with desired properties (Bernhard, & Tibbitt, 2021).

The development of hydrogels for biomaterials applications requires a selection of the cross-linking method. Physical crosslinking is required to develop materials with highly reversible dynamic properties, such as in self-healing hydrogels. Physically cross-linked hydrogels are of great interest for their potential applications in the areas of tissue engineering, drug delivery, cell encapsulation, adsorption, among others. However, since a matrix is formed by non-covalent crosslinking, these hydrogels may have poor mechanical properties. Therefore, more research is needed to further improve this approach (Alavarse, et al., 2022).

As mentioned above, the weakness in obtaining physically crosslinked hydrogels is the low degree of crosslinking, which can result in a matrix with poor mechanical properties and low stability (Singhal, et al., 2020). In this respect, research with natural polymers represents a more creative alternative for obtaining hydrogels with better properties to solve problems in the future. The selection of biopolymers must consider such aspects as behavior, solubility, density, crosslinking speed at different pH and temperature. In addition, depending on the final application, biodegradability, biocompatibility and side effects should also be considered (Ahsan, et al., 2021).

5. Final Considerations

The main approaches for the preparation of hydrogels by physical crosslinking have been revisited and reviewed throughout this work. Physical crosslinking approaches by freezing-thawing, crystallization, stereocomplex formation, ionic interaction, hydrogen bonds, and hydrophobic interactions are of great interest for designing new biomaterials. Biomedical applications of hydrogels were also discussed, mainly in drug delivery, cell culture, tissue engineering, wound coatings and dressings, and imaging diagnostics, among others. Despite the importance of the physical crosslinking method for obtaining hydrogels, this mechanism promotes only weak mechanical properties. In this sense, further research is needed to obtain hydrogels with chemical and mechanical characteristics suitable for use as biological material.

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