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Biohydrogels for medical applications: A short review

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Abstract: Hydrogels have been widely used as drug delivery systems for scaffold production (in different soft and hard tissue engineering, including bones), thanks to their biocompatibility and biodegradability. In addition to the possibility of synthesizing a wide range of hydrogels from various natural and synthetic polymers, they are suitable for scaffold production. Moreover, they can have antimicrobial, antitumor and analgesic functions due to their intrinsic properties or through addition of proper biologically active agents. In this article, different types of hydrogels are reviewed alongside their application areas. Different methods are applied for the preparation of hydrogels and their compositions, and are strongly correlated with the morphological and mechanical properties of the synthesized hydrogels, which had a great influence on the performances of the respective scaffolds. The most effective ways to produce the desired scaffolds are mold casting, especially, 3D printing and electrospinning due to the ability to manipulate and control the shape and size of the desired scaffold as well as their microstructure.

Keywords: processing routes; chemical composition versus medical applications; hydrogels.

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1. Introduction

Hydrogels are polymers of soft or wet structures that are arranged in three-dimensional hydrophilic polymeric matrices, films or microspheres that can hold large amount of water or other biological fluids, without being dissolved due to their hydrophobic cross-linked structures[1,2,3]. This in turn causes the hydrogels to be considered for various drug delivery methods. As hydrogels exhibit thermodynamic compatibility with water, they swell in aqueous media. In addition to the ability of synthesizing them from a large variety of synthetic polymers, they provide a wide range of application possibilities and choice of substances that could be used for their production and being loaded with a large number of different substances and molecules. Although these materials were initially obtained from natural resources, they were later synthesized as synthetic polymers and had a higher water absorption capacity, longer service life and larger spectra of chemical resources available[3]. Hydrogels are used in tissue engineering and tailored drug delivery mechanisms[4,5]. In soft tissue engineering, particularly, collagen, chitosan and bacterial cellulose are used in obtaining hydrogels that are able to speed up the healing process. Moreover, if proper biologically active agents are used, additional properties can be assured such as antimicrobial, antitumor and analgesic activities. As proposed by Murugan and Ramakrishna[6], four generations of bone grafting materials are known, i.e. metals and alloys (1st generation), polymers and ceramics (2nd generation), composite materials (3rd generation) and tissue engineered grafts (nanocomposite materials loaded with cells, growth factors, etc.) (4th generation). Collagen, chitosan and alginate, along with calcium phosphates (and especially hydroxyapatite) are suitable for bone grafting. Their composition and morphology are tailored to meet the biomechanical requirements[7-13].

2. Hydrogel types

2.1. PLGA – based hydrogels

PLGA – based hydrogels are made of hydrophobic polymeric networks, which could be constructed with poly(lactic acid) – PLA or poly(lactide-co-glycolide) – PLGA. The advantage of using PLGA is that its hydrolysis causes the production of lactic and glycolic acids. These two acids are monomers that are endogenous and easily metabolised, thereby causing a minimal systemic toxicity. Yet, they have limited water absorption capabilities.

2.2. Photopolymerized hydrogels

Photopolymerization is a method, requiring different spectra of light to start the process of linear polymer cross linked structure polymerization. This method is proposed to be used specifically for the production of biomaterial-based polymer networks for specific biomedical applications and, particularly, in tissue engineering due to their ability to entrap a wide range of cells and substances.

2.3. Nanomedicine – based hydrogels

Lipid nanocapsules (LNC) are nanocarriers composed of an oily core of triglycerides surrounded by a surfactant shell[2]. They have been under extensive studies to be used in drug delivery applications. That is due to their long-term stability, biocompatibility, ability to encapsulate a large array of different drugs and easy and low cost preparation procedure. They can be administered in a variety of methods such as subcutaneous, intravenous and local delivery. A new method has been developed constituting LNG and $GemC_{12}$. Even though this method is concerned with a hydrogel preparation, it has no polymeric constituents. The hydrogel structure is formed due to the location of the $GemC_{12}$. As this method has no other synthetic polymer present other than the $GemC_{12}$ at the LNG oil water interface, these hydrogels in turn reduce the risk of side effects.

2.4. Theranostic gelation hydrogels

Theranostic hydrogels are a class of polymeric hydrogels that are tailored to clinical and biomedical applications. This can help in a few aspects, such as assessing the biodistribution noninvasively, targeting the drug accumulation, a higher level of control on the drug release and increase the effectiveness and the therapeutical effects of the drugs via targeted drug release as well as the ability to predict the therapeutical response. An example for theranostic hydrogel is the pH/temperature sensitive magnetic nanohydrogel that contains an agent for MR and fluorescence imaging.

2.5. Thermo-reversible gelation polymers

Thermo-reversible Gelation Polymers (TGP) is another novel drug delivery system. They are able to make reversible hydrogen bonding with water at around body temperature. Although TPGs are in hydrogel form at body temperature, they become soluble at ambient temperature. They are mainly composed of polyethylene glycol (PEG) combined with a thermo responsive polymer and poly-N-isopropylacryl amide. They are biocompatible, non-cytotoxic and completely pathogen free[14].

2.6. Poly(acrylamide)and poly(acrylicacid)

Hydrogels prepared from polyacrylamide (PAAm) and polyacrylic acid (PAAc) have physically crosslinked networks generated via their hydrophobic association. They show no self-healing behaviour but exhibit a high adhesive capability when in contact with one another. That property is attributed to the hydrogen bonding between the amide and carboxyl groups of the PAAm and PAAc, respectively[5].

Table 1. Hydrogels and their applications**.**

Hydrogel	Medical Application	Reference
PLGA	Drug delivery, Tissue engineering, cancer treatment and imaging.	[31-33,34-37]
Photo-polymerizable	Tissue regeneration for bone, cartilage, and soft tissue, injectable controlled release devices for drug delivery.	[38-41]
LNC	Drug delivery, Chemotherapeutical agent delivery (glioblastoma),	[42-45]
Theranostic	Drug delivery (cancer treatment), diagnostics,	[46,47]
TGP	Food additives, pharmaceutical ingredients, agricultural products	[37]
PAAm PAAc	Drug delivery, prodrugs, cancer therapy.	[48]
Collagen	Tissue engineering, Drug delivery, cosmetic, pharmaceutic and food industry, etc.	[16-21]
Chitosan	Tissue engineering, drug delivery systems; wound dressing, antimicrobial agent	[30]
Alginate	Tissue engineering, drug delivery systems;	

2.7. Collagen

Collagen is a class of natural polymer/protein structure which is extensively used in tissue engineering of soft and hard tissues[15-19], being one of the most important components of these tissues. Collagen and its derivative, gelatine, is also intensively used in pharmaceutical, cosmetic and food industries[20]. The chemical structure allows it to be used as a platform for loading and delivering biological active agents. It can be applied with or without other organic and inorganic components along with an important number of biologically active agents capable of assuring specific functionalities (analgesic, anti-inflammatory, anti-tumoral and anti-infective properties[11,21-26]). All these properties are

coupled with its native properties such as good haemo- and tissue-compatibilities and ability to absorb high amount of water[27-29].

2.7. Chitin

Chitin and its deacetylated form chitosan are extensively used in biomedical applications because of their remarkable biocompatibility, high water uptake assuring healing conditions for the injuries and native antimicrobial activity due to their polycationic structure[30,31]. All types of hydrogels are summarized in Table 1 together with references corresponding to their application fields.

3. Methods of hydrogel preparation

Several methods are used in order to prepare hydrogels, the most important one of which is given below.

3.1. Conventional polymerization and crosslinking

Polymerization and covalently crosslinking are conventional methods for hydrogel preparation, which can be applied for the synthesis of large range of materials. These methods can be used to create specific hydrogels by combining different monomers or polymers to obtain hydrogels, having specific physio-, mechanic- and chemical properties[6,49,50]. Bio-hydrogels or their composite forms can be obtained using proper multifunctional cross-linking agents (Figure 1). The cross-linking reactions can be initiated using thermal, redox or photo (UV-VIS) initiators. Ionizing radiation techniques such as gamma and electron-beam are also used because of their remarkable practical advantages. The presence of solvent in the polymerization process provides a considerable advantage in terms of heat transfer since the polymerization reactions are highly exothermic. There are mainly four types of radical polymerization used in both industry and laboratory; bulk, solution, suspension and emulsion polymerizations, each having own practical advantages and disadvantages[7]. In the preparation of hydrogels aiming at medical applications, rather than synthetic monomers, the use of naturally formed polymers, such as collagens and alginates or variety of fermentation products such as hyaluronic acid and poly(hydroxy alkanoates), is especially desired to avoid possible local inflammations. Collagen based hydrogels are widely used for medical applications. It is extracted form bovine calf and processed to obtain hydrogels with favorable characteristics for tissue regeneration and drug delivery. Tannins and glutaraldehyde are especially used as crosslinking agents. Their concentrations assess some characteristics properties such as elasticity, swelling, water absorption, chemical stability and biocompatibility.

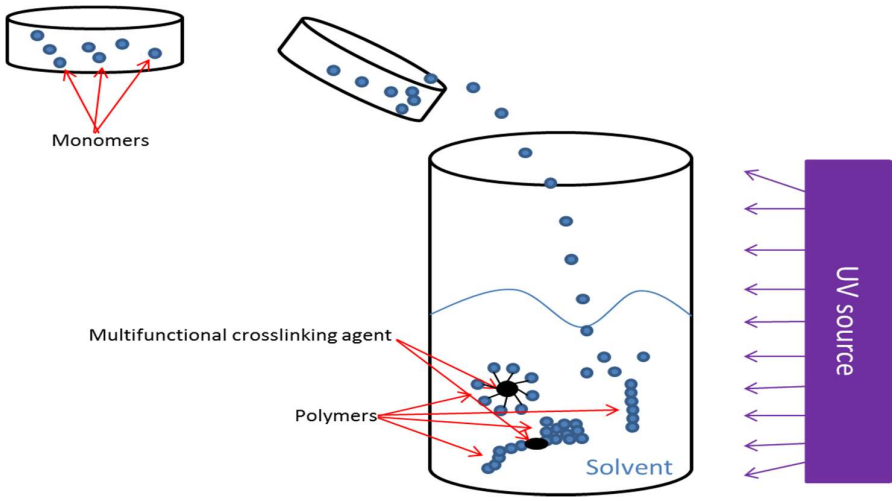

Figure 1. Conventional polymerization method.

3.2. Electro-hydrodynamic atomization

Electro-hydrodynamic atomization *(EHDA),* also known as electro-spraying technique, is a well-established technique and used for the generation of very fine uniform droplets under the influence of an electrical force. It is applied for the production and process of micro and nanoparticulate materials. As can be seen in (Figure 2a), this device consists mainly of five parts: a syringe pump, a syringe holding the material to be used, a needle/injector used as a nozzle, a power source to provide an electrical force and a grounded collector bed. The mechanism of EHDA device is the bulk fluid disburses into fine droplets, under the influence of an electrical field that appears to be coming out in a mist form (Figure 2b). Here, an important point is the fluid surface tension, which, if it exceeds the certain amount of electrical stress, fluid is atomized upon leaving the nozzle. Additionally, the physiochemical properties of the solvent will determine the size and surface roughness of the resultant particles, i.e. the vapor pressure and therefore the boiling point[34].

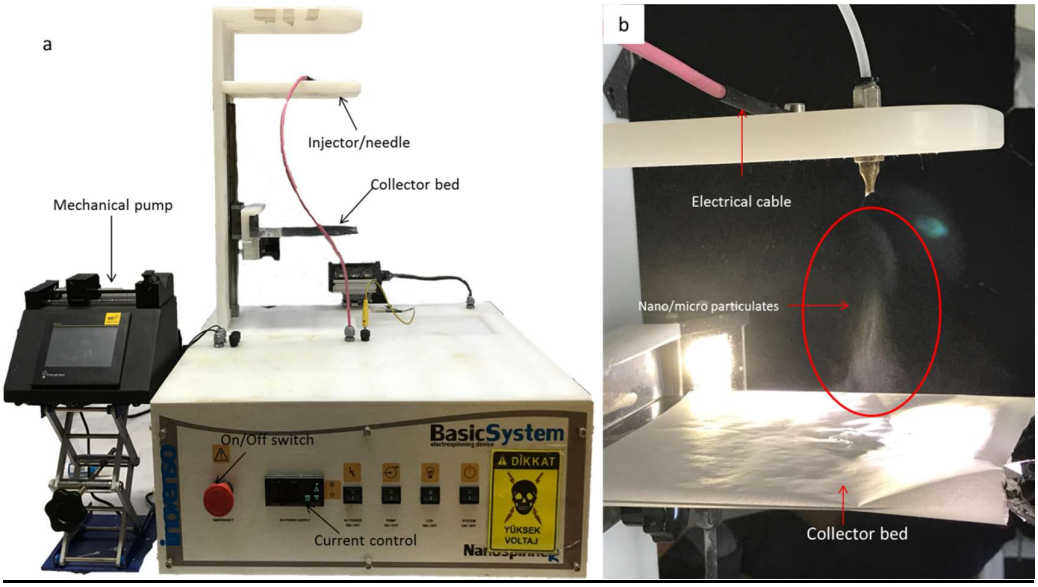

Figure 1. (a) EHDA device (b) particles produced during EHDA process.

Electrohydrodynamic method is a powerful technique to provide nano- and microparticles to allow an easy size control, high recovery and mild processing conditions by controlling some parameters such as conductivity of spraying liquids, flow rate, potential and distance between the needle and collector, etc.[50-51]. Starting from PCL, PEG and chitosan, by electrospinning at 12kV, efficient nano-encapsulation of albumin was obtained. The characteristics of the obtained micro and nanoparticles, according to the processing parameters, are presented in Table 2.

Table 2. Characteristics of the nano- and microparticles obtained by EHDA

Polymer	Dispersant wt%	Flow rate ml/h	Voltage kV	Distance cm	Diameter µm
Chitosan	TPP, 0.5wt% aq	0.2 - 1.0	10	3	1.3 – 13.4
	TPP 0.02wt% aq	0.2	8-14	0.5-7	2.5 – 19.4
	HPC 1wt% methanol	0.1 - 2	4-16	3-9	3.1 – 18.4
PCL[1]	PBS (pH=7.4)	0.1-0.4	10	3	22.5 - 11.4
PEG/PCL[2] in / out	SDS 1mM aq	in: 0.5-3 out: 0.5 - 2	10	3	0.12-0.70

PCL[1]: Mw = 42500 Da; 2 % w/v;
[2] co-axial methodology; PEG: Mw = 5000 Da; 3 wt%; PCL[**]: Mw = 10 000Da; 1wt%

Chitosan based micro and nanoparticles are obtained over 10 years by using electrohydrodynamic strategy, especially for drug delivery[50-52]. Based on the results obtained by Pancholi et al.[53], uniform spherical chitosan micro-particles of less than 10 µm can easily be obtained by electrospraying, highlighting excellent biocompatibility and biodegradability. Based on their results, the size can be tuned based on the viscosity and surface tension. The increasing viscosity led to the increasing particles size while decreasing the surface tension. Chitosan solution, having over 100 mPa viscosity, had the particles larger than 10 µm while at 80 mPa, the obtained particles reached 2.5 µm which, after drying, shrank to about 500 nm.

Recently, Pina et al.[54] used multi-needle electrohydrodynamic strategy to produce nanoparticles loaded with antitumoral agent (cisplatin). A three layered approach was used in order to obtain antitumoral nano/micro-spheres based on PLGA. The structure of these spheres includes an outer PLGA layer with mostly protective role, an intermediate layer of siRNA (loaded in chitosan) and an inner layer of cisplatin (loaded in PLGA). The presence of the siRNA and cisplatin can assure a combination therapy with improved anti-tumoral activity due to the synergy of the components.

3.3. Electrospinning

Electrospinning is a method similar to EHDA with the only difference in the microstructure of the resultant scaffold. The design of the electrospinner is similar to that of the EHDA device (Figure 5). When EHDA is used, the product comes out as spherical balls or clusters and with electrospinning they come out as fibers, followed by their interconnection to form a 2D network (the thickness is usually much lower comparing with the other dimensions)[55-56]. The electrospinned fibers have several desirable characteristics such as a large specific surface area, high flexibility that can be utilized in surface functionality, superior mechanical properties, adjustable porosity, tunable water uptake and air permeability as well as the ability to produce fibers of different shapes and sizes. On the other hand, electrospinning has a few disadvantages such as the hardship of production on a large scale that incorporates safety and toxicity of the solvent used and the high voltage used in the electrospinning process[55].

Chitosan is intensively used to obtain scaffolds based on micro and nano-fibrous structures. 3D printing as well as electrospinning, due the fluidity of the acidic chitosan solution, is hard to be used without a post-curing process (crosslinking). This process is devoted to reduce the fluidity and to stabilize the resulting strands (3D printing) / fibers (electrospinning). Genipin, along with the other agents, can be used for crosslinking the chitosan based fibers and the obtained scaffolds can be used even in tissue engineering because of the low toxicity and high ability to assure the adhesion and maturation of the osteoblast cells[57]. Asran et al.[58], starting form polyvinyl alcohol, collagen and hydroxyapatite, obtained nanofibrous biocomposite scaffolds mimicking some of the key features of natural bone at nanoscale level. Experimental models of 7 cm x 11 cm nanofibrous scaffolds were

obtained with controlled/adjustable fiber diameter, pore size and porosity (Table 3). In the case of PVA/COL/nHA, the average pore size is 650 µm, the porosity reaches 49.5% while the mechanical properties are suitable for the tissue engineering of spongious bones.

Table 3. Morphological and mechanical characteristics of the nanofibrous scaffolds.

Sample	Diameter of fibers [nm]	Mechanical properties / Elastic modulus [MPa] / [MPa]
PVA	160	0.22 ± 0.09 / 2.67 ± 0.78
PVA/nHA	176	NA
PVA/COL	245	0.62 ± 0.13 / 4.97 ± 2.41
PVA/COL/nHA	320	5% nHA: 1.03 ± 0.17 / 11.10 ± 3.38 10%nHA: 0.17 ± 0.05 / 0.10 ± 0.94

Electrospinning was also used for manufacturing pH-responsive composite hydrogels based on poly[styrene-*co*-(maleic sodium anhydride)] (SMA) and cellulose with nanofibrous structure[58]. Three compositions were obtained starting from SMA and cellulose acetate by varying their ratio from 10:90 to 20:80 and 40:60. In all cases, their solubilisation was conducted in N,N-dimethylacetamide/acetone 1:2 (v/v) and electrospinned at a feeding rate of 0.6 ml/h at 16 kV, while the tip-to-collector distance was 16 cm followed by drying at 80 °C and crosslinking thermally at 145 °C or chemically using diethylene glycol (3% relative to SMA). The chemically crosslinked SMA/cellulose exhibits a pH-dependent swelling/de-swelling behaviours. At acidic pH (2.5-5.5), the swelling is of ~1700 – 1900% and slightly increases to 2000% between 5.5 and 8.4. An important increase appears reaching up to 2700-2800% above pH 9.

Electrospinning can also be used in a two-syringe configuration as presented by Ji et al.[59]. They used a thiolated derivative of hyaluronic acid and poly(ethylene glycol) diacrylate (PEGDA) as crosslinking agents. The hyaluronic acid derivative was dissolved in a 2.5% (w/v) PEO solution (in Dulbecco's modified eagle's medium – DMEM). PEO was used to act as a viscosity controlling agent which was later removed by washing with water and filled in one syringe while the second syringe contains 9% (w/v) PEGDA solution in PBS (pH=7.4). The electrospinning process was performed using a T-shape three-way steel adapter. The spinning process was assured by a potential of 18 kV, and the needle was placed horizontally at 10 cm distance from the collector. The feeding rate was set at 1.2 for HA/PEO blend and 0.3 mL/h for the PEGDA crosslinking agent. The mean diameter of the fibers is 110 ± 28 nm (after removal of PEO, the fibers were not degraded even after prolonged washing). This scaffold, based on the biological assays and the swelling behaviour, seems to be adequate for tissue engineering of the soft-tissue. Most probably, these materials can also be loaded with biologically active agents able to induce faster healing. Moreover, they show analgesic and antibacterial properties. Drug delivery wound dressings obtained by electrospinning are increasingly exploited in medical applications[60-62]. Antibiotic (gentamicin sulfate and hydrophobic ciprofloxacin) loaded gelatine-based wound dressings were used for dermal regeneration of infected deep burns[60]. The use of the two antibiotics is justified based on their different release performance; gentamicin being released within 6 days while ciprofloxacin in over 3 weeks, after which the re-epithelization was practically complete.

Recently, Park et al.[63] manufactured poly(caprolactone)/calcium carbonate electrospinned network coated with chitosan to regulate the haemostatic properties and, based on the *in vivo* assays, it was found that these materials can be used when rapid blood coagulation is required.

3.4. 3D printing

3D printing is a method for synthesizing three-dimensional objects of various shapes and sizes from a digitalised model. 3D printing has the potential to fix or replace different tissues or organs. In contrast to other methods of scaffold fabrication that create a random scaffold with no apparent pattern, 3D printing allows a full control over the design of the scaffold in regard to shape, size and porosity. 3D

printers consist mainly of a material pump, thermal unit (heating or cooling ability), an injector/needle, movable head and a collector/print base (Figure 3). 3D printing implements an additive manufacturing (AM) that applies one consecutive layer over the other to reach the desired shape[64]. We can see the schematic representation of 3D liquid bio-printing in Figure 4. There are a lot of different additive processes including:

a. Selective Laser Sintering (SLS)
b. Stereo-Lithography (SLA)
c. Fuse Deposition Modeling (FDM)
d. Direct Metal Laser Sintering (DMLS)

Each of the process above differs in the materials they use as well as the type of the printing method. Different tissues and organs could be printed with the help of 3D printers such as: cartilages[65], bones[66], vessels[67,68], nerves[69], bladders[70] and many more. Additionally, for medical purposes, the created scaffolds were made of both natural and synthetic polymers. Natural polymers, such as collagen[71], chondroitin sulphate[72], chitin[73] and chitosan[74], are mainly used for tissue engineering and organ regeneration to the facilitate cell attachment and maintenance of differentiation. On the other hand, synthetic polymers are used in a large variety of applications as they are able to provide versatility regarding the control of their physiochemical properties and make different scaffolds[75-77]. Some examples of different synthetic polymers include poly(ε-caprolactone) (PLC), poly(lactic acid)(PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol)(PEG)[64]. Nowadays, a wide variety of hydrogels are studied for tissue engineering, including graphene oxides based hydrogels such as gelatine – poly(ethylene glycol)diacrylate – graphene oxide[78] for cartilage reconstruction and poly(N-acryloyl glycinamide) – nanoclay. The latter, due to the Mg^{2+} and Si^{4+} release, exhibits very good osteogenic differentiation of primary rat osteoblasts, making them recommended for bone grafting. Drug loaded hydrogels are also of great interest in the field of cancer treatment, especially to avoid recurrences[79,80].

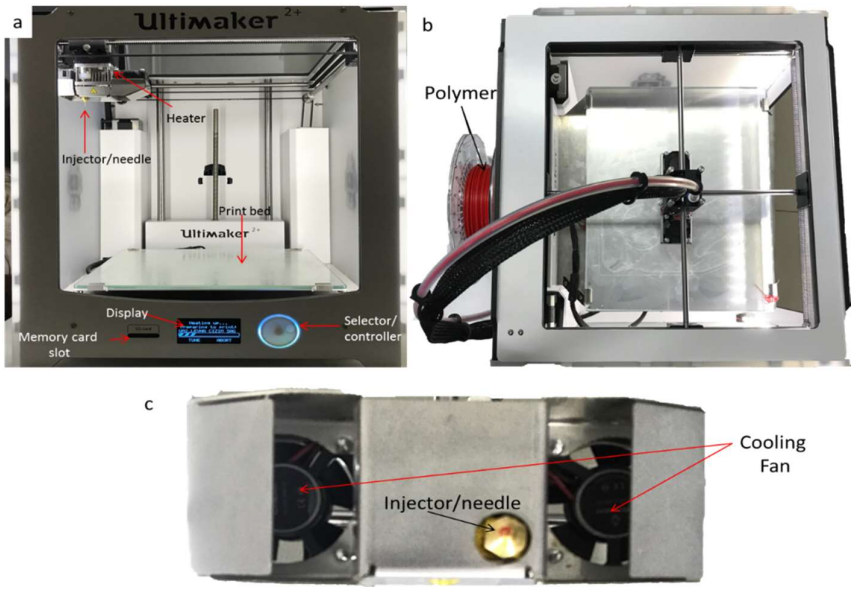

Figure 3. 3D printer (a) front view (b) top view (c) the moving injector.

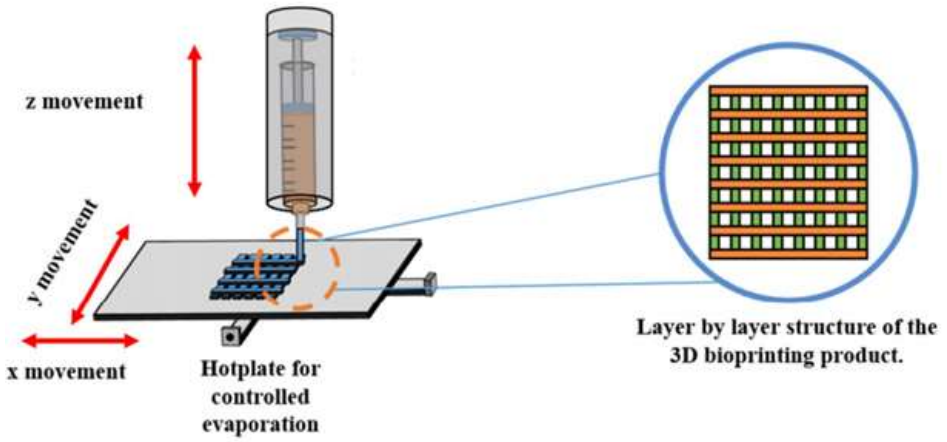

Figure 4. Schematic diagram of the 3D liquid bio printing.

Development of bone grafts is of great importance as the need for bone grafts is high. In this context, many research groups have been trying to develop bone grafting materials to tailor their morphology to obtain the desired properties. Alginate – polyvinyl alcohol – hydroxyapatite can be used to develop bone grafts[81] seeded with cells. For this reason, the rheological properties are very important since high pressure can destroy the mouse calvaria 3T3-E1 cells. Six different formulations were tested containing different amount of alginate (Alg), phosphate, calcium, hydroxyapatite (HA) and sodium chloride (Table 4). Based on the video images, the 3D printed samples, obtained from the formulations containing 2.5% alginate, seems to lead to a more stable 3D structure with a good printing. The 5th formulation led to the highest compressive modulus during the degradation study while the diameter change is minimal.

Table 4. Composition of the hydrogel.

Number	Content of %				
	Alg	NaH_2PO_4	$CaSO_4$	HA	NaCl
1	1.5	0.12	0.40	0	-
2	2.0	0.12	0.40	0	-
3	2.0	0.12	0.40	2.5	-
4	2.0	0.12	0.20	2.5	-
5	2.5	0.15	0.20	2.5	-
6	2.5	0.15	0.20	2.5	0.72
7	2.5	0.15	0.20	0	-

Nanocellulose-alginate hydrogels were manufactured by 3D printing in order to provide biomedical devices, wearable sensors and drug delivery systems[82]. The ternary systems, based on nanocellulose-alginate and glycerin, provide good printability, as the obtained materials presenting mm-large pores, which are extremely important as they can assure low irritation and pain induced by the compressive forces developed during healing. Moreover, the avidin-functionalized nanocellulose-alginate hydrogels can provide high immobilization ability via biotin-avidin interactions. Similarly, growth factors and antimicrobial agents (such as antibiotics) can be attached to develop drug delivery systems.

3D printed hydrogels, based on thiol-ene crosslinked polyglycidols and hyaluronic acid, containing amino and carboxy functionalized mesoporous silica nanoparticles, were obtained by 3D printing[83]. The printability of the hydrogels, loaded with two types of mesoporous silica nanoparticles

(amino and carboxy functionalized MSNs), was practically same but, the migration of the positively ($MSN-NH_2$) or negatively charged MSN (MSN-COOH) was very different when immersed in PBS. If the two strands, one containing $MSN-NH_2$ and the other containing MSN-COOH, are in contact, even after 1 day, the amino-functionalized MSN migrates but the carboxy-functionalized MSN does not migrate, regardless 1 day or 6 weeks of immersion in PBS. If the two strands are not in contact, after one day, no significant migration can be observed. On the other hand, after 6 weeks, the migration of the amino-functionalized MSN is important. The migration of the MSNs was monitored by using small amino and carboxy-functionalized MSNs, which were mixed with the corresponding hydrogels containing amino and carboxy-functionalized MSNs. Based on this study, the possibility of using printed scaffolds, loaded with positively and negatively charged drugs, can be used as sequential drug delivery systems.

Alginate/gelatine based hydrogels were also used in printing heterogeneous aortic valves with anatomical architecture by direct incorporation of two types of cells, meaning smooth muscle cells (SMC) for the root region and aortic valve leaflet interstitial cells (VIC) for the leaflet region[84]. A cellular 3D printed hydrogels exhibit lower mechanical properties comparing with the graft loaded with cells. The direct printing of the hydrogels encapsulating cells can be exploited as the viability is high enough, 81.4±3.4% for SMC and 83.2 ± 4.0% for VIC. On the other hand, if these artificial valves are maintained in adequate culture media, they can survive for at least 7 days.

3.5. Microfluidics

Microfluidics is a system that allows precise control and manipulation of very small volumes ranging from nL to pL in a reproducible fashion. Microfluidics platforms are generally made of a pump or an actuator, a valve, a drug reservoir and a membrane to control the drug release rate (Figure 5). These systems are used in a large variety of applications ranging from developing biomaterials that allow a controlled release of drugs to discover proteins or antibodies that ensure the specificity of the action[85]. These systems can be used in active and local drug delivery with planned release of drug in certain amount of time. Also it can be used for the administration of drugs that might carry the risk of cytotoxicity upon systematic administration. Instead of other drug administration methods that might incorporate a painful and hazardous needle injections this method is much more easy[86]. The particles in microfluidic systems are generally created via self-assembly, where two or more streams of different reagents are interfaced and the carriers are formed at the interface layer. The self-assembly is generally achieved via hydrodynamic flow focusing (HFF) or active or passive mixing. The size of the carriers is controlled by mixing, rated between the different streams[87], thereby creating quite advanced and complex drug carriers. In addition to that, microfluidic systems could be used for direct delivery of active molecules[88]. Using such a method increases the specificity of the drug delivery and ensures the drug reaching the target site, thereby decreasing the side-effects of the drug, caused by the interaction of the drug with other organs and tissues. Microfluidics has the ability to ensure a controlled convective drug delivery method, unlike the other methods that provide drug delivery via diffusive forces both in continuous and pulsatile manner. The platform reservoir could be refillable or disposable. The main aim of the system is controlled release of the drug from the platform, which could be a great challenge in microfluidic platforms. This is achieved via the pump being controlled and moved mechanically or by pressurising it to force the drug out[88] (Figure 6).

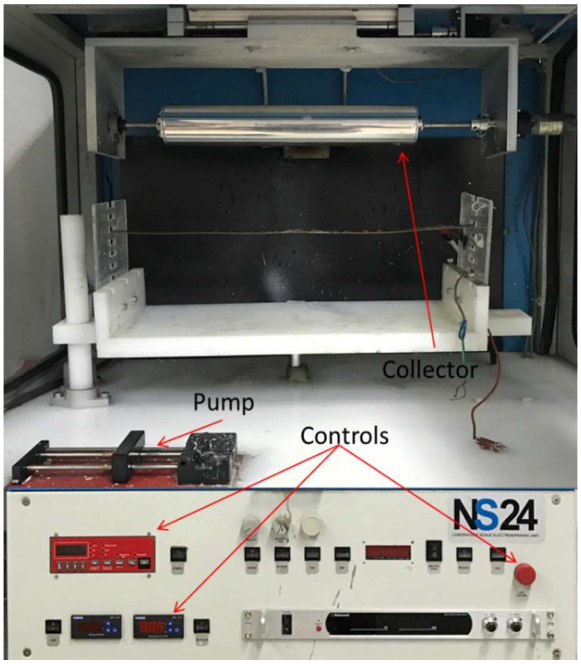

Figure 5. Electrospinning device.

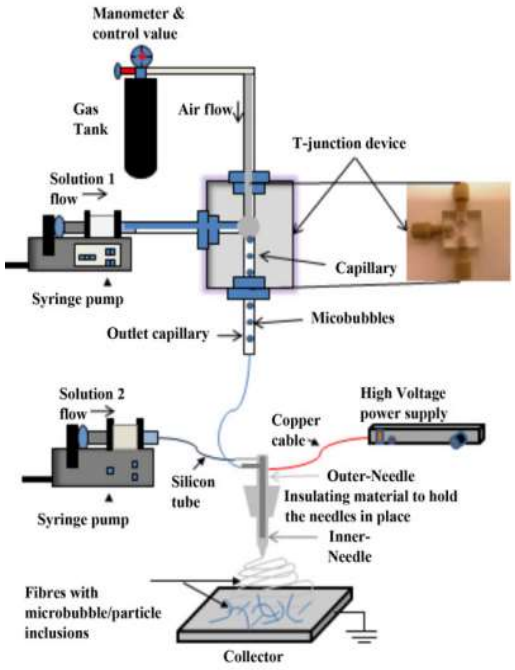

Figure 6. System design of microfluidics.

Microfluidic systems can be used in obtaining monodisperse hydrogel microsphere incorporating cells or biologically active agents. Alginate is extensively used in microfluidic approaches because of its fast and easy ionotropic gelation. The spherical alginate microspheres have found applicability in drug delivery, bio-sensing, microelectronics, bioanalysis cell delivery, etc.[90]. The size of the microcapsules can be tailored to accommodate fine nanoparticles, proteins as well as entire cells

or several cells, maintaining the cellular viability[90-92]. Moreover, these microcapsules are stable enough, both *in vitro* and *in vivo*[93]. Thus, they are suitable for a wide range of applications.

The presented methods were used in developing new or improved hydrogels for medical and nonmedical applications. Some of the most important methods applied for the processing of the biomedical-grade polymers and composites are presented in Table 5.

Table 5. Applicable materials for each device.

Method	Applicable Hydrogels	References
EHDA	chitosan, polycaprolactone (PCL), and poly(ethylene glycol) (PEG) were processed by EHDA in order to obtain biodegradable polymeric particles	[94-96] [25-29]
3D printing	methacrylated chondroitin sulfate (CSMA), thermo-sensitive poly (N-(2hydroxypropyl) methacrylamide-mono/dilactate)-polyethylene glycol triblock copolymer (M15P10), conductive graphene doped polylactic acid (G-PLA), pure PLA, stainless steel, ceramic beads, inconel alloy, iron, ABS, ASA, nylon 12, PC, PPSF/PPSU, PEI or ULTEM, PLA, TPU, titanium, aluminium, cobalt-chrome, Nickel based alloys, DC (100, 500) DL (350, 360), AB 001, GM 08, DM (210, 220), Rigid Polyurethane (RPU), Flexible Polyurethane (FPU), Elastomeric Polyurethane (EPU), CE (Cyanate Ester)	[97-99]
Microfluidics	N-alkylacrylamides, vinyl ether, alkylene oxide, -OH, -NH2, -CONH, -$CONH_2$, -COOH, -HSO_3, conductive polymer, poly(thiophene)s, poly(p-phenylene sulfide), poly(pyrrole)s, polyanilines, poly(acetylene)s; bismethacrylate-co-methacrylic acid sodium salt, charged colour pigments, azobenzene, spiropyran, triphenylmethane, cholesteric liquid crystal, carboxylic, poly(methacrylic acid), poly(methacrylic acid-g-ethylene glycol); amino groups, poly(N,N-dimethyl aminoethyl methacrylate); sulfonic acid, poly(n-(morpholino) ethylmethacrylate)-b-poly (4-(2-sulfoethyl)-1-(4-vinylbenzyl)pyridinium betaine), poly (ferrocenylsilane), poly (lactic/ glycolic) acid, poly (methyl methacrylate), polystyrene. Iron (III) oxide nanoparticles, cobalt ferrite ($CoFe_2O_4$), nickel ferrite, Poly(N-isopropylacrylamide) based polymer nanogels, silver nanoparticles, titanium dioxide nanoparticles, carbon black (paracrystalline carbon), conductive nanoparticles, gold nanoparticles; Iron (II, III) oxide nanoparticles	[100-129]
Electrospinning	Starch, polymers (organic/inorganic), metals, metal oxides, ceramics, sulfates.	[130,131]
Polymerization, polycondensation and crosslinking	Acrylamide, acrylic acid, etc. ethylene glycol, collagen, alginate, chitosan, …	[38]

4. Conclusions

Hydrogels are a versatile class of materials with various medical and nonmedical applications. Due to the presence of large volume of water, they are extremely well tolerated by human organism and consequently many applications of hydrogels are known, from soft to hard-tissue engineering, form regenerative to curative purposes. Hydrogels can be obtained starting from natural (collagen, chitosan, alginate, cellulose, etc.) or synthetic polymers (polylactic acid, poly(acrylic acid); poly(methyl methacrylate); poly(ethylene glycol), polycaprolactone, etc.). Moreover, ternary components can also be incorporated from cells and biological active agents to ceramic powders, graphene oxides and metallic nanoparticles. Besides the composition, the microstructure is an important issue, which must be considered when designing materials for tissue engineering. If only the classification of the materials is considered for bone grafting[7], the tendency of the last decades seems to be oriented to material design, which globally includes both composition and morphology. Therefore, a wide range of processing technologies were designed and are extensively used in optimizing the properties of the materials. 3D printing is a versatile processing technique, which is especially beneficial as it allows tuning the microstructure of the materials. In bone grafting, main factor affecting the homeostasis is microstructure of the main elements (Ca, P). In addition to that, mechanical properties of spongious and compact bony tissue can affect it. Electrospinning, for instance, is essentially suitable in developing 2D structure, such as membranes for wound healing (with pure regenerative but also curative role), transdermal drug delivery, loco-regional delivery of drugs, etc.

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