Medical Applications of Hybrid Hydrogels Containing Natural Polymers

Subjects: Materials Science, Biomaterials

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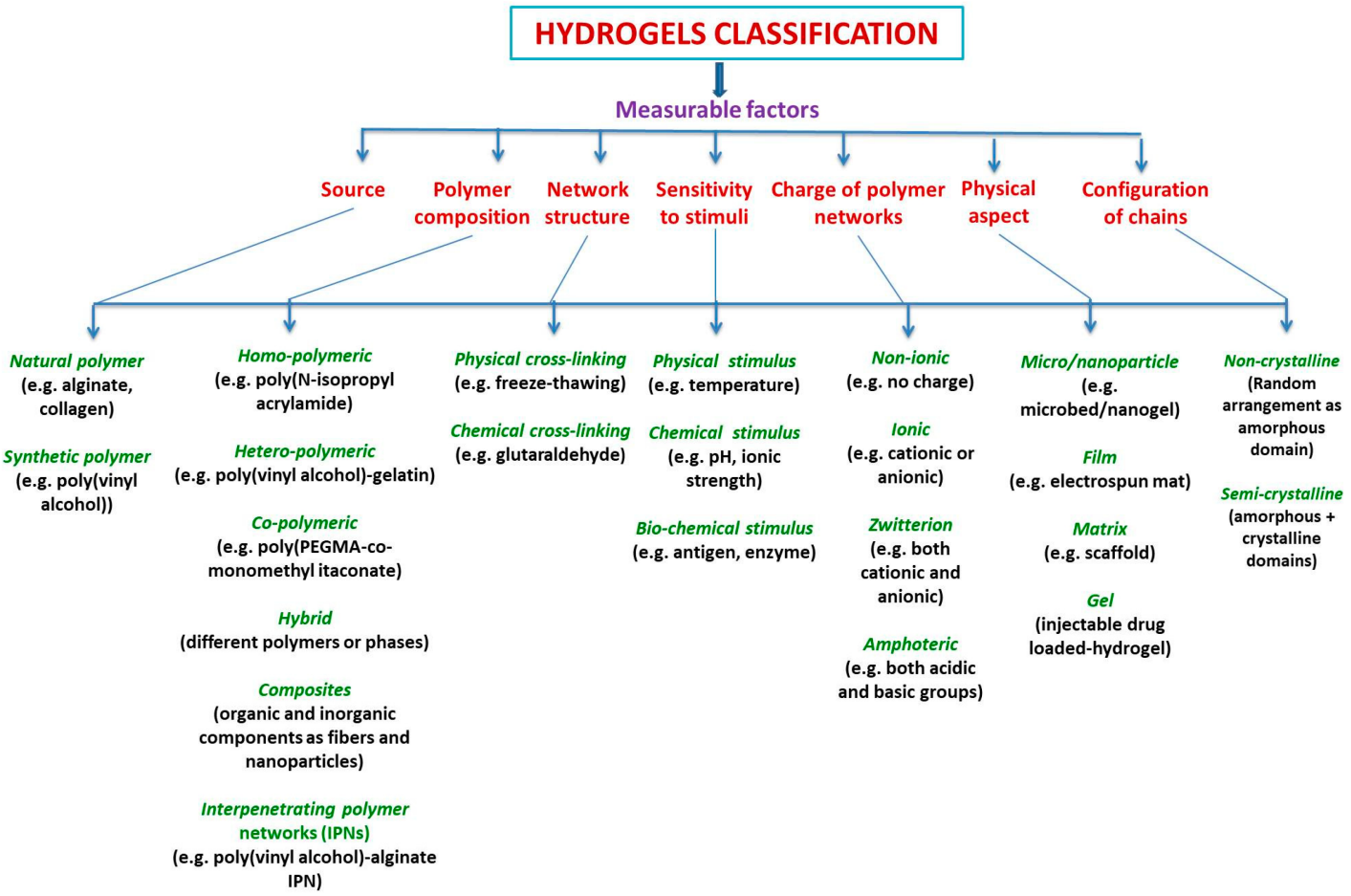
Hybrid hydrogels definition is still debatable. They are defined either as a complex composed chemically or physically cross-linking structures, or it refers to systems combining different polymers and/or with nanoparticles, such as plasmonic, magnetic, and carbonaceous nanoparticles, among others, or they are constituted by chemically, functionally, and morphologically distinct features from at least two different classes of molecules, which can include biologically active polymers as polysaccharides and/or proteins, peptides, or nano/microstructures, interconnected via physical or chemical means.

organic hybrid polymeric hydrogels | natural polymers | medical applications | homopolysaccharides | heteropolysaccharides | polypeptides | proteins | tissue engineering | Bone Tissue Engineering | cartilage Tissue Engineering | Wound Dressing | Drug Delivery | . Immunotherapy

1. Introduction

Hydrogels can be classified by taking into consideration many factors, such as source; preparation methods; network structure (as permanent (chemically crosslinked or irreversible), and non-permanent (physically crosslinked or reversible, hydrogen-bonded hydrogels); dimensions (macrogels, microgels, nanogels); sensitivity to stimuli (such as physical, chemical, and biochemical stimuli); charge of polymer network (nonionic, ionic, zwitterion, and amphoteric); physical aspect (micro-/nanoparticle, film, matrix, gel, etc.); configuration (amorphous and semicrystalline); composition (homopolymeric, multipolymeric or heteropolymeric, copolymeric, and interpenetrating polymer networks, hybrids, composites); degradability (biodegradable, bioabsorbable, bioerodible, and degradable in a controlled manner) (Scheme 1) [1][2].



Scheme 1. Classification of hydrogels [1][2].

Generally, hydrogels contain polar/charged functional groups which offer them hydrophilicity, water absorption capacity and, respectively, swelling in a certain medium, enhancement of their susceptibility to stimuli, etc. [3][4]. They can also differentiate in respect with their equilibrium swelling grade (SWD) as those low SWD hydrogels (20–50%), medium SWD hydrogels (50–90%), high SWD hydrogels (90–99.5%), and superabsorbent hydrogels (>99.5%) [5][6]. The hydrogels with high SWD show good permeability and biocompatibility [7] being preferred for use in the medical field.

Hybrid hydrogels definition is still debatable. They are defined either as a complex composed of hundreds of chemically or physically cross-linking nanogels [8], or it refers to systems combined with different polymers and/or with nanoparticles, such as plasmonic, magnetic, and carbonaceous nanoparticles, among others, or they are constituted by chemically, functionally, and morphologically distinct building blocks from at least two distinct classes of molecules, which can include biologically active polymers as polysaccharides and/or proteins, peptides, or nano/microstructures, interconnected via physical or chemical means [9]. Depending on the size and the nature of the building blocks, the hybridization can occur at molecular level or at microscopic scale [10][11](**Figure 1**).

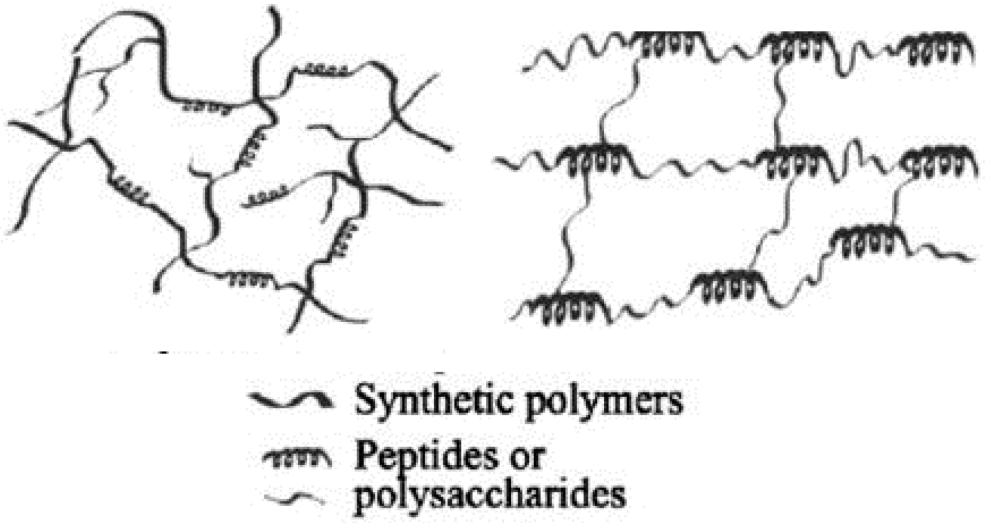


Figure 1. Schematic representation of organic hybrid hydrogels systems (adapted from [11]).

Each medical application involves the unique choice of a combination of the component materials, with the goal to match both desired structural and functional properties which must effectively produce an advanced polymeric system, with a new profile [12]. One of the most relevant examples is the combination protein/other polymers. Such combinations can be resulted by polymerization or conjugation (click chemistry) with synthetic polymers resulting compatible hybrid hydrogels both in vitro and in vivo as it was demonstrated by cell differentiation, proliferation, migration studies and drug delivery, tissue engineering, wound healing applications [13][14], respectively or sequestration of growth factors from the surrounding medium [15]. Commonly, the hybrid hydrogels are heterogeneous and this property is important to assure cell adhesion, organization, and cell–cell interactions required for medical applications [16][17][18][19].

1.1. Polymers Used in Hybrid Hydrogels

There are four main types of natural biodegradable polymers used in hybrid hydrogels described in this review—**Table 1**, including [20]: (1) homopolysaccharides, as: cellulose and derivatives, pullulan, dextran, starch, etc.; (2) heteropolysaccharides from which can be mentioned: chitosan/chitin and their derivatives [21], dextran, agarose, alginic acid and alginates, hyaluronic acid (HA), chondroitin and derivative sulphates, heparin, pectin, etc. (3) polypeptides/proteins, such as gelatin, collagen, albumin, fibrin and fibrinogen, soy and whey proteins, silk, Matrigel™, etc., and genetically engineered proteins [22][23][24] (calmodulin (a calcium-binding protein), elastin-like polypeptides, leucine zipper) [25]; (4) deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [26]. The protein/polysaccharide hybrid polymers like fibrin/cellulose, collagen/HA, gelatin/alginate and many others etc. were studied [27] and other many combination make now topics of undergoing researches. Lignin was also used [28][29]. Most of them are components of the extracellular matrix (ECM) in vivo. Their composition (bovine fibrinogen, rat tail collagen, etc.) may vary with source and processing method, being difficult to control their microstructures, properties and reproducibility between experiments.

Table 1. Natural polymers used in organic hybrid hydrogels for medical applications.

Polysaccharides		Polypeptides and Proteins	Polynucleotides and Others
Homopolysaccharides	Heteropolysaccharides		
Cellulose and derivatives (carboxymethylcellulose, hydroxyethyl cellulose; hydroxypropylcellulose methylcellulose hydroxypropylmethylcellulose; cellulose acetophphalate) • Pullulan and derivatives • Gelan • Curdlan • Scleroglucan • schizofillan • Starch and derivatives • Dextran • Dextrins and cyclodextrins • Carrageenan (K-, L-λ, etc) and derivatives (sulphates) • Glycogen • Inulin • Guar gum • Gum Acacia • Pectin	• Chitosan and derivatives • Chitin • Alginic acid and derivatives • Hyaluronic acid • Chondroitin and derivative sulphates • Xanthan gum • Heparin • Keratan sulphate • Dermatan sulphate • Pectin • Glycosaminoglycans (mucopolysaccharides) • Glucan and beta-glucan • Glucomanan • Laminarin • Proteoglycans • Agar • Gum Arabic	• Gelatin • Collagen • Albumins (bovine serum albumin, ovalbumin) • β-lactoglobulin • Elastin • Fibrin • Fibronectin • Resilin • Fibrinogen • Immunoglobulins • Soy Protein • Whey protein • Silk (silk fibroin and sericin) • Lactoferrin • Keratin • Zein • Casein	• DNA • RNA • Lignin

Polysaccharides		Polypeptides and Proteins	Polynucleotides and Others
Homopolysaccharides	Heteropolysaccharides		
[30][31]	• Gum tragacanth • Arabinixilans • Konjac glucomanan • Locust bean gum [32] [35][36][37][38]	• Synthetic proteins (Calmodulin, elastin-like polypeptides, leucine zipper) • Prolamins (gluten, gliadin) • Protamins and derivatives • Polylysines • Lysozyme • Histones • Enzymes • Myoglobin • Hemoglobin • Cytochrome C [33] • Proteic hormons • Interferon	[34]

ain types: mers are: (ethylene gels may AAm), 2-hydroxypropyl methacrylate (HPMA), acrylamide (AAm), acrylic acid (AAc) or macromers [37][38][39], methoxyl poly(ethylene glycol) (PEG), monoacrylates (mPEGMA or PEGMA), and diacrylates (PEGDA), ethylene glycol diacrylate (EGDA), Pluronic® polymers, etc. [39].

By combining the properties of synthetic and natural polymers to form hybrid hydrogels, a direct approach is created for bioactive hydrogel scaffolds for tissue engineering.

Comparatively with natural polymers, the synthetic polymers are easily synthesized even at large scale by polymerization, cross-linking, and functionalization (modification by block structures, by blending, copolymerization), their molecular structure, molecular weight, physical and chemical properties (mechanical strength, biodegradability [40][41]) are more reproducible, this aspect being critical for the medical applications

mainly scaffolding. Unfortunately, applications of synthetic hydrogels as biomaterials are limited by their absence of bioactivity. The protein-polymer hybrid networks with complex abilities, including bioactivity, stimuli-responsiveness, catalytic activity, or ability to regulate cell behaviors have been/are created to overcome this limitation, maintaining good mechanical properties of materials [42][43][44][45][46].

1.1.1. Microgel

The term microgel describes a variety of particles that differ substantially in structure, physico-chemical properties, preparation and application and is interchangeably with terms such as nanogel, microsphere and macrogel depending on the numerous particle types falling within the broad sphere of nano-/microparticle shapes and sizes [47][48][49][50].

1.1.2. Hybrid Nanogels

Hybrid nanogels later developed are highly crosslinked nano-sized hydrogel systems [47][48] with diameter less than 100 nm [49][50] having a non-fluid colloidal/polymer network that combine the properties of both hydrogels and nanomaterials. The nanoscale provides a large surface area for bioconjugation, long time of circulation in blood, and the possibility of being actively or passively targeted to the desired site of action (e.g., tumor sites) [10]. Hybrid smart hydrogels/nanogels show the ability to respond to biomedically relevant changes like pH, temperature, ionic force/concentration, redox environment, light, glucose, magnetic field, electrical field, chemicals or specific biomarkers etc., by changing their volume, refractive index, and hydrophilicity/hydrophobicity etc. Micro- and nano-sized hydrogels are faster in responding to changes in their environment than their macroscopic or bulk counterparts and can be used more efficiently in medical and sensor applications [51].

1.1.3. Multifunctional Hybrid Nanogels

Multifunctional hybrid nanogels found applications in medical field/nanomedicine for continuous monitoring by optical sensing to mentioned stimuli in complex samples such as blood and bioreactor fluids as well as for intracellular imaging, contributing to the explanation of intricate biological processes, the development of novel diagnoses and therapy toward clinical applications. [52].

1.1.4. Hybrid Polymer Nanogel/Hydrogels

Hybrid polymer nanogel/hydrogels include interpenetrated networks (IPNs) and core-shell particles. The core-shell strategy is especially useful for targeting therapy, while the interpenetration allows the development of multiresponssive nanogels and the control of the drug release profile [53].

1.1.5. Physical Hydrogels

Physical hydrogels result by ionic and physical interactions, such as hydrogen bonds, coordination bonds, electrostatic and hydrophobic interactions in certain conditions and physico-chemical interactions (stereo-complexation, charge condensation, or supramolecular chemistry) [54]. By changing the temperature, pH, ionic

strength or solvent composition, they form a homogeneous solution and re-gel when they return to their initial conditions, being reversible gels, generally unstable and mechanically weak [55]. The physical cross-links are also formed by crystallization, [56] between amphiphilic block and graft copolymers [57], and protein interactions [58]. Physically crosslinked hydrogels show stimuli-responsiveness and self-healing properties, but their mechanical strength is low and they often exhibit plastic flow [59].

1.1.6. Chemically or Covalently Crosslinked Hydrogels

Chemically or covalently crosslinked hydrogels with a permanently fixed shape at rest, exhibit a low fracture toughness and extensibility. Therefore, it is preferred to create both physically and covalently crosslinking hydrogels [60][61], resulting doubly-crosslinked hybrid gels that combine all mentioned properties [62]. Many double network (DN) hydrogels prepared by double chemically crosslinking or by hybrid physical/chemical crosslinking imply crosslinking agents, but they present toxicity which is an important disadvantage. Designing a new generation of DN gels comprising two non-covalent associated networks is a promising technique.

Kondo and coworkers [63] prepared a dually-crosslinked polymer gel with a very homogeneous network architecture, using a tetra-arm star-shaped poly(ethylene glycol) (PEG), PEG and poly(dimethylsiloxane) (PDMS) building blocks linked by orthogonal cross-coupling, The obtained network from hydrophilic and hydrophobic components regularly and uniformly distributed is non-covalent hydrophobic association whose strength is tuned by the molar ratio of the hydrophilic PEG and the hydrophobic PDMS segments [64].

1.1.7. Self-Assembling Hybrid Hydrogels

Self-assembling hybrid hydrogels containing peptides provide the desired biological functionality and biodegradability, are able to mimic biological structures and materials having direct biomedical applications, namely as carriers for drug and cell delivery (e.g., incorporation of bioactive sequences from natural proteins). To control mechanical, biocompatibility and degradation properties, the peptides are combined with polymeric networks [65][66] by chemical modification, covalently linking or non-covalent interactions between peptides and polymers [67].

Hybrid hydrogels self-assembled from graft copolymers via formation of coiled coil antiparallel heterodimers was also demonstrated [68], based on HPMA copolymers backbone and a pair of oppositely charged peptide grafts. The formation of these hybrid hydrogels was reversible [68]. A DNA/poly(lactic-*co*-glycolic acid) (PLGA) hybrid hydrogel (HDNA) was prepared for water-insoluble ophthalmic therapeutic delivery of dexamethasone and it may be applied in treatment of various eye diseases [69].

1.1.8. Interpenetrated and Semi-Interpenetrated Polymer Networks

To enhance the mechanical strength, the swelling/deswelling response, and to add new sensitivities to a nanogel, multicomponent networks as full IPNs and semi-IPNs (sIPNs) were prepared by simultaneous synthesis and sequential synthesis involving two or more polymers [70][71]. The reaction can take place in the presence of a crosslinking agent, in order to form a complete IPN or in the absence of the crosslinking initiator, to form a sIPN.

1.1.9. Core-Shell Polymer Networks

The most common techniques of synthesis of core-shell nanogels are the seed precipitation polymerization, crosslinking of amphiphilic micelles preformed by self-assembly or the reversible addition–fragmentation chain-transfer polymerization (RAFT) [72][73][74][75][76][77].

Several examples of hybrid polymeric hydrogel include:

(1) PEG-modified natural polymers [11][78][79], like fibrinogen, heparin (Hep), dextran, HA, and albumin;**1.1.10.**
(2) PNIPAAm-modified natural polymers, like collagen, chitosan, and alginate [80][81][82][83].**Supramolecular Hydrogel**

Supramolecular hydrogel are builded by blocks of peptides and polymers by the coupling/conjugation of specific peptide sequences (cell adhesive and/or enzymatically cleavable) to polymer chains. In such a way is obtained controlled cell responses (adhesion, migration, differentiation) because the components can self-assembly into hybrid hydrogels either, as peptide-polymer conjugates or combining individual components. These will determine the properties of the hydrogels (as stiffness, mesh structure, responsiveness, and biocompatibility) [84], cooperative folding/unfolding transitions control over the structure formation at the nanometer level. The new produced materials may possess unprecedented levels of structural organization and novel properties [85]. By optimizing the amino acid sequence, responsive hybrid hydrogels tailor-made for a specific application may be designed. Hybrid peptide/polymer molecular hydrogel design and synthesis showed significant research progress to mimic the natural proteins molecular architectures, dynamic responsiveness, and cellular functions, combined with tunability and processability provided by the synthetic polymer constituents.

2. Preparation Procedures for Polymeric Hybrid Hydrogels

2.1. Routes to Obtain Hybrid Hydrogels

Crosslinking techniques can be: (i) physical crosslinking (achieved by using repeated freezing/thawing cycles and led to cryogels) by ionic interaction, complex coacervation or H-bonding; (ii) chemical crosslinking or grafting by polymerization, co-polymerization, chemical conversion (using crosslinking agents such as borates, glyoxal, glutaraldehyde, etc.), and (iii) irradiation crosslinking or grafting (electron beam or gamma radiation, depending on irradiation dose). The properties of hydrogels can be controlled by different parameters, such as structures, by cross-linking type, end density, and synthesis of polymers, while in the case of physical hydrogels, by environment conditions (as pH, temperature, ionic strength etc.).

Chemically cross-linked gels are obtained by radical polymerization/crosslinking, emulsion, reverse microemulsion, inverse miniemulsion, heating, irradiation (ultraviolet, high-energy radiation, especially gamma and electron beams), photolithographic chemical reactions via crosslinker as di-sulfide crosslinking, ionic, click chemistry (such as azide-alkyne cyclo-addition reactions, thiol-ene couplings, Diels-Alder reactions and tetrazine-norbornene chemistry), Schiff base crosslinking with a huge ensemble of reactions, such as Michael type reaction, Michaelis-

Arbuzov reaction, and nucleophile addition [86], and enzymatic cross-linking [87]. Both chemical and physical cross-linking approaches are employed for hydrogels preparation [2].

A breakthrough toward the synthesis of complex structures with a high degree of functionality and compositional variety is the utilization as synthesis ways the controlled/living radical polymerization technique such as the catalytic atom (group) transfer radical polymerization (ATRP), degenerative chain transfer polymerization represented by iodine-mediated polymerization (RITP), and reversible addition-fragmentation chain transfer polymerization (RAFT) [88]. A new strategy of hybrid hydrogels synthesis entails the non-covalent attachment of genetically engineered coiled-coil protein motifs to hydrophilic synthetic HPMA copolymer backbone. The physical crosslinking was established by self-assembly of the coiled-coil domains [89].

2.1.1. Chemical Modifications

Chemical modifications involve a plenty of ligands which can be used for targeted drug delivery, stimulus responsive drug release or preparation of complex materials. The cross-linking of the hybrid network and conjugating proteins to the gel backbone as a platform for immobilizing functional proteins was reported by Lim et al. [90].

2.1.2. Functionalization

Hybrid hydrogels/nanogels can also be surface functionalized with specific ligands to achieve targeted therapy and reduce toxicity [91]. Functionalization is also important in order to create different types of macro/micro/nanogels morphologies, as hairy microgels, core-and-shell, hallow, multilayer microgels, [92] etc.

2.1.3. Stealth Functionalization

Hybrid nanosystems/nanogels for drug delivery and biomedical purposes need a non-secondary requirement, as their biocompatibility necessary both to reduce the inflammatory or the immune response of the organism, and to improve blood circulation lifetime, biodistribution, and bioavailability of the carried drugs and also to overcome the self-defense mechanisms present in the bloodstream of the host organism. To achieve this requirement the hybrid nanogels must be specifically designed. A very wide variety of architectures result by their decoration, modification, and functionalization, [93], or they can be modified by conjugation with both organic [94] and inorganic [95] types of nanoparticles and nanostructures. The morphologies of hybrid nanogels vary both with the particle type and the assembly technique, each component being either core or shell, of different size and architecture [96]. These variable morphologies may be obtained by chemical reactions or through physical crosslinking based on hydrogen bonds, ionic interactions, and other intermolecular bonds. Therefore, a proper surface decoration and its biocompatibility, is a parameter capable of strongly influencing the biodistribution together with the dimensions, the surface charge and the ligands interaction. Many stealth functionalizations exploit hydrophilic polymeric chains, as polyethylene glycols or chitosan.

2.1.4. PEGylation

PEGylation is a solution to increase the bioavailability of the decorated nanostructures and to extend the circulating lifetime [97]. After this modification a protein corona is formed around the antifouling PEG functionalization [98]. It will create a hindered zone around the nanoparticles and reduces the wrapping by plasma proteins and the subsequent uptake by macrophages PEGylation depends on many factors such as hydrophilicity of the PEG chains, molecular weight (MW) which vary from 2000 to 13,000 Da.

2.2. Processing Methods

Processing methods include [1]: solution casting/drying, theta gelation, freezing or freezing/pressurizing, freeze drying, emulsion freeze drying, inverse microemulsion polymerization technique, solution blowing, electrospinning, coagulation treatment, CO_2-in-water emulsion, sol-gel method/thermal annealing, CO_2 bubbles template freeze drying, high hydrostatic pressure [HHP] method, supercritical gel-drying. Other new synthesis methods include the implementation of click chemistry reactions [99], photo-patterning, and rapid prototyping, 3D printing for the facile production of hybrid hydrogels, self-assembly [100][101], the use of biological molecules and motifs to promote a desired cellular outcome, and the tailoring of kinetics and transport behavior to obtain desired biomedical outcomes [102]. 3D bioprinting of hydrogels is performed in accordance with the native tissue architecture therefore it is expected to result in a new generation of engineered tissues. Bakarich et al. [103] fabricated by a new 3D-printing approach an interesting material with good mechanical performance based on κ-carrageenan and poly(oxyalkylene amine) (Jeffamine) based ionic-covalent entanglement hydrogels. The carrageenan induced a fast gelation, a structural integrity to the hydrogel system and thermoresponsiveness, while the epoxy-amine reaction to form covalent bonding takes place an ambient temperature for covalent bond formation.

Hydrogels and their products can be obtained in a wide range of shapes as temporary or permanent shape, shape memory, smart shape memory, quadruple-shape, sponges, soft or rigid, stretchable, films, sheets, bilayer, micro/nanoparticles with defined shapes, ultrathin microcapsules, matrix, scaffolds, hollow cube, hemisphere, pyramid, cylindrical, twisted bundle, patches for wound dressing, artificial ear, nose, and many others.

3. Properties

The specific physico-chemical key properties of the hybrid hydrogels are: remarkable thermodynamic stability, elevated capacity of solubilization, mildness, density, swelling/deswelling, high-water content and permeability, low surface tension and relative low viscosity, stiffness, mesh structure and size, responsiveness, biocompatibility and biodegradability (so avoiding its accumulation in the organs), non-immunologic response and capability of undergoing vigorous sterilization techniques [48], as well as their tunable viscoelasticity and structural similarity to the ECM. Their properties can be fine-tuned through selection of the hydrogel components (chemical composition), hydrophobicity/hydrophilicity ratio, and cross-linking strategy, crosslinking density etc. Hydrogels are commonly considered as highly biocompatible, owing to the high-water content and also to the physico-chemical similarity with the native ECM. Chemically cross-linked synthetic polymeric hydrogels have higher mechanical properties compared to self-assembling (physically crosslinked) systems, thanks to the high molecular weight of polymer

materials, but they lack biological functionality, while self-assembling hydrogels, formed through physical cross-links, allow minimally invasive implantation in the body.

3.1. Swelling

The swelling of hydrogels is a process occurring in three steps, namely: (a) diffusion of water molecules into hydrogel network, (b) hydration of polymeric chains and their relaxation and (c) expansion of crosslinked polymeric network. The primary and secondary bound water is uptaken by the network by its interaction with the polar and hydrophobic sites, respectively and then the network is imbibed with additional water which is named free water. Finally at an infinite dilution to a maximum, level equilibrium water content is reached. The determination of swelling behavior is the main assay to establish the hydrogel quality, as it is also a means to evaluate other properties as: crosslinking degree, mechanical properties, degradation rate, etc. Swelling properties of the stimuli responsive hydrogels are significantly changed by the modification in parameters of the surrounding environment (i.e., temperature, pressure, pH, solvent composition, ionic strength, electrical potential, etc.). The polymeric hybrid hydrogels exhibit biodegradability and biocompatibility, high permeability, to oxygen, nutrients, and to water-soluble metabolites, being promising carriers and for cells encapsulation. They resemble with natural soft tissues [41][104] being very useful in regenerative medicine, for tissue scaffold or therapeutic transfer systems, promoting cell attachment and proliferation [2].

3.2. Mechanical Properties

The mechanical properties can be varied and tuned by changing the crosslinking degree, or lowered by heating. To seed osteoblast cells, it is necessary a more stiff material than in the case of adipocyte culture, as for this is also requirement for the development of a heterogeneous prosthetic device, as substitute for the intervertebral disc. The elastic nature of hydrated gels has been found to minimize irritation to the surrounding tissues after implantation.

3.3. Responsiveness

Generally, hydrogels have weak mechanical properties and a slow or delayed response to external stimuli. Novel hydrogel designs substantially enhanced mechanical properties and by creating the superporous and comb-type grafted hydrogels fast responses to external stimuli were obtained as also was done by development of self-assembling hydrogels from hybrid graft copolymers with property-controlling protein domains, and genetically engineered triblock copolymers containing hydrogels.

The low interfacial tension between the gel surface and body fluid minimizes protein adsorption and cell adhesion, reducing the chances of negative immune reactions [105].

3.4. Porosity and Permeation

The average pore size, the pore size distribution, and the pore interconnections included together in the parameter called « tortuosity » are important factors for a hydrogel matrix. They are influenced by the composition and the

crosslink density of the hydrogel polymer network. Pores can show different morphologies: they can be closed, open as a blind end or interconnected, again divided in cavities and throats.

Net charge of the polyelectrolyte hydrogel is determined by the initial concentration of the cationic and/or anionic monomer.

Crosslinking influences all the other properties of the hydrogels. By controlling the crosslinking degree, the materials with tunable and optimized properties destined to different applications can be obtained [106].

The micro-/nanogels are valuable materials as drug-delivery carriers because they show high loading capacity, good stability, and reversible volume change in response to environmental stimuli (such as pH, temperature, and glucose level) [93].

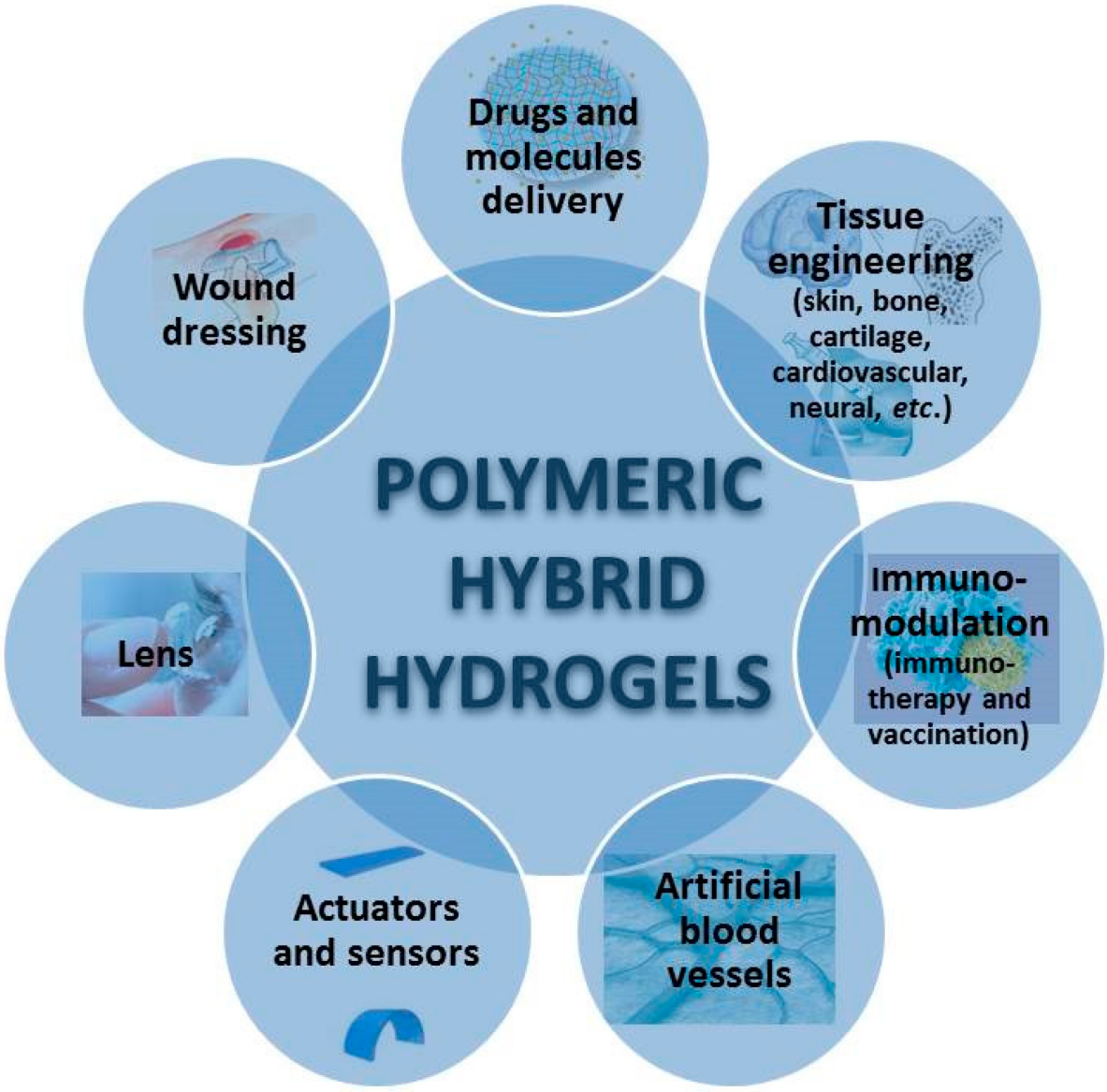
4. Applications

Hydrogels remain the most appealing candidates for tissue engineering scaffolds. The development of hybrid hydrogels constituted from different polymers is based on numerous resources and they are applied for regenerative medicine, tissue engineering (including: bone regeneration [107][108][109][110], cartilage tissue, vascular tissue, cardiac tissue, cardiovascular tissue, meniscus tissue, human prostate tissue, skin tissue/wound, and other tissues), wound healing, artificial cornea, drug/gene delivery, cancer cells, nucleus pulposus bioelectronic interfaces due to their structural similarity to the natural ECM, inherent biocompatibility, tunable viscoelasticity, tunable physical and mechanical properties, and their ability to form scaffolds for different tissues, high-water content and high permeability for oxygen and essential nutrients [11]. Biomedical applications of hydrogels as the first materials developed for uses inside the patient started from the decade of 70 s [111].

It is considered that the development of the hydrogels for medical applications known three steps [100][112]. The first generation of hydrogels is characterized by various crosslinking procedures involving the chemical modifications of a monomer or polymer with an initiator to develop materials with high swelling and good mechanical properties. The second generation of materials is that capable to respond to specific stimuli (temperature, pH, ionic strength, different external fields or concentration of specific bioactive molecules etc.), known as smart hydrogels. Finally, the research for the third generation of hydrogels was focused on the investigation and development of hybrid, stereo complexed materials (e.g., PEG-PLA interaction) with a wide spectrum of tunable properties and trigger stimuli [113][114]. This last stage aimed to develop the so called “smart hydrogels” with a variety of possible applications. Hybrid hydrogels based on both natural and synthetic polymers offer infinite possibility to cells encapsulation, as matrices for repairing and regenerating a wide variety of tissues and organs [115], are capable of responding to biological signals in vivo or remote triggers and other many possible applications in biomaterials, biomedicine and nanomedicine [116].

Other important applications are [102] (Scheme 2): wound dressing/healing, treatment of severe burns, drug delivery/controlled release, injectable hydrogels, vaccines, cancer treatment, autoimmune disease,

neurodegenerative disease, anti-inflammatory, ophthalmology, etc.



Scheme 2. Biomedical applications of hydrid hydrogels based on natural and synthetic polymers.

Particularized examples of medical applications of hybrid hydrogels containing different classes of natural polymers as: homo and hetero polysaccharides, proteinsl, nucleic acids and lignin are described.

Some selective research studies have been summarized especially those from the last two decades, for the preparation of natural polymers-containing hybrid hydrogels and their potential in a wide range of medical applications. It was described both advantages and disadvantages of each hydrogel applied in different medical applications. Desired hybrid hydrogels may be developed for targeted applications by making changes in composition, use of specific biomolecules, antimicrobial agents, use of suitable cells, and selecting suitable synthesis routes and processing techniques. The successful use of a polymeric hybrid hydrogel consists in creating a three-dimensional micro-/nano environment that represents a synthetic ECM for the cells, which should provide

biodegradability, biocompatibility, pore interconnectivity to assure the penetration and absorption of nutrient, modulation of proliferation for successful reconstruction of organs, cell-adhesion and regeneration certain tissue. In the most recent researches, injectable hydrogels and 3D-bioprinted hybrid hydrogels allow successful their interaction with the cells of damaged tissues. The hybrid nano hydrogel materials are able to convert external stimuli signals to heat, highly oxidative species etc., which are helpful for combinatorial therapies and theranostics. By a simple hybridization of the components of the hybrid hydrogels smart multiresponsive materials can be obtained by synergistic combination of the best properties of both components, useful toward applications in nanomedicine which exhibit an excellent targetability, minimal side effects in treatments and diagnostic. The industrial application of the new hybrid hydro/nanogels materials is in its first steps and it need more relevant clinical data concerning their safety and efficacy *in vivo.*

References

1. Kumar, A.; Han, S.S. PVA-based hydrogels for tissue engineering: A review. Int. J. Polym. Mater. Polym. Biomater. 2017, 66, 159–182.
2. Budama-Kilinc, Y.; Cakir-Koc, R.; Aslan, B.; Özkan, B.; Mutlu, H.; Üstün, E. Chapter 12: Hydrogels in Regenerative Medicine. In Biomaterials in Regenerative Medicine; Dobrzański, L.A., Ed.; IntechOpen: London, UK, 2018; pp. 277–301.
3. Durmaz, S.; Okay, O. Acrylamide/2-acrylamido-2-methylpropane sulfonic acid sodium salt-based hydrogels: Synthesis and characterization. Polymer 2000, 41, 3693–3704.
4. Ekici, S.; Saraydin, D. Synthesis, characterization and evaluation of IPN hydrogels for antibiotic release. Drug Deliv. 2004, 11, 381–388.
5. Peppas, N.P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 2000, 50, 27–46.
6. Swami, S.N. Radiation synthesis of polymeric hydrogels for swelling-controlled drug release studies. Ph.D. Thesis, Western Sydney University, Sydney, Australia, 2004. Available online: http://handle.uws.edu.au:8081/1959.7/698 (accessed on 7 January 2020).
7. Katime, I.; Novoa, R.; Zuluaga, F. Swelling kinetics and release studies of theophylline and aminophylline from acrylic acid/n-alkyl methacrylate hydrogels. Eur. Polym. J. 2001, 37, 1465–1471.
8. Zhang, T.; Yang, R.; Yang, S.; Guan, J.; Zhang, D.; Ma, Y.; Liu, H. Research progress of self-assembled nanogel and hybrid hydrogel systems based on pullulan derivatives. Drug Deliv. 2018, 25, 278–292.
9. Kopeček, J.; Tang, A.; Wang, C.; Stewart, R.J. De novo design of biomedical polymers. Hybrids from synthetic macromolecules and genetically engineered protein domains. Macomol. Symp. 2001, 174, 31–42.

10. Molina, M.; Asadian-Birjand, M.; Balach, J.; Bergueiro, J.; Miceliac, E.; Calderon, M. Stimuli-responsive nanogel composites and their application in nanomedicine. Chem. Soc. Rev. 2015, 44, 6161–6186.
11. Jia, X.; Kiick, K.L. Hybrid multicomponent hydrogels for tissue engineering. Macromol. Biosci. 2009, 9, 140–156.
12. Myung, D.; Waters, D.; Wiseman, M.; Duhamel, P.E.; Noolandi, J.; Ta, C.N.; Frank, C.W. Progress in the development of interpenetrating polymer network hydrogels. Polym. Adv. Technol. 2008, 19, 647–657.
13. Jonker, A.M.; Löwik, D.W.P.M.; van Hest, J.C.M. Peptide- and protein-based hydrogels. Chem. Mater. 2012, 24, 759–773.
14. Lau, H.K.; Kiick, K.L. Opportunities for multicomponent hybrid hydrogels in biomedical applications. Biomacromolecules 2015, 16, 28–42.
15. Maisani, M.; Pezzoli, D.; Chassande, O.; Mantovani, D. Cellularizing hydrogel-based scaffolds to repair bone tissue: How to create a physiologically relevant micro-environment? J. Tissue Eng. 2017, 8.
16. Joseph, C.A.; McCarthy, C.W.; Tyo, A.; Hubbard, K.; Fisher, H.; Altscheffel, J.; He, W.; Pinnaratip, R.; Lui, Y.; Lee, B.P.; et al. Development of an injectable nitric oxide releasing poly(ethylene) glycol-Fibrin adhesive hydrogel. ACS Biomater. Sci. Eng. 2019, 5, 959–969.
17. Hoffman, A.S. Hydrogels for biomedical applications. Ann. N. Y. Acad. Sci. 2001, 944, 62–73.
18. Yang, G.; Lin, H.; Rothrauff, B.B.; Yu, S.; Tuan, R.S. Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering. Acta Biomater. 2016, 35, 68–76.
19. Patel, D.; Sharma, S.; Screen, H.R.C.; Bryant, S.J. Effects of cell adhesion motif, fiber stiffness, and cyclic strainon tenocyte gene expression in a tendon mimetic fiber composite hydrogel. Biochem. Biophys. Res. Commun. 2018, 499, 642–647.
20. Kopeček, J. Hydrogel Biomaterials: A Smart Future? Biomaterials 2007, 28, 5185–5192.
21. Liang, Y.; Liu, W.; Han, B.; Yang, C.; Ma, Q.; Song, F.; Bi, Q. An in situ formed biodegradable hydrogel for reconstruction of the corneal endothelium. Colloids Surf. B: Biointerfaces 2011, 82, 1–7.
22. Ehrick, J.D.; Deo, S.K.; Browning, T.W.; Bachas, L.G.; Madou, M.J.; Daunert, S. Genetically engineered protein in hydrogels tailors stimuli-responsive characteristics. Nat. Mater. 2005, 4, 298–302.
23. Sengupta, D.; Heilshorn, S.C. Protein-engineered biomaterials: Highly tunable tissue engineering scaffolds. Tissue Eng. Part B: Rev. 2010, 16, 285–293.

24. Foo, W.P.C.T.; Lee, J.S.; Mulyasasmita, W.; Parisi-Amon, A.; Heilshorn, S.C. Two-component protein-engineered physical hydrogels for cell encapsulation. Proc. Natl. Acad. Sci. USA 2009, 106, 22067–22072.
25. Sakai, S.; Hirose, K.; Taguchi, K.; Ogushi, Y.; Kawakami, K. An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. Biomaterials 2009, 30, 3371–3377.
26. Xing, Y.; Cheng, E.; Yang, Y.; Chen, P.; Zhang, T.; Sun, Y.; Yang, Z.; Liu, D. Self-assembled DNA hydrogels with designable thermal and enzymatic responsiveness. Adv. Mater. 2011, 23, 1117–1121.
27. Davidenko, N.; Campbell, J.J.; Thian, E.S.; Watson, C.J.; Cameron, R.E. Collagen–hyaluronic acid scaffolds for adipose tissue engineering. Acta Biomater. 2010, 6, 3957–3968.
28. Raschip, I.E.; Hitruc, G.E.; Vasile, C.; Popescu, M.C. Effect of the lignin type on the morphology and thermal properties of xanthan/lignin hydrogels. Int. J. Biol. Macromol. 2013, 54, 230–237.
29. Raschip, I.E.; Hitruc, E.G.; Vasile, C. Semi-interpenetrating polymer networks containing polysaccharides. II. Xanthan/lignin network: A spectral and thermal characterization. High Perform. Polym. 2011, 23, 219–229.
30. Hejčl, A.; Sedý, J.; Kapcalová, M.; Toro, D.A.; Amemori, T.; Lesný, P.; Likavcanová-Mašínová, K.; Krumbholcová, E.; Prádný, M.; Michálek, J.; et al. HPMA-RGD hydrogels seeded with mesenchymal stem cells improve functional outcome in chronic spinal cord injury. Stem Cells Dev. 2010, 19, 1535–1546.
31. Beamish, J.A.; Zhu, J.; Kottke-Marchant, K.; Marchant, R.E. The effects of monoacrylated poly(ethylene glycol) on the properties of poly (ethylene glycol) diacrylate hydrogels used for tissue engineering. J. Biomed. Mater. Res. Part A 2010, 92, 441–450.
32. Zustiak, S.P.; Leach, J.B. Hydrolytically degradable poly (ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. Biomacromolecules 2010, 11, 1348–1357.
33. Silva, A.K.A.; Richard, C.; Bessodes, M.; Scherman, D.; Merten, O.-W. Growth factor delivery approaches in hydrogels. Biomacromolecules 2008, 10, 9–18.
34. Varghese, S.; Elisseeff, J.H. Hydrogels for musculoskeletal tissue engineering. Adv. Polym. Sci. 2006, 203, 95–144.
35. Jiang, Z.; Hao, J.; You, Y.; Liu, Y.; Wang, Z.; Deng, X. Biodegradable and thermoreversible hydrogels of poly (ethylene glycol)-poly (ε-caprolactone-co-glycolide)-poly (ethylene glycol) aqueous solutions. J. Biomed. Mater. Res. Part A 2008, 87, 45–51.
36. Deshmukh, M.; Singh, Y.; Gunaseelan, S.; Gao, D.; Stein, S.; Sinko, P.J. Biodegradable poly (ethylene glycol) hydrogels based on a self-elimination degradation mechanism. Biomaterials

2010, 31, 6675–6684.

37. Schmedlen, R.H.; Masters, K.S.; West, J.L. Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. Biomaterials 2002, 23, 4325–4332.
38. Ossipov, D.A.; Brännvall, K.; Forsberg-Nilsson, K.; Hilborn, J. Formation of the first injectable poly (vinyl alcohol) hydrogel by mixing of functional PVA precursors. J. Appl. Polym. Sci. 2007, 106, 60–70.
39. Higuchi, A.; Aoki, N.; Yamamoto, T.; Miyazaki, T.; Fukushima, H.; Tak, T.M.; Jyujyoji, S.; Egashira, S.; Matsuoka, Y.; Natori, S.H. Temperature-induced cell detachment on immobilized pluronic surface. J. Biomed. Mater. Res. Part A 2006, 79, 380–392.
40. Zhu, J. Bioactive modification of poly (ethylene glycol) hydrogels for tissue engineering. Biomaterials 2010, 31, 4639–4656.
41. Geckil, H.; Xu, F.; Zhang, X.; Moon, S.; Demirci, U. Engineering hydrogels as extracellular matrix mimics. Nanomedicine 2010, 5, 469–484.
42. Matsumoto, T.; Isogawa, Y.; Tanaka, T.; Kondo, A. Streptavidin-hydrogel prepared by sortase A-assisted click chemistry for enzyme immobilization on an electrode. Biosens. Bioelectron. 2018, 99, 56.
43. Ito, F.; Usui, K.; Kawahara, D.; Suenaga, A.; Maki, T.; Kidoaki, S.; Suzuki, H.; Taiji, M.; Itoh, M.; Hayashizaki, Y.; et al. Reversible hydrogel formation driven by protein-peptide-specific interaction and chondrocyte entrapment. Biomaterials 2009, 31, 58–66.
44. Ramirez, M.; Guan, D.; Ugaz, V.; Chen, Z. Intein-triggered artificial protein hydrogels that support the immobilization of bioactive proteins. J. Am. Chem. Soc. 2013, 135, 5290.
45. Krishna, O.D.; Kiick, K.L. Protein- and peptide-modified synthetic polymeric biomaterials. Biopolymers 2010, 94, 32–48.
46. Pamfil, D.; Vasile, C.; Tarțău, L.; Vereștiuc, L.; Poieată, A. pH-Responsive 2-hydroxyethyl methacrylate/citraconic anhydride-modified collagen hydrogels as ciprofloxacin carriers for wound dressings. J. Bioact. Compat. Polym. 2017, 32, 355–381.
47. Dorwal, D. Nanogels as Novel and versatile pharmaceutics. J. Pharm. Pharm. Sci. 2012, 4, 67–74.
48. Yadav, H.K.S.; Al Halabi, N.A.; Alsalloum, G.A. Nanogels as Novel Drug Delivery Systems-A Review. J. Pharm. Pharm. Res. 2017, 1, 1–8.
49. Tahara, Y.; Akiyoshi, K. Current advances in self-assembled nanogel delivery systems for immunotherapy. Adv. Drug Deliv. Rev. 2015, 95, 65–76.

50. Bencherif, S.A.; Siegwart, D.J.; Srinivasan, A.; Horkay, F.; Hollinger, J.O.; Washburn, N.R.; Matyjaszewski, K. Nanostructured hybrid hydrogels prepared by a combination of atom transfer radical polymerization and free radical polymerization. Biomaterials 2009, 30, 5270–5278.
51. Sahiner, N.; Godbey, W.T.; McPherson, G.L.; John, V.T. Microgel, nanogel and hydrogel–hydrogel semi-IPN composites for biomedical applications: Synthesis and characterization. Colloid Polym. Sci. 2006, 284, 1121–1129.
52. Wu, W.; Zhou, S. Hybrid micro-/nanogels for optical sensing and intracellular imaging. Nano Rev. 2010, 1, 5730.
53. Lohani, A.; Singh, G.; Sankar Bhattacharya, S.; Verma, A. Interpenetrating Polymer Networks as Innovative Drug Delivery Systems. J. Drug Deliv. 2014, 2014, 583612.
54. Hoare, T.R.; Kohane, D.S. Hydrogels in drug delivery: Progress and challenges. Polymer 2008, 49, 1993–2007.
55. Ebara, M.; Kotsuchibashi, Y.; Narain, R.; Idota, N.; Kim, Y.J.; Hoffman, J.M.; Uto, K.; Aoyagit, T. Smart Biomaterials; Springer: Tokyo, Japan, 2014; ISBN 978-4-431-54400-5.
56. Amini, A.A.; Nair, L.S. Injectable hydrogels for bone and cartilage repair. Biomed. Mater. 2012, 7, 024105.
57. Jin, R. Chapter 2: In-Situ Forming Biomimetic Hydrogels for Tissue Regeneration. In Biomedicine; Lin, C., Ed.; INTECH Open Access Publisher: London, UK, 2012; pp. 35–58.
58. Augst, A.D.; Kong, H.J.; Mooney, D.J. Alginate hydrogels as biomaterials. Macromol. Biosci. 2006, 6, 623–633.
59. Czarnecki, S.; Rossow, T.; Seiffert, S. Hybrid Polymer-Network Hydrogels with Tunable Mechanical Response. Polymers 2016, 8, 82.
60. Sun, J.-Y.; Zhao, X.; Illeperuma, W.R.K.; Chaudhuri, O.; Oh, K.H.; Mooney, D.J.; Vlassak, J.J.; Suo, Z. Highly stretchable and tough hydrogels. Nature 2012, 489, 133–136.
61. Lin, P.; Ma, S.; Wang, X.; Zhou, F. Molecularly engineered dual-crosslinked hydrogel with ultrahigh mechanical strength, toughness, and good self-recovery. Adv. Mater. 2015, 27, 2054–2059.
62. Narita, T.; Mayumi, K.; Ducouret, G.; Hébraud, P. Viscoelastic properties of poly(vinyl alcohol) hydrogels having permanent and transient cross-links studied by microrheology, classical rheometry, and dynamic light scattering. Macromolecules 2013, 46, 4174–4183.
63. Kondo, S.; Hiroi, T.; Han, Y.S.; Kim, T.H.; Shibayama, M.; Chung, U.I.; Sakai, T. Reliable hydrogel with mechanical “fuse link” in an aqueous environment. Adv. Mater. 2015, 27, 7407–7411.

64. Sletten, E.M.; Bertozzi, C.R. Bioorthogonal chemistry: Fishing for selectivity in a sea of functionality. Angew. Chem. Int. Ed. 2009, 48, 6974–6998.
65. Kopeček, J.; Yang, J. Smart Self-Assembled Hybrid Hydrogel Biomaterials. Angew. Chem. Int. Ed. 2012, 51, 7396–7417.
66. Rodriguez, L.M.D.L.; Hemar, Y.; Cornish, J.; Brimble, M.A. Structure–mechanical property correlations of hydrogel forming β-sheet peptides. Chem. Soc. Rev. 2016, 45, 4797.
67. Baker, B.M.; Chen, C.S. Deconstructing the third dimension–how 3D culture microenvironments alter cellular cues. J. Cell Sci. 2012, 125, 3015–3024.
68. Yang, J.; Xu, C.; Wang, C.; Kopeček, J. Refolding hydrogels self-assembled from N-(2-hydroxypropyl)methacrylamide graft copolymers by antiparallel coiled-coil formation. Biomacromolecules 2006, 7, 1187–1195.
69. Ren, N.; Sun, R.; Xia, K.; Zhang, Q.; Li, W.; Wang, F.; Zhang, X.; Ge, Z.; Wang, L.; Fan, C.; et al. DNA-Based Hybrid Hydrogels Sustain Water-Insoluble Ophthalmic Therapeutic Delivery against Allergic Conjunctivitis. ACS Appl. Mater. Interfaces 2019, 11, 26704–26710.
70. Mundargi, R.C.; Patil, S.A.; Kulkarni, P.V.; Mallikarjuna, N.N.; Aminabhavi, T.M. Sequential interpenetrating polymer network hydrogel microspheres of poly(methacrylic acid) and poly(vinyl alcohol) for oral controlled drug delivery to intestine. J. Microencapsul. 2008, 25, 228–240.
71. Liu, X.; Guo, H.; Zha, L. Study of pH/temperature dual stimuli-responsive nanogels with interpenetrating polymer network structure. Polym. Int. 2012, 61, 1144–1150.
72. Blackburn, W.H.; Dickerson, E.B.; Smith, M.H.; McDonald, J.F.; Lyon, L.A. Peptide-Functionalized Nanogels for Targeted siRNA Delivery. Bioconjug. Chem. 2009, 20, 960–968.
73. Schachschal, S.; Balaceanu, A.; Melian, C.; Demco, D.E.; Eckert, T.; Richtering, W.; Pich, A. Polyampholyte Microgels with Anionic Core and Cationic Shell. Macromolecules 2010, 43, 4331–4339.
74. Zhang, W.; Yao, R.; Tao, W.; He, H.; Shui, S. Preparation of monodisperse HPMC/PAA hybrid nanogels via surfactant-free seed polymerization. Colloid Polym. Sci. 2013, 292, 317–324.
75. Nayak, S.; Lee, H.; Chmielewski, J.; Lyon, L.A. Folate-Mediated Cell Targeting and Cytotoxicity Using Thermoresponsive Microgels. J. Am. Chem. Soc. 2004, 126, 10258–10259.
76. Dickerson, E.; Blackburn, W.; Smith, M.; Kapa, L.; Lyon, L.A.; McDonald, J. Chemosensitization of cancer cells by siRNA using targeted nanogel delivery. BMC Cancer 2010, 10, 10.
77. Lapeyre, V.; Ancla, C.; Catargi, B.; Ravaine, V. Glucose-responsive microgels with a core-shell structure. J. Colloid Interface Sci. 2008, 327, 316–323.

78. Zieris, A.; Prokoph, S.; Levental, K.R.; Welzel, P.B.; Grimmer, M.; Freudenberg, Y.; Werner, C. FGF-2 and VEGF functionalization of starPEG–heparin hydrogels to modulate biomolecular and physical cues of angiogenesis. Biomaterials 2010, 31, 7985–7994.

79. Jin, R.; Moreira Teixeira, L.S.; Krouwels, A.; Dijkstra, P.J.; van Blitterswijk, C.A.; Karperien, M.; Feijen, J. Synthesis and characterization of hyaluronic acid–poly (ethylene glycol) hydrogels via Michael addition: An injectable biomaterial for cartilage repair. Acta Biomater. 2010, 6, 1968–1977.

80. Li, F.; Griffith, M.; Li, Z.; Tanodekaew, S.; Sheardown, H.; Hakim, M.; Carlsson, D.J. Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration. Biomaterials 2005, 26, 3093–3104.

81. Nistor, M.T.; Vasile, C.; Tatia, R.; Chiriac, A. Hybrid collagen/pNIPAAM hydrogel nanocomposites for tissue engineering application. Colloid Polym. Sci. 2018, 296, 1555–1571.

82. Nistor, M.T.; Chiriac, A.P.; Nita, L.E.; Vasile, C. Characterization of the semi-interpenetrated network based on collagen and poly(N-isopropyl acrylamide-co-diethylene glycol diacrylate). Int. J. Pharm. 2013, 452, 92–101.

83. Cheaburu, C.N.; Vasile, C. Responsive freeze-drying interpolymeric associations of alginic acid and PNIPAM. II. Transition and temperature dependence on pH and composition. Cell. Chem. Technol. 2008, 42, 207–212.

84. Radvar, E.; Azevedo, H.S. Supramolecular Peptide/Polymer Hybrid Hydrogels for Biomedical Applications. Macromol. Biosci. 2019, 19, 1800221.

85. Vandermeulen, G.W.M.; Klok, H.A. Peptide/protein hybrid materials: Enhanced control of structure and impropved performance through conjugation of biological and synthetic polymers. Macromol. Biosci. 2004, 4, 383–398.

86. Gulrez, S.K.H.; Al-Assaf, S.; Phillips, G.O. Hydrogels: Methods of Preparation, Characterisation and Applications. In Progress in Molecular and Environmental Bioengineering. Analysis and Modeling to Technology Applications; Carpi, A., Ed.; IntechOpen: Rijeka, Croatia, 2011.

87. Khademhosseini, A.; Langer, R. Microengineered hydrogels for tissue engineering. Biomaterials 2007, 28, 5087–5092.

88. Barner-Kowollik, C. Handbook of RAFT Polymerization; Wiley-VCH Verlag GmbH & Co.: Weinheim, Germany, 2008; ISBN 978-3-527-31924-4.

89. Wang, C.; Kopeček, J.; Stewart, R.J. Hybrid hydrogels crosslinked by genetically engineered coiled-coil block proteins. Biomacromolecules 2001, 2, 912–920.

90. Lim, S.; Jung, G.A.; Muckom, R.J.; Glover, D.J.; Clark, D.S. Engineering bioorthogonal protein–polymer hybrid hydrogel as a functional protein immobilization platform. Chem. Commun. 2019,

55, 806–809.

91. Sierra-Martin, B.; Fernandez-Barbero, A. Multifunctional hybrid nanogels for theranostic applications. Soft Matter 2015, 14, 8205–8216.
92. Sanson, N.; Rieger, J. Synthesis of nanogels/microgels by conventional and controlled radical crosslinking copolymerization. Polym. Chem. 2010, 1, 96577.
93. Kabanov, A.V.; Vinogradov, S.V. Nanogels as pharmaceutical carriers: Finite networks of infinite capabilities. Angew. Chem. Int. Ed. 2009, 48, 5418–5429.
94. Wu, H.Q.; Wang, C.C. Biodegradable smart nanogels: A new platform for targeting drug delivery and biomedical diagnostics. Langmuir 2016, 32, 6211–6225.
95. Karg, M. Functional Materials Design through Hydrogel Encapsulation of Inorganic Nanoparticles: Recent Developments and Challenges. Macromol. Chem. Phys. 2016, 217, 242–255.
96. Eslami, P.; Rossi, F.; Fedeli, S. Review Hybrid Nanogels: Stealth and Biocompatible Structures for Drug Delivery Applications. Pharmaceutics 2019, 11, 71.
97. Grover, G.N.; Rao, N.; Christman, K.L. Myocardial Matrix-Polyethylene Glycol Hybrid Hydrogels for Tissue Engineering. Nanotechnology 2014, 25, 014011.
98. Foster, J. Chapter 12: PEGylation and BioPEGylation of Polyhydroxyalkanoates: Synthesis, Characterisation and Applications. In Biopolymers; Elnashar, M., Ed.; IntevhOpen: Rijeka, Croatia, 2010; pp. 243–256.
99. Jiang, Y.; Chen, J.; Deng, C.; Suuronen, E.J.; Zhong, Z. Click hydrogels, microgels and nanogels: Emerging platforms for drug delivery and tissue engineering. Biomaterials 2014, 35, 4969–4985.
100. Chirani, N.; Yahia, L.H.; Gritsch, L.; Motta, F.L.; Chirani, S.; Faré, S. History and Applications of Hydrogels. J. Biomed. Sci. 2015, 4, 32.
101. Zhou, M.; Smith, A.M.; Das, A.K.; Hodson, N.W.; Collins, R.F.; Ulijn, R.V.; Gough, J.E. Self-assembled peptide-based hydrogels as scaffolds for anchorage-dependent cells. Biomaterials 2009, 30, 2523–2530.
102. Palmese, L.L.; Thapa, R.K.; Sullivan, M.O.; Kiick, K.L. Hybrid hydrogels for biomedical applications. Curr. Opin. Chem. Eng. 2019, 24, 143–157.
103. Bakarich, S.E.; Balding, P.; Gorkin, R.; Spinks, G.M. Printed ioniccovalent entanglement hydrogels from carrageenan and an epoxy amine. RSC Adv. 2014, 4, 38088–38092.
104. Tan, H.; Marra, K.G. Injectable, Biodegradable Hydrogels for Tissue Engineering Applications. Materials 2010, 3, 1746–1767.
105. Peppas, N.A.; Hilt, J.Z.; Khademhosseini, A.; Langer, R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Adv. Mater. 2006, 18, 1345–1360.

106. Weber, L.M.; Lopez, C.G.; Anseth, K.S. Effects of PEG hydrogel crosslinking density on protein diffusion and encapsulated islet survival and function. J. Biomed. Mater. Res. A 2009, 90, 720–729.

107. Fujioka-Kobayashi, M.; Ota, M.S.; Shimoda, A.; Nakahama, K.; Akiyoshi, K.; Miyamoto, Y.; Iseki, S. Cholesteryl group- and acryloyl group-bearing pullulan nanogel to deliver BMP2 and FGF18 for bone tissue engineering. Biomaterials 2012, 33, 7613–7620.

108. Shimoda, A.; Chen, Y.; Akiyoshi, K. Nanogel containing electrospun nanofibers as a platform for stable loading of proteins. RSC Adv. 2016, 6, 40811–40817.

109. Shimoda, A.; Sawada, S.-i.; Akiyoshi, K. Intracellular protein delivery using self-assembled amphiphilic polysaccharide nanogels. In Intracellular Delivery II: Fundamentals and Applications; Prokop, A., Iwasaki, Y., Harada, A., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 265–274.

110. Molinos, M.; Carvalho, V.; Silva, D.M.; Gama, F.M. Development of a Hybrid Dextrin Hydrogel Encapsulating Dextrin Nanogel As Protein Delivery Systems. Biomacromolecules 2012, 13, 517–527.

111. Lee, S.C.; Kwon, I.K.; Park, K. Hydrogels for delivery of bioactive agents: A historical perspective. Adv. Drug Deliv. Rev. 2013, 65, 17–20.

112. Buwalda, S.J.; Boere, K.W.; Dijkstra, P.J.; Feijen, J.; Vermonden, T.; Hennink, W.E. Hydrogels in a historical perspective: From simple networks to smart materials. J. Control. Release 2014, 190, 254–273.

113. Yom-Tov, O.; Neufeld, L.; Seliktar, D.; Bianco-Peled, H. A novel design of injectable porous hydrogels with in situ pore formation. Acta Biomater. 2014, 10, 4236–4246.

114. Abebe, D.G.; Fujiwara, T. Controlled thermoresponsive hydrogels by stereocomplexed PLA-PEG-PLAprepared via hybrid micelles of premixed copolymers with different PEG lengths. Biomacromolecules 2012, 13, 1828–1836.

115. Sefton, M.V.; May, M.H.; Lahooti, S.; Babensee, J.E. Making microencapsulation work: Conformal coating, immobilization gels and in vivo performance. J. Control. Release 2000, 65, 173–186.

116. Wang, L.; Guo, S.; Dong, J.; Cui, J.; Hao, J. Microgels in biomaterials and nanomedicines. Adv. Colloid Interface Sci. 2019, 266, 1–20.