Chapter 17: Biomedical applications

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Abstract

Hydrogels are used in biomedical applications thanks to their high-water content, porosity, and their ability to easily modify their properties (mechanical, chemical, microstructure, etc.). Hydrogels are the materials that most resemble the extracellular matrix of mammals. In recent years, magnetic hydrogels have become especially important. These are the result of combining magnetic nanoparticles with different hydrogel matrices. Among its properties, they have the ability to be remotely controlled modifying their physical properties, such as stability, stiffness and temperature (magnetic hyperthermia). Such unique characteristics make magnetic hydrogels very promising in biomedical applications such as, tissue engineering, drug delivery, biosensors, and cancer therapy. At this respect, this chapter focuses on the main biomedical applications of magnetic hydrogels and the most important discoveries on the subject.

Keywords: magnetic particle; magnetic hydrogel; tissue engineering; drug delivery; injectable; biosensors; anisotropic hydrogel.

1. Introduction

Hydrogels are three-dimensional (3D) hydrophilic networks of flexible chains swollen by water or biological fluids. From the fundamental viewpoint, hydrogels are unique systems, which surprisingly combine a soft solid-like macroscopic appearance, with a highly porous microscopic structure. Because of this, provided the required biocompatibility, hydrogels recreate the extracellular matrix (ECM) of living tissues, more so than any other class of synthetic biomaterials. Thus, hydrogels find widespread applications in the biomedical field.[1,2]

In the search of hydrogels with improved controllability, actuation and response properties, the development of intelligent hydrogels that respond to external stimuli such as temperature, pH, electric field, specific analytes and enzymes has received considerable attention in the last two decades.[3] An important category of stimuli-responsive hydrogels are magnetic hydrogels or ferrogel[4] (i.e., the combination of hydrogels with magnetic micro- and/or nanoparticles) that are able to respond to an external magnetic field, modifying their properties (microstructure, mechanical behaviour). This quality makes these materials unique since their mechanical properties can be controlled remotely.

Ferrogels are of particular interest for many applications in biomedicine, such as drug delivery, artificial muscles and tissue engineering. In fact, ferrogels present some interesting characteristics as compared with nonmagnetic hydrogels:

i. It is possible to act at a distance on ferromagnetic materials, including ferrogels, by means of applied magnetic fields. Furthermore, the human tissues are permeable to the usual magnetic fields, which are in addition innocuous for human body functions and tissues, at least for the stationary or extremely low frequency fields reported for applications of these materials.[5] As a consequence, ferrogels can be manipulated and guided inside the human body by noncontact magnetic forces externally applied.
ii. The ferromagnetic character of hydrogels allows visualization and in-vivo follow-up by magnetic resonance imaging.[6]
iii. The mechanical properties of ferrogels can be modified in a controllable manner up to several orders of magnitude by means of magnetic fields.[7-9]

The properties of magnetic hydrogels rely on several factors, including the type of gelator and magnetic particle (MP) used, the gelator and MP concentration, and the size and distribution of the MPs within the hydrogels. Moreover, MPs are solid substrates that allow coating and functionalization for different purposes. The nature of the coating shell can modulate the interactions between the nanoparticles and the polymer filaments that form the gels, having a direct impact on the properties of the hydrogels.[10] Furthermore, MP with the appropriate surface chemistry can conjugate drugs, proteins, enzymes, antibodies, or nucleotides to be used for numerous applications.

Various methods have been developed to fabricate magnetic hydrogels: (Figure 17.1).

i. The simplest one is the immersion of a nonmagnetic hydrogel in a magnetic suspension, waiting for the absorption of the magnetic particles on the polymer network (Figure 17.1a).[11] The main drawback of this approach is that the magnetic particles can be desorbed rather easily, as the solvent is drained out or diluted.
ii. Another approach is the *in situ* synthesis of the magnetic particles inside the hydrogel (Figure 17.1b).[12] The main hindrance of this approach is to provide a biocompatible coating to the magnetic particles under the synthetic conditions, to render a ferrogel with the required biocompatibility for biomedical applications. In addition, it could be difficult to eliminate from the ferrogel the toxic chemicals originating during the synthesis. Therefore, this method does not seem appropriate for applications in which biocompatibility is required.
iii. A third approach consists of mixing the particles with hydrogel monomers prior to polymerization (Figure 1c).[13-15] This approach presents several advantages. For example, the particles can be properly functionalized before mixing with the hydrogel monomers, to provide them with biocompatibility, to charge them with a drug, cell or growth factors, and/or to serve as additional points of cross-

linking for the polymer chains. In particular, this could even give rise to hydrogels in which the cross-linking is provided only by the particles.[16,17] Note that by bounding the particles to the polymer network, the problems of particle losses during manipulation, drying or swelling of the hydrogels are prevented. Alternatively, the particles can be simply encapsulated within the polymer network if they are not properly functionalized to interact with the monomers.[18] Another advantage of mixing the particles with the monomers prior to polymerization is to get a microstructural ordering by alignment the particles and the polymer fibers by application of an external magnetic field.[13,19]

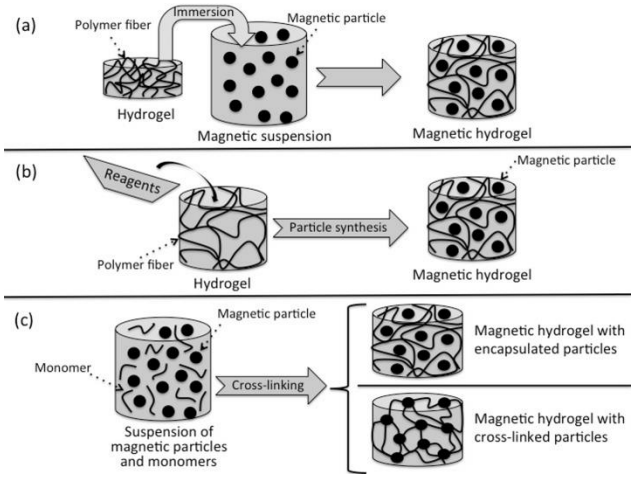

Figure 17.1. Different strategies for the preparation of magnetic hydrogels. (a) Blending method: a nonmagnetic hydrogel is immersed in a magnetic suspension, absorbing the magnetic particles during swelling.[18] (b) In situ precipitation method: magnetic particles are synthesized inside a nonmagnetic hydrogel.[20] (c) Grafting-onto method: cross-linking of polymers precursors is performed in the presence of magnetic particles, which may result just in the encapsulation of the particles or their cross-linking with the polymer fibers.[21]

As a consequence of the above-mentioned advantages of magnetic hydrogels over nonmagnetic ones in biomedical applications, much effort has been devoted in the last few years to the design, construction, characterization and application of these soft magnetic materials. (Figure 17.2)

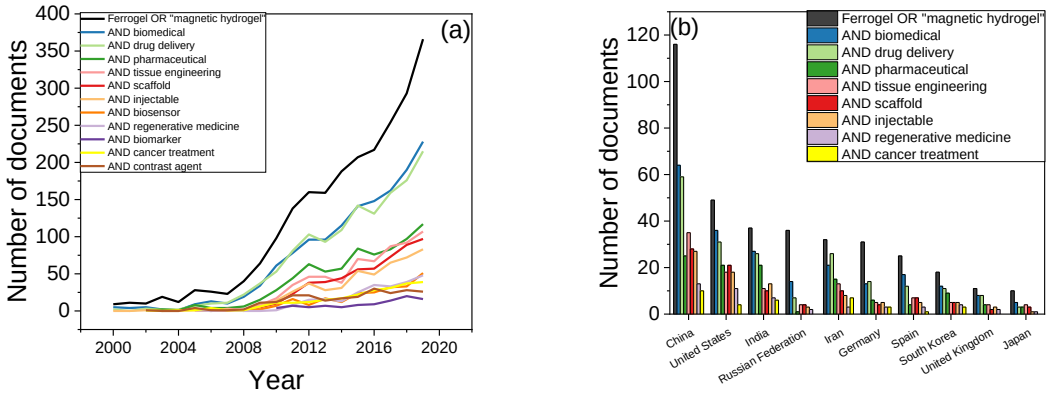

Figure 17.2. Number of documents related with magnetic hydrogels and their biomedical applications published **(a)** per year; **(b)** per country in 2019. Database: SCOPUS.

However, despite progress, understanding how to control cellular organization and vascularization precisely in complex tissue constructs is still in a preliminary state. Furthermore, all examples reported in the literature of magnetic hydrogels for biomedical applications are mainly based on polymers gelators where long-chain polymer molecules (such as collagen, alginate, chitosan, etc.) form the network required for gelation, through either covalent or non-covalent crosslinking. These molecules tend to form relatively robust networks (particularly those with covalent crosslinking), but as a consequence, they are usually unresponsive to stimuli. Therefore, novel magnetic hydrogels able to modify, in a major degree, or modulate reversibly their mechanical properties are needed.

2. Drug Delivery Applications

Dose amount and posology are key factors to know for the correct treatment and control of an illness. Equally important is controlling the rate of diffusion and the effective drug concentration at the site of action. Many drugs use delayed-release kinetics to control their administration within the therapeutic window, but controlling the effective dose over long periods is complicated. Other drugs are insoluble in water or have short half-lifes. The inability to accurately control these factors can lead to toxic effects due to an inadequate drug administration, limiting the therapeutic uses of some drugs.[22]

The increasingly frequent use of hydrogels as drug delivery vehicles is explained by their biocompatibility and the ability to modify their physical and chemical properties easily.[23] Hydrogels can be made from natural or synthetic polymers, such as, polysaccharides, proteins, peptides, surfactants, etc. Additionally, hydrogels can be made with different pore sizes and mesh sizes, and the connection between polymer chains can be made through covalent (chemical crosslinking) or noncovalent interactions (physical crosslinking). All these factors modify the way and the rate at which a drug can be released from inside them. Moreover, hydrogels can allow the

administration of several drugs simultaneously, create hydrophobic or hydrophilic environments (particularly attractive for biopharmaceuticals drugs), add nanoparticles, combine them with other materials and a wider variety of other options.[24,25]

In recent years, the use of magnetic nanoparticles embedded in a polymeric network has been proposed as a possible drug delivery vehicle. Magnetic nanoparticles have high interest due to their clinical applications. Thanks to their unique physical characteristics and their ability to act at the cellular level, they are being used in hyperthermia, in magnetic resonance imaging, acting as contrast agents, as a vehicle for the distribution of drugs and the detection and diagnosis of diseases, specially cancer.[26]

One of the advantages of these magnetic scaffolds is that they can control the distribution of drugs using an external magnetic field, and can simulate more realistically the physiological needs of bioactive agents. For example, Zhao *et al.*, have developed a magnetic alginate scaffold capable of modifying its dimensions and shape by the application of an external magnetic field., allowing the release of biological agents on demand.[27]

Combining the design and composition of the magnetic hydrogel with different magnetic stimuli allows the tuning of the drug release profile. An example of a magnetic hydrogel is the one reported by Chen *et al.*, combining polyvinyl alcohol with Fe_3O_4 magnetic nanoparticles (MNP). When this hydrogel is subjected to an intermittent on and off magnetic field, a decrease in the porosity of the hydrogel is observed when the field is activated, reducing the release of the drug.[28] In another example, Cezar *et al*., designed a biphasic alginate gel with superparamagnetic iron oxide nanoparticles inside, which by applying a static magnetic field, it was possible to release the drug more effectively in the site on the action.[29]

Remotely controlling the frequency and intensity of the applied magnetic field improves the delivery capacity of the drug, especially important in cancer treatment, reducing the side effects that it causes. This is the basis for the work of Kennedy *et al*., where the same biphasic alginate gels were used to insert the drug mitoxantrone and study the melanoma cells survival in two different ways: by continuous drug delivery or by intermittent delivery, controlling both processes by magnetic stimulation.[30]

2.1. Hyperthermia

One of the advantages of the inclusion of magnetic particles whithin the hydrogels is the potential application in hyperthermia treatments. Due to superparamagnetism shown by magnetic nanoparticles (MNP), a heat generation appears in response to alternating magnetic fields. This charactheristic of MNPs, combined with the controlled release of antitumor drugs from hydrogels, makes magnetic hydrogels promising materials for hyperthermia cancer therapy.[31]

An example of this approach can be found in a recent work by Wu *et al.*, where they designed an injectable magnetic hydrogel using Fe_3O_4 nanoparticles with a polyethylenglycol (PEG) and α-cyclodextrin layer. These particles first generated 42 ºC of heat to the tumor and then produced hydroxyl radicals which enhanced the tumor oxidative stress levels.[32]

Other example of the combined application of hyperthermia and drug delivery is provided in a recent work by Zhang *et al.*, where they described the fabrication of injectable magnetic hydrogels composed of poly(organophosphazene) polymers, positively charged tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and hydrophobic superparamagnetic iron oxide nanoparticles.[33] It was observed a significant *in vivo* tumor reduction when hyperthermia and TRAIL release was combined, without damage to other organs. Moreover, thanks to the presence of magnetic particles, the process was monitored by magnetic resonance imaging.

Similarly, magnetic hydrogels formed by doxorubicin, ferumoxytol and medical chitosan were produced by Chen *et al.* with promising applications in hyperthermia and tumor synergistic treatment.[34] This work shows the simultaneous transport and release of different drugs, as they combine different anticancer agents in a multidrug delivery complex formulation.

2.2. Drug Delivery Patents

Some patents relate to the use of magnetic hydrogels in drug delivery applications.

- Invention EP2533759 A2 refers to the use of magnetic polyvinyl alcohol (PVA) hydrogels as magnetically sensitive drug carriers for treatment or targeted delivery.[35] In this context, magnetically sensitive means that the compositions of the invention experience a change in motion or a change in velocity when exposed to a magnetic field. In this way, the therapeutic agents can target the specific organs and tissues intended for the release of the drug and exercise its function.

- Invention CN105561320 A describes the use of magnetic chitosan hydrogels for cancer treatment.[36] It is reported that the magnetic hydrogel permits a controlled release of anticancer drugs (e.g., doxorubicin) by application of external magnetic fields. This, in combination with the use of surface bound tumor necrosis factors, allows obtaining negligible side effects, according to *in vivo* experiments with mice that demonstrate inhibition of cancer cell growth.

- Invention CN102727445 A relates to magnetic alginate and chitosan hydrogel beads loaded in this case with matrine as a drug to be administered orally for the treatment of gastrointestinal cancers.[37] These beads present dual sensitivity to both pH and magnetic field. Firstly, by its sensitivity to the pH the rate of release of the matrine will depend mainly on the digestive tract site. Secondly, the magnetic character of the beads can be used to guide them and achieve targeted

drug delivery characteristic, reducing adverse drug reactions and side effects. As a consequence, and according to the invention, these beads effectively enhance the clinical outcomes and reduce the adverse side effects of the matrine, improving the patient medication compliance.

- A similar invention is reported in patent US2012179031 A1, where magnetic pills based on magnetic alginate hydrogel spheres containing a therapeutic agent are proposed for localized drug delivery within the gastrointestinal tract.[38] These magnetic pills can be guided by external fields to the desired localization where the water-soluble capsule is dissolved releasing the drug. Some oher patents also relate to the use of magnetic hydrogels for drug delivery applications.[39,40]

3. Tissue Engineering Applications

Tissue engineering is a multidisciplinar field of knowledge in which engineering and life sciences joint forces for the development of *de novo* tissues subtitutes to repair or reconstruct tissues and organs through the combination of suitable scaffolds, supporting cells and bioactive molecules.[41] While tissue engineering has currently succeeded in fabricating thin or avascular tissues, it still remains a challenge the development of complex functional organs.[42] Due to their adequate properties, such as biocompatibility, high water content, softness and flexibility, hydrogels are ideal materials in tissue engineering.[1] Among them, magnetic hydrogels are considered smart materials which can be remotely controlled via external magnetic fields, providing a tunable 3D scaffold for the growth, migration and differentiation of cells. Moreover, the presence of magnetic particles in the hydrogel, provides more advantages as long as they can be assembled in order, to form complex constructs, and can be functionalized with growth factors or other biological agents, to promote cell adhesion and proliferation.

3.1. Scaffolds

Designing the substrate (scaffold) onto which new tissue may grow has become a critical part of the development process of tissue engineering. Some of the most important features of scaffolds are a suitable porosity, for the migration of cells and the removal of waste, and an active contrability of cell growth. These critical factors can be potentially achieved using magnetic hydrogels.

For example, Bonhome-Espinosa *et al*., have shown that fibrin hydrogels greatly modify their internal structure even for a minimal amount of magnetic nanoparticles, as they become attaching nodes for fibrin fibers. The changes in the fibrin assembly give rise to a more porous and robust hydrogel in comparison with the non-magnetic one.[10]

Furthermore, external magnetic fields can be applied to magnetic hydrogels in order to control the assembly and the microstructure, creating complex tissue contructs[43] and to

organize engineered cell tissues.[44] Likewise, the application of cyclic magnetic fields results in mechanical stresses that mimic the biological behaviour of some tissues.[45]

In this sense, Lopez-Lopez *et al.*, have recently generated artificial magnetic tissues prepared by cell culture in magnetic hydrogels, with mechanical properties that can be reversibly tuned by noncontact magnetic forces.[13,19] Within the same field, the same authors have succeeded in the generation of biocompatible core-shell magnetic nanocomposites to use as magnetic phase in the preparation of magnetic hydrogels. They have demonstrated that the core–shell architecture is doubly advantageous, allowing a reduction of gravitational settling in water media and an enhancement of the magnetic response, which are key factors to get control on the microstructure of the resulting magnetic hydrogels. Furthermore, they have demonstrated excellent biocompatibility of the core-shell nanocomposites *ex vivo* and *in vivo.*[14] In addition, they have reached significant progress in the theoretical modelling of the mechanical properties of these complex systems.[46-48]

Further, recent studies indicate that when magnetic scaffolds are used in tissue engineering, the presence of MNP (i.e., approx. diameter 50-100 nm) stimulates adhesion, proliferation, and differentiation of cells *in vitro*, and even bone formation *in vivo*.[49-52] Recently, a method for promoting osteogenesis using MNP and eletromagnetic fields has been patented.[53] It has also been demonstrated the combinatory effects of a static magnetic field with the use of scaffolds containing MNP on bone regeneration.[54,55]

The functionalization of magnetic particles with biological agents has turned out to be an excellent vehicle for achieving better cell growth. The coating of magnetic particles with certain compounds such as PVA or PEG, enhances the hydrogel biocompatibility, biodegradability and mechanical properties.[14,56,57] Concerning the cell adhesion, the attachment of Arg-Gly-Asp (RGD) to the particles surface is a widely used way of improvement, as the RGD integrins of cells plays an important role in their adhesion into the scaffolds.[58] Similarly, the addition of well-known inorganic substances present in natural tissues leads to important enhancements. For example, hydroxyapatite in bones plays a key role in biomineralization, biocompatibility and osteo-conductivity.[59,60] For instance, it has been recently shown that magnetic nanoparticles conjugated with nerve growth factor significantly promote neurite outgrowth increasing the complexity of neuronal branching trees.[61]

Another reported advantage of magnetic scaffolds is that subjecting them to time-varying magnetic fields generate stresses at the microscopic level to the tissue forming cells, which is of special interest for the growth of mechano-responsive tissues.[45,62]

3.2. Anisotropic hydrogels

Most hydrogels are synthesized by the polimerization or self-assembly of molecular components homogeneously distributed in aqueous media. Therefore, the resulting

polymeric networks are conventionally isotropic. However, many of the biological systems comprise a well-defined hierarchical structure anisotropically oriented up to the macroscopic lenght scale. This is the case of muscle tissues,[63,64] skin[65,66] and articular cartilage.[67,68] Anisotropy plays an essential role in processes such as the transport of matter, surface lubrication and the transmission of forces or the adaptive response to external stresses. A representative example is muscle contraction resulting from the anisotropic disposition of actin and myosin in the muscle sarcomere.[63,64] Likewise, the existence of non-isotropic structures in the culture media performs a great influence on the proliferation, migration and differentiation of cells.[69-72]

Taking these aspects into account, anisotropic hydrogels represent an excellent way to explore the biomimetic applications of anisotropic constructs, since they can be media that accurately reproduce the ECM. Therefore, they can promote tissue generation *ex vivo* and *in vivo* in a more efficient way.[69]

However, despite their attractiveness for tissue engineering applications, they are not easy materials to obtain if there is a biocompatibility requirement. There are different procedures to obtain anisotropic hydrogels. Most of them involve the use of different directional stimuli such as: external forces (compression or mechanical shear, magnetic or electrical forces), gradients of temperature or ionic concentration.[73]

In order to synthesize hydrogels with oriented polymer chains, a unidirectional stimulus is required. The most common method to achieve this involves mechanical gel deformation,[74] although other methods that require nanoparticles have been described.[13,75]

Magnetic fields can also be applied to produce anisotropic hydrogels by aligning the MNP embedded in the hydrogel. Magnetic fields can be remotely (without contact with the sample) and non-destructively applied. Furthermore, they can homogeneously penetrate the entire volume of the sample. Therefore, magnetic orientation is easily applicable to the synthesis of large-scale anisotropic hydrogels with considerable thickness or large dimensions.

Recently, Contreras-Montoya *et al.*, have prepared anisotropic magnetic hydrogels based on the supramolecular self-association of small peptides in the presence of MNP.[76] Optical images revealed the formation of MNP aggregates arranged in columns parallel to the applied magnetic field. These hydrogels present an anisotropic stiffness that is modulated by the orientation of the MNP. The same group have also generated anisotropic magnetic hydrogels based on fibrin and alginate, with elastic moduli more than an order of magnitude greater than those of non-magnetic hydrogels.[77,78] Results have shown that the presence of functionalized MNP does not compromise the *ex vivo* and *in vivo* biocompatibility.[10,14,19]

However, given the technical difficulty in obtaining these types of materials, especially through procedures that provide biocompatibility and scalability, the number of studies to date is very limited.

3.3. Tissue Engineering Patents

Some patents relate to the use of ferrogels in tissue engineering applications.

- Invention US20040147015 A1[45] describes a method of growing artificial tissues for animal or human replacement, particularly but not exclusively mechano-responsive tissues such as bone, cartilage, ligament and tendons, by using magnetic scaffolds or hydrogels. In this invention, the magnetic particles of the hydrogel are embedded in the scaffolds in order to subject the growing tissue forming cells to mechanical stresses during their culture. For this aim the growing tissues are subjected to time-varying magnetic fields that provoke the movement of the magnetic particles and consequently generate stresses to the tissue forming cells.

- Invention US2012214217 A1[44] refers to a method for 3D manipulation of cells, and for the formation of an organized engineered cell tissue. For this aim, magnetically labeled cells were mixed with a cross-linkable hydrogel to form a cell-hydrogel mixture. Then, an external magnetic field was applied in order to arrange the magnetically labeled cells, and the hydrogel was eventually cross-linked to form the organized engineered cell tissue. According to inventors, this method allows the production of organized tissues *in situ* with specific cellular organization that mimic the native tissue, and it is indicated for many types of tissues, including bone, cartilage, tendons, ligaments and skin.

- Invention KR20160031683 A[79] relates to the preparation of scaffolds made of magnetic nanofibers produced by electrospinning of solutions of mixtures of polymer and magnetic nanoparticles. The field of application of scaffolds of this invention is bone regeneration by tissue engineering. Advantages of this magnetic nanofiber scaffold stand on the enhancement of mechanical properties as a consequence of the presence of the magnetic nanoparticles, at the same time that the biocompatibility is preserved.

- Invention CN104841020 A[80] discloses the preparation of supramolecular 3D ordered structures with embedded magnetic particles, to be used as 3D scaffolds for tissue engineering. The 3D ordered structure is controllable as the application of a magnetic field allows an accurate positioning and fixation of the magnetic particles. Furthermore, it is possible the introduction of specific growth factors at specific sites within the ordered structure.

- In relation to regenerative medicine, invention JP2007185107 A[81] reports the use of magnetic hydrogel thin films as repair material of lesions. Magnetic hydrogel thin films can be moved and fixed by magnetic forces.

4. Injectable Hydrogels

Injectable hydrogels are becoming increasingly important in biomedicine. The interest stands on the fact that injectability is one of the main requirements for minimally invasive procedures, particularly in tissue engineering and drug delivery applications. A principal advantage of such systems is ease of application using a syringe. Two main

alternatives coexist for the delivery of the product by a syringe: (i) in situ gelling hydrogels, (ii) shear-thinning (thixotropic) hydrogels.

The first approach relies on the gelation post-injection of a liquid-like mixture that includes the gel precursors. This can take place in response to a stimulus, such as temperature, pH, ionic strength, a specific molecular recognition event, or a change in solvent composition.[82-86]

In the case of shear-thinning hydrogels the strategy involves the formation of a solid hydrogel with the desired mechanical, morphological, and biological properties *in vitro* that can flow like a low viscous material under stress due to its shear-thinning property. After injection, the hydrogel should experience a fast recovery of the elastic modulus (self-healing). Protein- and peptide-based hydrogels, hydrogels from blends, colloidal systems, hydrogels based on cyclodextrins and block copolymers, and decellularized extracellular matrix-based hydrogels generally show shear-thinning behavior.[87] Those derived from amino acids or peptides are of special importance due to their inherent biocompatibility and biodegradability. Unfortunately, in spite of the clear advantages of shear-thinning hydrogels, they are typically less robust under physiological conditions than chemically cross-linked systems.

For example, Shi *et al.* recently created a magnetic hydrogel formed by bisphosphonate (BP)-modified hyaluronic acid (i.e., HA–BP) cross-linked by BP groups and the surface of iron oxide nanoparticles. The iron-BP link leads to self-healing injectable magnetic hydrogels which heats under cyclic magnetic fields, finding thus promising anticancer treatment applications.[88]

Nevertheless, despite the efforts devoted to the improvement of injectable hydrogels, the development of robust, injectable magnetic hydrogels is an on-going challenge. Furthermore, the generation of novel magnetic hydrogels with injectable behaviour for biomedical applications still remains an almost virgin field of research. The generation of this kind of hydrogels would allow the combination of all the characteristics mentioned above for magnetic hydrogels with those of injectable hydrogels.

4.1. Injectable Magnetic Hydrogels Patents

Some patents relate to the use of injectable magnetic hydrogels applications.

- Invention WO 2019/040224 Al[89] relates the preparation of injectable hydrogels to create 3D printed structures with high cell viability to their use in tissue engineering as tissue constructs with complex shapes (femur, ear, skull…). They fabricate hydrogels by creating microgels crosslinked with a first agent, which optionally contains cells dispersed. Then, these microgels are 3D printed and droplets are capable of being crosslinked with a second different agent into a bulk hydrogel, forming a complex 3D printed biological structure. They also explore a magnetic assembly approach as a robust and facile method to assemble magnetic beads into tube shapes.

- Invention WO 2017/087754 A2[90] presents an injectable magnetic hydrogel that, in contact with the tissue and under an electromechanical signal, applies cyclic mechanical compressions to the damaged tissue promoting its regeneration. This method reduces inflammation and fibrosis of the tissue, while increasing the level of oxygen available, the metabolic waste removal and the blood perfusion to a tissue (for example, muscle).

- Similarly, invention CN110591126 A[91] presents a preparation method of injectable magnetic hydrogels formed by a gelatin-ferric oxide particles solution in water crosslinked by glutamine transaminase. These magnetic hydrogels can be implanted through an injection needle and relieve muscle atrophy and muscle fibrosis by the application of regular magnetic fields to the injection position.

- In relation to drug delivery, invention US 2019/0298852 A1[92] reports a magnetic glycol chitosan–based hydrogel which exhibits self-healing behaviour under physiological conditions. The delivery rate of the carried substance can be regulated by the application of magnetic fields. Thus, these hydrogels can be used as delivery vehicles for a physiologically active substance such as a therapeutic agent.

- The invention described in US 9,675,561 B2, AU 2019201669 A1 and EP 3 417 876 A1[93-95] provides macroporous ferrogels based on dramatically deformable and compressed elastic alginate hydrogels which results in injectable sensitive scaffolds. The loaded ferrogels with biological agents lead to triggering release of drugs and cells in a controlled way under the application of magnetic fields. It is easily administered with subcutaneous injection and greatly increases the efficacy of vaccine therapy for many cancer types, such as melanoma and breast cancer. In other provided examples, the hydrogel comprises a cell adhesion composition chemically linked (covalently attached) to the polymer, such as an RGD amino acid sequence. Therefore, the invention features an injectable macroscopic scaffold comprising a high density of open interconnected pores, wherein the hydrogel is characterized by shape memory following deformation by compression or dehydration for minimally invasive administration.

- Another biomedical application is provided by invention CN109364018 A.[96] This injectable magnetic hydrogel is liquid at room temperature, it is crosslinked after injection at body temperature and can be stable at hyperthermia temperatures (42 ºC – 45 ºC). The obtained magnetic hydrogel can heat up under the application of an alternating magnetic field to reach the hyperthermia temperature.

5. Biosensors and Biomarkers

Another reported application of magnetic hydrogels is their use as biosensors and

biomarkers. Biosensors are an accurate, fast, highly sensitive diagnostic method and allow real-time monitoring, which specifically improves the detection and diagnosis of diseases, especially used in the detection of cancer biomarkers.[97]

The magnetic hydrogel as a diagnostic element constitutes a versatile and profitable platform and can be used for different applications in the field of diagnosis.[98]

The use of magnetic hydrogels to create biosensors that respond to external stimuli is increasingly used. This hydrogel must have great stability and hardness and be able to adequately detect the component of interest and translate this interaction into a quantifiable signal.[99]

In addition to the hydrogel microstructure, the MNP used plays a crucial role in a biosensor, since the changes in the response of the interaction will depend on them.[100]

In this sense, Wang *et al.*, combined the enzyme-like activity of Fe_3O_4 and the temperature-sensitive properties of poly(N-isopropylacrylamide) to create magnetic microgels in order to develop a nonenzymatic switchable bioelectrocatalysis sensor.[101] Moreover, Kurlyandskaya *et al.*, have developed a magnetoimpedance biosensor prototype to detect acrylamide, based on ferrogels including γ-Fe_2O_3 magnetic nanoparticles.[102] Similarly, the same research group has recently developed a series of magnetic hydrogels for their use in biosensor applications; such as polyacrylamide hydrogels with maghemite Fe_2O_3 as magnetic phase,[103] and polyacrylamide ferrogels with micron sized magnetic particles of magnetite and strontium hexaferrite.[104]

5.1. Biosensors and Biomarkers Patents

Some patents relate to the use of magnetic hydrogels as biosensors and biomarkers.

- Within this field of application, invention KR20100070095 A[105] relates the preparation of a hydrogel with encapsulated magnetic nanoparticles with a biomarker fixed in their surface. Application as a biosensor, drug delivery system and contrast agent are also mentioned in this invention. According to this invention, by encapsulating the biocompatible biomarkers fixed on the surface of magnetic nanoparticles, degeneration and leakage of the biomarkers are prevented, as well as it is possible to vary the rate of reaction of the biomarker.

- In the invention US2013245402 A1,[106] the magnetic particles within the pH-sensitive poly (methacrylic acid-co-acrylamide) hydrogel were arranged in such a way so that magnetic properties of the hydrogel were altered by changes of thickness or volume of the hydrogel. These changes resulted in response to a variation in a condition, such as moisture, pH or presence of glucose, so that these changes can be detected.

- In an invention of Kimberly-Clark Corporation (CA 2121514 A1) the use of magnetic hydrogels as disposable absorbent products of body liquids is presented.[107] The method uses a magnetic field to collect the magnetic hydrogels after absorption.

The absorbent compositions of the invention are suited to absorb many biological liquids, such as water, saline, urine, menses, and blood. Applications in products such as diapers, adult incontinent products, bed pads, napkins and tampons are mentioned.

- Magnetic hydrogels have also been proposed for biodegradable stents. For example, invention CN102371006A[108] refers to a magnetic biodegradable stent consisting of a dispersion of magnetic nanoparticles within a polymeric network. According to inventors, this magnetic stent can be expanded by the warning heat produced by the magnetic nanoparticles under an external alternating magnetic field. Then, once the stent has experienced thermoplastic deformation it can be fixed by a rack until it cools down. It is reported that this approach can effectively reduce retraction and collapse of the stent, which led to a restenosis problem.

- In invention RU2232002 C1[109] magnetic hydrogels formed by polyacrylates, vinyl polymers, polyorganosiloxanes, collagen copolymer or silicon rubber, are proposed as ophthalmic implants for the use in the treatment of various types of eye diseases. A distinctive characteristic of this invention is that dispersed magnetic powder is a permanent rare earth magnet material (samarium-cobalt or neodymium-iron-boron), and, as such, the proposed implants should also be permanent magnets.

6. Closing Remarks

As we have discussed in this chapter, magnetic hydrogels have great potential in biomedical applications such as drug delivery, tissue engineering, injectability, and as biosensors. Thanks to the ability of ferrogels to modify their internal microstructure by the application of an external magnetic stimulus, specific functions and properties can be obtained, such as achieving anisotropy in a tissue, pulsatile release of drugs, hyperthermia and even detecting a specific substance.

Concerning the applications of ferrogels, we have commented the most relevant bibliography, including articles and patents, classified into biomedical and pharmaceutical applications. This bibliography includes applications as scaffolds for tissue engineering purposes, ordered structures and thin films as repair materials in surgeries, medical stents, ophthalmological implants, artificial muscles, magnetic catheter, systems (particularly pills) for targeted drug delivery, biomarkers and biosensors and disposable absorbent products of body liquids. For most of these applications biocompatibility of the particles is a requirement. All these applications benefit from the magnetic character of ferrogels, which allows actuation and control at a distance by noncontact magnetic forces. In addition, the large surface-to-volume relation of nanoparticles makes possible effective functionalization of the particles for an enhanced application.

For the near future, advances concerning core properties and functionalization of the particles are expected. In particular, new particle shapes, such as fiber-like or plate-like, should confer enhanced properties to the ferrogels. Furthermore, combination of magnetic and nonmagnetic synthetic materials within the hydrogels would also make possible the preparation of new ferrogels with novel properties. In addition, the applications of ferrogels should expand in a parallel way to the expansion of the applications of hydrogels and magnetic nanoparticles. In fact, the inclusion of magnetic particles within hydrogels not only can enhance the properties of the hydrogels, but also of the particles, by combination of the characteristics of both materials.

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