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HYDROGELS IN REGENERATIVE MEDICINE: A CONCISE REVIEW

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ABSTRACT: Hydrogels were the primary biomaterials developed for human use. Hydrogels are playing an increasing role in regenerative medicine attributable to their growing functional sophistication. Polymer scaffolds have many diverse applications within the field of drug delivery, tissue engineering, and implantation. They are used as dispensing devices for bioactive molecules and as three-dimensional (3D) structures that provide stimulants that organize the cells and guide the desired original tissue development. Hydrogels are used as a scaffolding material since they are structurally identical to the extracellular matrix of various tissues, mostly manufactured under mild conditions, and can be introduced in a minimally invasive way. Hydrogel materials formed a gaggle of polymeric materials. The hydrophilic arrangement enables them to retain huge amounts of water in their three-dimensional (3D) backbone. As a result, hydrogels are used as scaffolding substrates for delivery of the drug and growth factor transmission, modifications in tissue engineering, and many other applications. In this review, we describe the properties of hydrogels, types of polymers, classification, fabrication methods and application.

INTRODUCTION: Hydrogels are polymers with a cross-connected network consisting of up to 90% water. Wichterle and Lim first developed such kinds of polymers as hydrogels within the 1960s, having earlier been found by Du Pont de Nemours in 1936. In 1987 Peppas published a detailed review of hydrogels in biomedical applications [1, 2, 3, 5]. Hydrogels are engineered to fulfill requirements like swelling and mechanical properties so that they have great potential for a wide range of biomedical applications, from contact lenses to controlled drug delivery and tissue engineering [4].

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A hydrogel is a three- (3D) network of hydrophilic polymers which will swell in water and retain significant quantities of water while retaining the structure because of the chemical or physical interlinking of individual polymer chains [5]. Another description defines hydrogels as a kind of substance that exhibits high swelling capability without altering its structure, volume, or shape. Based on the application, hydrogel is flexible and straightforward to shape [6].

Because of their substantial water content, hydrogels often exhibit a degree of flexibility somewhat the same as natural tissue. Network hydrophilicity is because of the existence of hydrophilic groups, including -NH2, -COOH, -OH, -CONH2, -CONH- and -SO3H.5,7 In reaction to certain physical and chemical stimuli, hydrogels undergo a major volume form transition or a gel-sol form transition. Physical stimuli involve temperature, electric and magnetic fields, solvent

composition, light intensity, and pressure, while pH, ions, and particular chemical compositions are included within the chemical or biochemical stimuli [5]. Hydrogels developed by photo-polymerization were extensively investigated as biomaterials in applications like tissue engineering scaffolds, drug delivery carriers, thrombosis prevention, postoperative adhesion formation, and biosensor coatings. The photo-polymerization method enables the hydrogel to be produced *in-vitro* or *in-vivo* by means of a free radical pathway in a very minimally invasive manner from less viscous solution of monomer, oligomer, or low molecular mass polymer (macromer) [8]. Significant progress has been made within the design and manufacture of hydrogels for different applications from both natural and artificial sources, including regenerative medicine, drug/gene supply, stem cell, and cancer research and cell therapy [9].

Regenerative medicine is characterized as the area of translation science concerned with "the process of replacing or regenerating human cells, tissues or organs in order to preserve or restore normal function" [10, 11, 12]. Regenerative medicine not only holds promise as a way of compensating for donor shortages but also as a way of increasing the quality of treatment. Transplants or medical prosthetics are currently available in many situations, but they furnish only a short-term solution compared to the stable, undamaged physiological state. It is, therefore, necessary for regenerative medicine researchers to keep in mind the present medical options and consistently attempt to improve them.

Because of their inherent structural and compositional similarities with the extracellular matrix and their comprehensive structure for cell proliferation and survival, hydrogels have received long attention in terms of material needs in regenerative medicine, like those required for tissue scaffolds or as therapeutic delivery systems. Over the past few decades, several kinds of hydrogel with dramatically different chemical and physical characteristics are produced from a different variety of chemical components and employing a number of synthetic strategies. This expansion of information of hydrogel enables scaffolding properties like cell attachment, molecular reaction, structural integrity, biodegradability, bio-compatibility, and solute transport to be carefully designed to fulfill the proliferative needs of the construct [13]. Hydrogels used as acellular regenerative substrates include therapeutic agents like growth factors or drugs (*e.g.,* genes), which play an important role in offering both spatial and temporal control of the agent delivery. The hydrogel also can act as a protective shield to maintain the drug's action and will also ready to fill a gap, thereby providing some spatial support to the encircling regenerating tissue [14].

In this review, we first introduce the properties, polymers used in fabrication, and classification of hydrogels for regenerative engineering. Then we explore various fabrication methods for hydrogel. Furthermore, important regenerative engineering approaches focused on hydrogel are highlighted for treating various tissues, like musculoskeletal, neurological, and cardiac systems. In the future, we assume that nano and micro-fabricated hydrogels can play a significant role in regenerative medicine to cure multiple forms of tissues [15].

Physical and Chemical Properties of Hydrogels:

Cross-Linking and General Characteristic of Hydrogels: The water content of hydrogels governs its specific physicochemical properties such as mildness, density, and low surface tension of hydrogel [16]. Hydrogel's mechanical properties involve swelling rate, pore size. The mechanical characteristics of hydrogels can influence the material's stability and cell behaviour, including cell spreading, migration and differentiation by influencing the signal conversion process; for example, mechanical signals are transformed into biochemical signals [17].

Hydrogels may also have various physical forms such as solid moulds forms, pressed powder matrices, microparticles (wound therapies), coatings, membranes or disks, encapsulated solute [16]. Different cross-linking strategies, including chemical and physical methods, were used to build polymer networks and to shield 3D structures in aqueous media [14, 16]. Physical interactions between the polymer chains in the physically cross-linked gels prevent hydrogel degradation, whereas the covalent bonds in the chemically cross-linked gel between the polymer chains form a stable hydrogel [16].

Physically Cross-linked Hydrogel: Cross-linking of ionic interaction and physical interactions such as hydrogen bonds, coordination bonds, and hydrophobic interactions is the result of physical gel called hydrogels. These hydrogels change the composition of the solvent, pH, or temperature to form a homogeneous solution and re-gel when they come back to their initial conditions. Due to this property, the physical gel is also known as a reversible gel. They are usually fragile and mechanically weak [16]. A variety of environmental stimuli (pH, temperature, ionic strength) and a number of physicochemical interactions (hydrophobic interactions, charge condensation, hydrogen bonding, stereo-complexation, or supra molecular chemistry) may be used to establish the physical cross-linking of polymer chains [18].

Chemical Cross-linked Hydrogels: Chemical cross-linked hydrogels are considered permanent since they are cross-linked between their chains by strong chemical bonds and are not re-dissolved by altering temperature, pH, or solvent composition. There are permanent junctions between cross-linked chemical networks. Chemical cross-linking hydrogels include permanent covalent bonds that leading to enhance the mechanical strength of the gel, and these hydrogels are more stable than physical cross-linking. However, the introduction of chemical cross-linkers may cause toxicity problems.16 Cross-linking of hydrogel can occur with a method such as radical polymerization, enzymatic reactions, click chemistry, thermo-gelation and cross linking by radiation.19

Polymer used in Hydrogels Fabrication:

Natural Polymers: There are three types of natural polymers. First proteins such as collagen, silk, gelatin, fibrin, genetically engineered proteins like calmodulin (a calcium-binding protein) [13, 16, 20, 21, 22, 23]. Second polysaccharides like hyaluronic acid, chitosan, and dextran [16, 17, 21, 22, 23, 24]. Last is modified polysaccharides or Protein/ poly-saccharide hybrid polymers like dextran methacrylate and laminin/chitosan [8, 16].

Synthetic Polymers:

Biodegradable Synthetic Polymers: The most popular substitute for poloxamer-based copolymers is poly (L-lactic acid), poly (L- glycolic acid), or its copolymers.16,18 Several vinylic monomers are 2-hydroxyethyl methacrylate, N-isopropylacrylamide, 2-hydroxypropyl methacrylate, acrylamide, acrylic acid.5,7,16,21 Various macromers includes; N, N′-methylenebis poly (ethylene glycol), and methoxy poly (ethylene glycol) (PEG), monoacrylate (mPEGMA or PEGMA) [5, 13, 16, 21, 25].

Non-Biodegradable Synthetic Polymers: Triblock copolymers of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) structures are modified through chemical cross-linking with poly (vinyl alcohol) (PVA) [16, 25, 26].

Types of Hydrogels: Depending on the origin, hydrogels can be divided into those derived from natural polymers and those derived from synthetic polymers.

Natural Hydrogels: Naturally derived hydrogels are divided into two categories as Protein-based materials and polysaccharide-based materials [19].

Protein-Based Hydrogels:

Collagen: Collagen is the main protein in human connective tissue, containing 25% -33% of total protein. It is made from repeating peptides (primarily proline, hydroxyproline, and glycine) [6]. Collagen is a natural biomaterial generally used in biology and medicine that present primarily in the skin, bone, cartilage, blood vessels, teeth, and tendons. Collagen has excellent biological properties, like those of low antigenicity, bio-degradability, biocompatibility, and adaptability to cells [27]. Collagen is the main extracellular matrix protein in the body, and its role is to provide mechanical strength for opposing action forces to avoid eventual repetitive plastic deformation and, at the same time, the type of collagen and the orientation of fibers describes the different types of cell in tissue disposition [19]. Collagen gels can be produced in situ, and can be easily used for cells and growth factors as a natural delivery tool. Collagen was used to produce hydrogels for regeneration of the vocal cord, reconstruction of the spinal cord passage, and faults in cartilage [13]. Collagen fibers are the term for collagen's quaternary structure and are created by self-assembled fibers. Till now, 29 collagen forms have been identified, but collagen I is the most widely used and studied [19]. Collagen was also used in

ophthalmological drug delivery systems, as a regulating medium for transdermal delivery, and in skin maintenance in sponges for wounds. Additionally, collagen was used as artificial blood vessels. Collagen can serve as a good carrying model system in the brain. Collagen gels are used in the spinal cord and peripheral nervous system as nerve guiding substances [13].

Fibrin: Fibrin is a protein that is specially engaged in the natural phenomenon of tissue repair and in the cascade of coagulation. Fibrinogen is the inactive form of fibrin, and when it gets activated, it plays an important role in the regeneration of damaged tissue by producing an extensive network of fibers [19]. The key advantage of fibrin gels is that fibrinogen can be derived from the plasma, thereby minimizing the chances of a reaction from the foreign body. In addition, fibrin was used to deliver chondrocytes in a knee injury model in combination with other gels, such as Hyaluronic acid-based gels [13]. Fibrin-based hydrogels are widely used in cardiac tissue engineering, but poor mechanical strength is the major barrier [19]. In the medical field, fibrin is used as a glue consisting of fibrinogen and thrombin solutions which form a clot when combined. Fibrin glue is mainly used to control bleeding and to bind tissues during surgery [13].

Polysaccharide-based Hydrogels:

Alginate: Alginate is a natural, non-mammalian polysaccharide that produces a gel through ionic cross-linking in the presence of divalent cations.28 Alginate is a polysaccharide portion of the cell walls of brown algae and some capsules of bacteria [19]. Alginate is a linear chain copolymer of the residues of D-mannuronic acid (M) and L-glucuronic acid (G), commonly used for cell encapsulation [13, 29, 30]. From the biological point of view, alginate exhibits non-toxic and non-inflammatory attributes for medical applications; in vivo degradation does not occur, although cell adhesion is weak and mechanical properties are inadequate. Applications of alginates have been studied in different tissues such as liver, nerve, skin, and cartilage [19]. Alginate gels were often used for the encapsulation of cells (*e.g.*, osteoblasts and chondrocytes) for cartilage repair, and chondrocyte and alginate solutions were injected into anatomically structured implants. For surgical dressings and drug delivery applications, alginate was also used [13]. Because of its biocompatibility and biodegradability, gel-forming abilities, and low cost, Alginate has broad applications for manufacturing hydrogels [6].

Chitosan: Chitosan is a linear polysaccharide made up of β-(1–4) bound D-glucosamine with randomly distributed groups of N-acetyl-D-glycosamine obtained partially deacetylating chitin, the main structure of exoskeletons arthropods such as shells of crabs and shrimp [19, 23, 31]. Chitosan structure is identical to glycosaminoglycans found in the extracellular matrix. This biocompatible, cationic polymer dissolves probably up to a pH of 6.2 in water [31]. Physical and mechanical characteristics are directly associated with the molecular weight and degree of deacetylation of chitosan molecules. The benefits of using chitosan in hydro-gel preparations are such as antibacterial properties, straightforward to sterilize, low cost, bio-active, biocompatible, and their degradability can be regulated by altering the deacetylation level. This form of hydrogel is influenced easily by the parameters like pH and temperature. Its weak mechanical properties are among its drawbacks, but this issue can be resolved by cross-linking with chemical materials or gelatin. Chitosan is a very good candidate in the development of hydrogels employed in drug delivery systems and provided its controllable degradability and its short duration in physiological conditions [19]. In another research, chitosan and gelatin were combined to create an in-situ gel for cell seeding and/or drug delivery. In particular the group assessed the difference between two different enzymes in cross-linking [1].

Synthetic Hydrogels:

Poly (ethylene glycol): Poly (ethylene glycol) is water-soluble and biocompatible, having characteristics that restrict immunogenicity, antigenicity, protein binding, and cell adhesion. PEG homopolymer is a polyether that can be polymerized by condensation from ethylene oxide. Because of their fascinating ability to inhibit serum protein adsorption, PEG first became popular as a surface coating for biomaterials in the 1970s [13]. PEG polymers can be cross-linked covalently using a number of hydrogel-forming approaches. An especially attractive technique of cross-linking PEG chains is the use of acrylate-terminated PEG

monomers by photopolymerization. PEG hydrogels are passive components of the cell environment in the presence of proteins, as they resist protein adsorption. However, several techniques for modifying PEG gels have made PEG gels a flexible arrangement for many subsequent conjugations. For example, sequences of peptides were inserted into PEG gels to cause degradation or alter cell adhesion [25]. Bio-active alteration of PEG hydrogels may promote cellular function for tissue engineering applications [29]. Due to their inert nature, photocurable hydrogels that are PEG-based are commonly used to encapsulate cells into scaffolds. The confidentiality characteristics of PEG are well recognized, and thus, these gels can be used in encapsulated cell scaffolds to avoid unwanted interactions between the polymer and encapsulated cells. Compared with many other synthetic hydrogels, PEG scaffolds have been most popular in applications of tissue engineering that does not require vascularization of the scaffold, such as skin, and cartilage [13].

Some Common Types of Hydrogels:

Stimuli-sensitive Hydrogels: Stimulus-sensitive hydrogels are commonly referred to as "environmentally friendly", "smart," or "intelligent" hydrogels due to their ability to absorb, transmit or process a stimulus and to react by producing a useful result [32]. "Smart" hydrogels or stimuli-sensitive hydrogels are very different from normal hydrogels in that they can "feel" changes in environmental factors such as pH and temperature and react by raising or decreasing their swelling, ionic strength, solvent form, electrical and magnetic fields, light and chelating species existence [21, 32, 33]. The combined effect of two or more stimulus-responsive mechanisms into one hydrogel system results in the formation of unique hydrogel class, called double-responsive hydrogel. This dual-response hydrogel can respond to more than one external stimulus simultaneously and independently. For example, poly(N- isopropylacrylamide- co-propyl acrylic acid) and copolymers of poly(N-isopropylacrylamide- co-propyl acrylic acid) have been documented to show a sharp response to minimum pH and temperature signals. Biochemical stimuli include responses to proteins, antigens, ligands, and other biochemical substances [32]. Currently, stimulus-responsive hydrogel formed using biocompatible thermally responsive polymers, which facilitated cancer cell destruction [34]. Hydrogels which contain functional proteins as a portion of their structure, have enormous potential applications. Calmodulin (CaM) has been recently identified as the biological element in CaM-phenothiazine hydrogels that react to stimuli [33]. Stimuli-responsive hydrogels were studied extensively for various applications such as actuators, sensors, valves, and modulators for delivery of drug [35].

Temperature-sensitive Hydrogels: One of the responsive hydrogels systems studied most widely is Temperature- responsive hydrogels [25, 53]. Temperature-responsive hydrogels are developed from polymers that demonstrate a temperature-induced transformation from a polymer-water preferential interaction state to a polymer-polymer preferential interaction state. Temperature-responsive polymers show a phase change between polymer and solution at a critical solution temperature, based upon the structure of the polymer chain [32]. Temperature-sensitive polymers key feature is the existence of hydrophobic groups [5]. Poly(N-isopropylacrylamide) (PNIPAM), poly (vinyl methyl ether) (PVME), and poly (N-vinyl caprolactam) (PNVC) are the examples of temperature-sensitive hydrogels.32,36

pH-Responsive Hydrogels: Another commonly researched stimulus-responding hydrogel is pH-responsive polymer [32, 35]. Any pH-sensitive polymer structurally contains acidic groups such as carboxylic and sulfonic acids or basic group, such as ammonium salts that react by gaining or losing protons to the pH changes in their environment [5, 36, 37].

Polyelectrolytes are polymers that have these ionizable groups in large numbers. In specific environmental conditions, anionic polyelectrolytes such as poly (acrylic acid) (PAA) are deprotonated, and then electrostatic repulsion between chains increase significantly, allowing water molecules to penetrate, causing extreme swelling of the hydrogel [5]. Weak poly acids and Weak poly-bases two forms of pH-responsive hydrogels that exist. Poly (acrylic acid) is the standard example of weak poly acids. By comparison, such as poly (N, N′-diethylaminoethyl methacrylate) in weak poly bases [32]. Poly(acrylamide) (PAAm), PAA, poly

(methacrylic acid) (PMAA), poly (diethylaminoethyl methacrylate) (PDEAEMA), and Poly (dimethylaminoethyl methacrylate) (PDMAEMA) are the most widely observed ionic polymers for pH-responsive actions [5].

Hybrid Systems: Hybrid hydrogels are typically referred to as hydrogel structures that contain materials from at least two distinct types of molecules, such as synthetic polymers and biological macromolecules, which are linked either covalently or non-covalently. The combination of peptide domains and synthetic polymers will create new materials with superior characteristics to those of separate components. Proteins and protein modules have well-defined and homogeneous shapes, stable mechanical properties, and cooperative folding/unfolding transformations compared to synthetic polymers. The peptide domain can impose a level of control over structural development at the nanometer level; the synthetic component can contribute to the hybrid material's biocompatibility.

The synergistic combination of two types of structures will create new substances with unprecedented structural, organizational levels and novel characteristics. The physical crosslinking of the coiled-coil domains were created by self-assembly. Recently it has been proved that graft copolymers are self-assembled into hybrid hydrogels. A new HPMA-based hybrid hydrogel system consisted of a hydrophilic polymer skeleton and a pair of oppositely charged peptide grafts [33].

In recent years, hydrogels formed by a covalent cross-linking of polymers to promote targeted delivery of drugs have been achieved. The hydrogel-nanoparticles is a hybrid system developed in three different ways.The first approach is to entrap a hydrogel inside a nanoparticle, the second approach is to build a 3D hydrogel network with the nanoparticles by crosslinking the latter using hydrophobic interactions or combining nanoparticles with opposite charges and the last approach is to combine the nanoparticle with the hydrogel covalently [34].

Drug Release Mechanisms from Hydrogel Devices: Hydrogel release mechanisms are not similar to release mechanisms from hydrophobic polymers. These models are based on the controlled release rate-limiting stage and are thus classified as Diffusion-controlled, Swelling-controlled, Chemically-controlled.

Diffusion-controlled: Diffusion-controlled is the most commonly used mechanism for explaining the release of drugs from hydrogels. Fick's diffusion law with either constant or diffusion variables is typically used in diffusion-controlled release models [21, 38]. Diffusion-controlled delivery of drugs with hydrogels uses reservoir or matrix systems that enable diffusion-based release of drugs through a hydrogel mesh or water-filled pores [34]. A reservoir delivery mechanism involves a drug-containing core that is covered with a hydrogel membrane, typically available as capsules, tubes, spheres, or slabs.

The drug concentration in the core of the system is higher to allow a steady rate of release [39]. The drug release is time-independent in reservoir system [34]. In matrix systems, the drug is uniformly distributed or dissolved in the hydrogel's three-dimensional structure. The release of drugs is accomplished via the macromolecular mesh or pores, and in this case, the initial release rate is proportional to the square root of time instead of remaining constant [39]. The drug release is time-dependent in matrix system [34].

Swelling-controlled: Swelling-controlled release takes place when drug diffusion is more rapid than swelling of the hydrogel. Modelling of this mechanism generally include shifting boundary conditions where particles are released at the rubber like interface and glassy stages of swollen hydrogels [21, 38]. In swelling-controlled release systems the drug is spread as in a matrix system inside a glassy polymer, and when the polymer comes in contact with a bio-fluid, it begins to swell [34, 39]. The substance then extends beyond its boundary, enabling drug diffusion by relaxing polymer chains. This method is also called Case II transport and exhibits continuous, time-independent release kinetics. It is also known as 'anomalous transport,' which combines the diffusion with swelling-controlled release [34, 39]. Regulation of swelling properties of hydrogel can be used as a tool to stimulate the release of drugs [25].

Chemically-controlled: Chemically-controlled release is employed to characterize the discharge of molecules determined by the reactions that occur within a delivery matrix. The foremost common reactions that happen in hydrogel delivery systems are the cleavage of polymer chains through hydrolytic or enzymatic degradation or reversible or irreversible reactions that occur between the polymer network and release able drugs [21, 38]. Afterward, it is further classified as (i) Purely kinetic-controlled release in during which the breakdown of polymers (bond-cleavage) is the rate-determining step, and therefore, the term of diffusion is taken into account negligible; and (ii) Reaction-diffusion-controlled release where both reaction conditions (*e.g.* polymer degradation, protein-drug interaction) and diffusion conditions must be included within the model for predicting drug release accurately. The reaction-diffusion-controlled release is especially fascinating as more synthetic hydrogel systems engineered with drug-binding ability are employed in drug delivery and tissue engineering [21].

Fabrication Methods: Fabrication of hydrogel scaffolds include various techniques such as Photolithography or Photo-patterning, Emulsi-fication, Bioprinting, Micro-moulding, and Microfluidics.

Photolithography or Photopatterning: One of the techniques that have gained success in making micro fabricated hydrogels is photolithography or photo-patterning. This technique was originally designed for the manufacture of micron-sized features for applications in micro-electro-mechanical systems (MEMS) [9]. This approach is reproducible for monomers with groups that can be photopolymerized (*e.g.,* methacrylate) [40]. Photo-lithography is a method in which photo- cross-linkable hydrogels are placed beneath a mask that regulates the light exposure of a hydrogel precursor film to specific regions. Where the light is revealed, the photo-crosslink able hydrogel will crosslink to create mask-shaped structures. Structures extending sub-micrometer to millimetre scale can be produced with photo-lithography. It has been shown that photo-lithography is compatible with the different polymers. Normally a photo-initiator is used to form a polymerized network which forms free radicals upon light irradiation and begins the polymerization reaction [13]. The entire thickness of the hydrogel layer is normally crosslinked during the photo-patterning process without any control over the cross-linking depth. Their aspect ratio (*i.e.*, the ratio between their height and width) is another significant property that can restrict the dimensions of the microfabricated hydrogel construction. Since hydrogels are typically not mechanically solid, features of a high aspect ratio may fail. Photo-patterning is a flexible technique that enables precise spatial control over the microenvironment of the cells. Moreover, it enables the manufacturing of 3D cell-laden constructs or patterning of various cells by sequential photo-patterning of hydrogels containing different types of cells. The technique of photo-patterning is simple to use and does not need sophisticated equipment [9].

Emulsification: Historically, emulsification was used to produce hydrogel microspheres by incorporating hydrogel precursors in a hydrophobic medium (such as oil) and breaking up the hydrogel stage into small droplets by stirring. The size of the microgels can be regulated, depending on the agitation conditions. Emulsified spherical microgels were used for various micro-encapsulation techniques, including immuno-isolation. The microgel is utilized in these methods to separate transplanted cells from the host's immune system while allowing the exchange of oxygen and nutrients as well as metabolic products secreted by cells between the hydrogels and the surrounding environment. Emulsification can also be utilized to encapsulate ESCs as an in vitro culture inside microgels to create more controllable environments for differentiation. Though emulsification is a relatively easy method, there are a number of possible limitations to it. For example, the structure of the resulting gels is typically restricted to spheres, and the resulting spherical gels will often have some degree of diversity despite being able to regulate the resulting sizes [13].

Bioprinting: Bio-printing is a computer-assisted design-based technique that integrates biomaterials loaded with cells to create complex 3D functional tissue structures. Bio-inks play a significant role in this strategy, as they offer a supportive scaffold and also maintain the viability and function of printed cells [15]. Bio-printing techniques allow fast printing with high cell viability of micro-and macro-scale

3D structures [9]. Additionally, bio-inks can be deposited simultaneously using numerous material printing to mimic tissue interfaces with slowly altering composition and structure [15]. Broad diversity of bio-printing systems was developed to prepare and regulates the 3D hydrogel design and architecture [9].

Inkjet Bioprinting: In the 1980s, attempts were initially documented to use inkjet printers as devices for dispensing cells and biological materials. To deposit both collagen and fibronectin suspensions with cells, Klebe used a popular Hewlett-Packard desktop printer to shape simplified analogs of tissues. In inkjet bio-printing, a container, similar to ink cartridges, dispenses drops by heating and vaporizing in the range of 1 to 100 pl, while either a bubble or a piezoelectric actuator pushes the liquid drop out. The integration of bio-printing with other commonly used manufacturing techniques, such as electrospinning, has also attracted considerable attention. Usually, inkjet printers are commonly used in the manufacturing of many devices, from light-emitting diodes to high-resolution full-color flat panel displays. A fascinating area that has emerged currently is the use of inkjet printers as instruments for developing biochemical sensing devices. In the creation of such biosensors, inkjet printers are used to form electrically conducting traces (*e.g.,* electrical contact and electrode) or sensing layers (*e.g.,* polymer film, enzyme or antibody spot, and colorimetric reagent). All these innovations are benefited from developments achieved in printed electronics [9].

Laser-Assisted Bioprinting: A laser-induced forward transfer (LIFT) method is used in popular laser-guided bio-printers to migrate a biological material from a source film to a non-absorbent surface near the film. Based on the rheological characteristics of liquid films and the thickness of the metallic absorbing layer, printing is achieved by means of jet formation, which occurs above a limit of laser energy. However, the need for a high-energy transfer system has been linked to a decrease in cell survival after deposition. One of the latest possible applications of the LIFT methodology is the manufacturing of cardiac patches developed with Matrigel-treated polyester urethane urea (PEUU) [9].

Drop-Based Bioprinting: Drop-based bioprinting is becoming more common due to the speed at which it can create scaffolds and biomaterials with detailed 3D structures. Actually, this speed makes it difficult for the technique to be applied to other polymer systems, as it needs gelation time to be faster than, or at least identical to, the drop deposition time. Using a 3D printer-based device, an alginate bio-ink printing technique deposits alginate pre-polymer onto a gelatin surface that serves as a calcium reservoir. The alginate pre-polymer is then injected dropwise into the surface and forms a gel as the calcium ions diffuse into the droplet. An alternate drop deposition algorithm enables the printing of vessel-like structures to mitigate droplet spreading effects on structures. While drop-based printing gives a fast method of manufacturing large structures, it requires rapid kinetics of polymerization, which limits the polymers that can be used [41].

Micro-moulding: Micro-moulding is a technique that uses a prefabricated mould to shape and then cross-link precursors of the hydrogel into desired forms. The emergence of biological micro-electro-mechanical system (BioMEMS) technology has led to greater micro-molding adaptation. Micro-moulding was primarily used in the manufacture of structures such as heat- cross-linkable polymers like collagen, agarose, and gelatin, as well as photo-cross-linkable polymers (*e.g.,* methacrylate PEG and HA).

One micro-molding task has been to produce harvestable microstructures from chemically cross-linkable hydrogels, such as alginate and chitosan. The gel precursor is initially developed using the hydrogel mould in this process, and a crosslinking agent is then added to the mould to crosslink the resulting structures into a new gel. Moulding was also used to produce hydrogel structures at nanoscale. To do this, it is necessary to use moulds that can dehydrate the hydrogels in regions that come in contact between the mould and the substrate [13].

Microfluidics: Microfluidics is the last technique that is used to build micro-scale structures from hydrogels. Recently a various strategy was used to develop microscale hydrogels through the creation of single or multi-phase flows within microfluidic

channels. Frequently, Hydrogel precursors and cells flow *via* microchannel controlling the resulting hydrogel form. By placing these cell-laden microgels on each other, complex 3D structures have been formed, in which multiple cell types can be patterned relative to each other to re-create tissue as complex. In addition, two-phase systems in a hydrophobic medium consisting of hydrophilic droplets are used to produce hydrogel droplets with controllable physical characteristics. As the suspension of the hydrogel precursor flows through the channel, a gel is created by exposure to a crosslinking agent. Example, Microfluidic channels were used to produce micro-engineered hydrogels using processes in which a stream of gel precursors was exposed to light that passed through a mask and was microscopically focused. As the fluid is exposed to light, the hydrogel will crosslink to create microgels that will be afterward collected at the outlet of the microchannel. By using this technique, it is possible to encapsulate cells in hydrogels of controlled shapes [13]. Microfluidic devices have been commonly used to micro-fabricated structures of hydrogel and could be used for tissue-engineered vessels, lungs, and cartilage. For example, Microfluidic devices with bifurcated patterns were used to create highly functionalized vascular structures based on polystyrene [32].

Applications of Hydrogels: The hydrogels with various features are widely used as wound dressing, drug delivery, dental equipment, implants, and tissue engineering.

Applications of Hydrogels in Tissue Engineering: Tissue engineering is described as a combination of materials, engineering, and cells for improvement or replacement of biological organs [5]. Tissue engineering is an interdisciplinary field utilizing the concepts of engineering and life sciences that seek to improve, preserve and renew tissues and organs functions [16]. When parts or the whole of some tissues or organs fail, there are many recovery choices, including repairing, replacing with a synthetic or natural substitute, or regeneration. Hydrogels have increasingly been researched as tissue engineering matrices. Hydrogels intended to be used as tissue engineering scaffolds may contain pores that are large enough to accommodate living cells, or they will be engineered to dissolve or degrade away, releasing growth factors and forming pores through which living cells may penetrate and proliferate [42]. In tissue engineering scaffolds, both the artificial and naturally derived materials may be used to construct hydrogels [5]. Because of their various controllable properties, similarity to tissues, and their ability to shape scaffolds for different tissues due to their tunable physical and mechanical characteristics, hydrogels are widely used in biomedical and bioengineering fields. Hydrogels used in tissue engineering have low viscosity prior to injection and allow rapid gel formation in the tissue's physiological environment. It's widely employed in bone, cartilage, vascular tissue, meniscus, tendon, skin, cornea, and soft tissue [16]. Different types of hydrogels can be used, depending on the type of desired tissue [5].

Bone Tissue Engineering (BTE): The bone is a vascularized and dynamic tissue with the potential to recover naturally on an injury. However, in large bone deficiencies, the repair mechanism cannot be adequate [16]. The large bone lesions caused by injuries, illnesses, cancers, or other disorders be still a difficult clinical issue. In tissue engineering, cells such as stem cells, neural cells, and osteoblasts are a significant component [43]. Bone tissue engineering is focused on the utilization of 3D matrices, which promote both cellular growth and bone regeneration. The matrices of hydrogel may comprise biological substances such as cells and growth factors. One of the studies is about cells being encapsulated in alginate and/or collagen hydrogel scaffolds to determine the impact of different natural skeletons on this coculture method. A researcher has found that the natural cell-binding capabilities of hydrogels such as collagen are favored over the cell-cell proximity between the two types of cells [16].

Cartilage Tissue Engineering: Articular cartilage damage is one of the most common forms of orthopedic disease in the clinic, and is challenging for surgeons due to articular cartilage self-repair restrictions [43].

Simply, articular cartilage is a tissue comprised of one type of cell (chondrocytes) incorporated in an extracellular matrix (ECM). However, the structure is more complex, and based on depth, the three ECM contents, involve three depth-related layers

such as a superficial layer, middle layer, and deep layer [13]. Traumatic and degenerative articular cartilage injuries are major causes of disability. More than 40 million Americans are estimated to be suffering from osteoarthritis today [23]. The requirement for tissue-engineered cartilage is enormous and significant clinical importance [16]. Hydrogel architecture has advanced in recent years to improve cartilage repair. Many of the applications of hydrogels include the creation of enhanced network cross-linking (e.g., double networks), novel hydrogel manufacturing technologies (*e.g.,* 3D printing), and better implementation of biological markers (e.g., controlled release) [16]. The stem-cell therapy has been implemented into cartilage injury management. Because of nano-HAP's high biocompatibility, strong toughness, and cell adhesive capacity, several types of literature have indicated that nano-HAP exhibits effective repair capability for articular cartilage [43].

Neural Tissue Engineering: Neural tissue damages caused by either neurodegenerative disorders or high-energy trauma significantly affect the quality of life of patients around the world [43]. There is a need for axon outgrowth, migration, and the creation of a connective neural network to regenerate neural tissues. The construction of these networks is challenging as axonal guidance is sensitive to the scaffold's various physicochemical characteristics, such as mechanical properties, topography, and various factors of neurogenic growth that can bind to the scaffold. The neuronal cells of mammals show restriction of regrowth performance and functional healing, presenting a critical clinical challenge for surgeons [15]. With the encapsulation of Schwann cells and neural progenitor cells, hydrogels are becoming more widely used to facilitate neural regeneration through cell-based trophic support [20]. Nanofibers embedded in hydrogel scaffolds can serve as topographical and biological indicators for the regeneration of the neurons. For example, McMurtrey manufactured a unique 3D neural tissue using the integrated electrospun poly-caprolactone (PCL) nanofibers in hydrogel scaffolds [15].

Applications of Hydrogels in Drug Delivery: Conventional drug delivery can lead to toxicity and adverse effects due to the need of high dosages and repeated administration to induce a therapeutic effect, and this can decrease efficacy and patient compliance. In recent years, scientists have decided to focus on controlled drug delivery systems to reduce toxicity and to improve therapeutic efficacy [16]. Structural-modification amenability of hydrogels makes them in different shapes and sizes. This attribute is especially important for drug delivery applications with the goal of designing the hydrogels according to the target sites to which the drugs are delivered. Based on the route of drug administration, the hydrogel-based dosage forms may have various designs and shapes [34]. Hydrogels were studied extensively as the carrier for drug delivery systems. They have low interfacial tension; therefore, they have a limited propensity to adsorb fluid body proteins. In addition, molecules of various sizes are able to enter and exit hydrogels (entering is termed as drug loading and existing is called drug release), making them suitable for dry or swollen polymeric structure as drug delivery systems for nasal, oral, ocular, buccal, parenteral, epidermal administration routes [16].

Ocular Delivery: The eye has protecting mechanisms, such as tear drainage, blinking capacity, and low corneal permeability. These are several physiological barriers that prevent the successful delivery of drugs to the eye [16]. Conventional eye drops have issues with sustained drug delivery, such as there is a significant loss of medication immediately due to eye drainage [34].

It has been reported that nasolacrimal drainage losses 75 percent of the ophthalmic solution, and the bioavailability of the ocular drug is low [44]. Scientists were inspired to come up with such a system that delivers the medication in the eye for a longer period of time [16]. Dextenza is the very latest formulation of ocular therapeutic hydrogel approved by the FDA (3 December 2018) for human use. This is used for ophthalmic surgery and is the first intracanalicular implant developed by Ocular Therapeutix company for drug delivery [34].

Application of Hydrogel in Wound Dressing: The searchable database of the FDA (1979 to the present) shows that more than 510,000 applications were granted for the use of hydrogels in the treatment of wounds than for any other application of medical devices [20]. There is a lot of literature

available that depicts the use of hydrogels in wound healing [44]. A wound is a skin injury or cut that may be due to trauma or medical/physiological problems. Wounds can be categorized, based on the number of skin layers and on the region of the skin affected, as superficial (if only the epidermis is engaged), partial-thickness (if the epidermis and deeper dermal layers are damaged), and full-thickness wounds (when subcutaneous fat and deeper tissue has been harmed) [39]. Hydrogels are generally used as wound dressing components because they are flexible, durable, non-antigenic, and permeable to water vapor and metabolites, while covering the wound securely to prevent bacterial infection [45].

A perfect wound dressing should maintain a moist environment at the wound interface, allow the exchange of gases, create barriers for micro-organisms, and eliminate extreme exudates. It should also be manufactured from a biomaterial readily available which is non-toxic and non-allergic, unattached and easily removed without damage, needs minimal processing, has anti-microbial features, and speeds up wound healing. Hydrogels are useful products for dressing wounds and treating severe burns [16]. The use of hydrogel dressing in such applications provides many benefits, such as they are permeable to oxygen to prevent necrosis of remaining and freshly developing tissue. Capable of maintaining moisture at the injury site to facilitate healing. Compatible with significant wound healing therapeutic agents, including antimicrobials, steroids, and growth factors. Capable of stimulating cellular response and infiltration with minimal response from foreign bodies and subsequent scarring [20]. Hydrogels are used in dry necrotic injuries, non- exudate, non-infected injuries, superficial injuries [16].

CONCLUSION AND FUTURE ASPECTS: Hydrogels are essentially bio-compatible because of their hydrophilicity and internal similarity to our own anatomical structure or ECM. They are highly customizable as 3D networks, with a very broad selection of available constituents, techniques for synthesis, and methodologies for manufacturing. For these purposes, the use of hydrogels was significant in many biological and clinical applications, including drug delivery and tissue engineering. Hydrogels play a significant role in the biomedical and nanotechnological fields. The future success of hydrogels is premised on synthesizing new polymers or modifying natural polymers to overcome certain biological and medical problems. Many scientific researches demonstrate that hydrogel studies have a promising future. In the investigation of these biomaterials, new strategies for hydrogel design are enhanced. Quick response, self-assembly, strong and good mechanical properties, and super porous hydrogel are just a few examples of biomaterials with a bright future.

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