ACS OMEGA

http://pubs.acs.org/journal/acsodf

Review

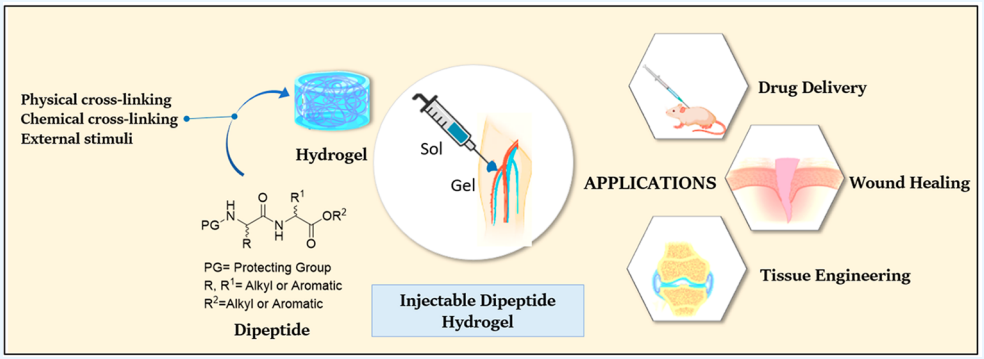
Emerging Role of Injectable Dipeptide Hydrogels in Biomedical Applications

Neeraj Kulkarni, Prajakta Rao, Govinda Shivaji Jadhav, Bhakti Kulkarni, Nagaraju Kanakavalli, Shivani Kirad, Sujit Salunke, Vrushali Tanpure, and Bichismita Sahu*

Cite This: *ACS Omega* 2023, 8, 3551−3570

Read Online

ACCESS | Metrics & More | Article Recommendations



ABSTRACT: Owing to their properties such as biocompatibility, tunable mechanical properties, permeability toward oxygen, nutrients, and the ability to hold a significant amount of water, hydrogels have wide applications in biomedical research. They have been engaged in drug delivery systems, 3D cell culture, imaging, and extracellular matrix (ECM) mimetics. Injectable hydrogels represent a major subset of hydrogels possessing advantages of site-specific conformation with minimal invasive techniques. It preserves the inherent properties of drug/biomolecules and is devoid of any side effects associated with surgery. Various polymeric materials utilized in developing injectable hydrogels are associated with the limitations of toxicity, immunogenicity, tedious manufacturing processes, and lack of easy synthetic tunability. Peptides are an important class of biomaterials that have interesting properties such as biocompatibility, stimuli responsiveness, shear thinning, self-healing, and biosignaling. They lack immunogenicity and toxicity. Therefore, numerous peptide-based injectable hydrogels have been explored in the past, and a few of them have reached the market. In recent years, minimalistic dipeptides have shown their ability to form stable hydrogels through cooperative noncovalent interactions. In addition to inherent properties of lengthy peptide-based injectable hydrogels, dipeptides have the unique advantages of low production cost, high synthetic accessibility, and higher stability. Given the instances of expanding significance of injectable peptide hydrogels in biomedical research and an emerging recent trend of dipeptide-based injectable hydrogels, a timely review on dipeptide-based injectable hydrogels shall highlight various aspects of this interesting class of biomaterials. This concise review that focuses on the dipeptide injectable hydrogel may stimulate the current trends of research on this class of biomaterial to translate its significance as interesting products for biomedical applications.

1. INTRODUCTION

Hydrogels are hydrophilic cross-linked polymeric three-dimensional networks that can hold a large amount of water and biological fluids.[1] They have diverse applications in biomedical research as drug delivery systems, diagnostics, tissue engineering, wound healing, optics, imaging, infectious disease control, and bacterial management.[2−5] However, challenges associated with these hydrogel-based scaffolds include tedious surgical processes and post operative complications. Hydrogels with shear thinning and self-healing properties that can be injected directly in the gel form at the site of action are even more attractive due to their minimal invasiveness. Various polymeric matrices form an injectable hydrogel through appropriate cross-linking. However, quite few of them trigger adverse reactions associated with inflammation and immunogenicity. Moreover, removal of unwanted side products during the cross-linking process is quite cumbersome.[6−8] Therefore, biocompatibility

Received: August 30, 2022
Accepted: December 30, 2022
Published: January 20, 2023

ACS Publications

© 2023 The Authors. Published by American Chemical Society

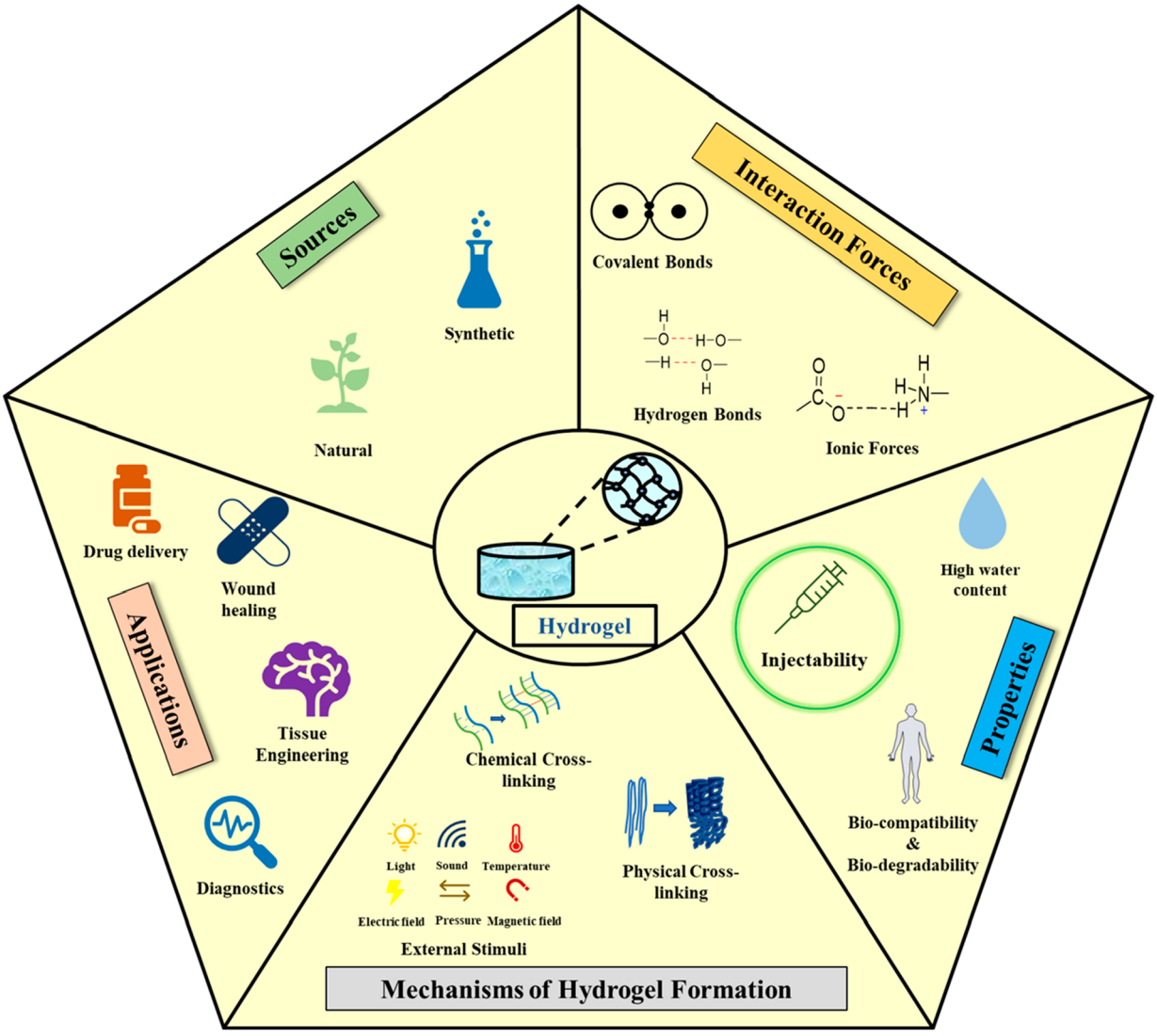


Figure 1. Illustration of interaction forces, properties, sources, applications, and mechanism of the formation of hydrogels.

and nontoxicity are considered to be utmost important criteria for injectable hydrogels.

Peptides are ubiquitously present in biological systems and play an important role in various biological signaling processes.[9,10] Peptides are next generation biomaterials with interesting amalgamation of requisite properties such as biocompatibility, nontoxicity, biodegradability, tunable physicochemical properties, and stimuli-responsiveness. Hydrogels prepared from peptides have high water content, microporous structure, cyto-compatibility, injectability, and tissue-like elasticity. Therefore, peptide-based hydrogels are being explored as scaffolds for 3D cell culture media,[11] sustained drug release,[12] wound healing,[13] and tissue engineering.[14] Various peptide-based hydrogels are now commercially available as products including Puramatrix, HydroMatrix, Biogelx, and, more recently, PGD-HydroGels (PeptiGelDesign).[15,16] The challenges associated with these peptide-based hydrogels are cost of production and stiff synthetic versatility.

Dipeptides are minimalistic peptides that can also form hydrogels through molecular self-assembly.[17] Dipeptide-based hydrogels have been investigated as important tools in drug delivery, tissue regeneration, 3D cell culture, and imaging.[18−20] Dipeptide hydrogelators result from the molecular preorganizations and noncovalent interactions between peptide strands.[17] Therefore, this new class of peptides is promiscuous for imparting shear thinning and self-healing properties in response to external stimuli. Low cost and easy synthetic tunability to control the inherent properties of hydrogels make them more desirable for practical applications.[21] Aligning the growing research interest in peptide-based injectable hydrogels and numerous reports on injectable dipeptide hydrogels, this review may help biomedical researchers obtain more insights on the importance and applications of dipeptide injectable hydrogels.

2. PEPTIDE HYDROGELS AND THEIR APPLICATIONS IN BIOMEDICAL RESEARCH

Peptide-based hydrogels are emerging as next generation biomaterials owing to their unique cytocompatibility and biosignaling. Peptide hydrogels have various biomedical applications including drug delivery, tissue engineering, biosensing, wound healing, etc.[22] A three-dimensional network of hydrogels is achieved through chemical, physical, and enzymatic cross-linking or through molecular self-assembly, which involves cooperative noncovalent interactions such as

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

electrostatic interactions,[23] hydrogen bonds,[24] $\pi-\pi$ stacking,[25] and hydrophobic interactions[23] of peptides to form hydrogels commonly known as self-assembled peptides (SAPs).

Most of the peptide-based hydrogels are formed via molecular self-assembly constructed on secondary structural motifs such as β-hairpins, α-helically coiled coils, α-helices, and β-sheets.[26,27] The self-assembly process can be programmed at the molecular level and achieved via a bottom-up approach.[28] The secondary structure of the peptide is determined by its primary sequences; therefore, over the decades, the in-depth understanding of the molecular forces involved in supramolecular interactions of the 20 naturally occurring amino acids even led to the design of low molecular weight hydrogelators of two amino acids.[29] Although reversibility and lack of potentially harmful chemical reactions is advantageous, physically cross-linked hydrogels are susceptible to change in pH or ion concentration and the effect of bodily fluids which hampers mechanical as well as physiological in vivo stability of the hydrogel.[30] Green and colleagues designed amphiphilic and anionic β-sheet peptide Pro-Asp-$(Phe\text{-}Asp)_5$-Pro having the ability to self-assemble into a hydrogel that can induce calcium−phosphate biomineralization and thus can be used in bone tissue regeneration. They exemplified that integrin-binding peptide RGD and other cell-binding motifs when combined with β-sheet peptide Pro-Asp-$(Phe\text{-}Asp)_5$-Pro seem to enhance cell signaling and have applications in osteoblast cell culture.[27]

Enhanced mechanical properties and stiffness, which are comparatively less in physically cross-linked hydrogels, can be achieved through chemical cross-linking either intramolecularly or intermolecularly with different polymers. Chemical cross-linking gives the benefit of accessibility to varied chemical cross-linking reagents which have diversified functionalities, size, and reactivity.[31] This type of cross-linked hydrogel offers better stability and mechanical strength and reduced degradation time in comparison to that of physically cross-linked hydrogels.[32] Enhanced rheological properties of hydrogels are achieved through short peptide−chromophore−peptide triblock peptides through PEG-based guest molecules.[33] The natural product-based molecule genipin was used as a cross-linker in Fmoc tripeptide hydrogelators.[34] Most widely used cross-linkers include chemical reactions such as Michael addition reaction[35] and click chemistry.[36] Click reactions have applications in the preparation of the hydrogels which are used for synthesizing DNA ligands. Here, irreversible formation of DNA G-quadruplex-selective 1,4-triazole ligands was shown with utilization of an in situ click chemistry reaction.[37,38] In order to enhance the mechanical stability, Wang and co-workers developed a post-self-assembly cross-linking method by modifying the hydrogelators with an acrylate group (NapFFK-acrylic acid) for the formation of hydrogels through chemical cross-linking.[39]

Peptides can also form a hydrogel via enzyme-triggered cross-linking in physiological conditions.[30] Various enzymes derived from plant and animal sources, horseradish peroxidase (HRP), glucose oxidase (Gox), and laccase, are examples of sources. For example, thermolysin facilitates the formation of peptide bonds through reverse hydrolysis. A series of Fmoc-protected dipeptide amphiphiles that were responsive to alkaline phosphatase were prepared and tested for their antimicrobial properties.[40]

Taking advantage of the fact that peptides can undergo structural changes in the presence of external stimuli, responsive hydrogels have been developed for specific applications such as advanced drug delivery and tissue engineering.[41] The external stimuli may include pH, redox environment, light, heat, and enzymes. Peptides containing acidic or basic amino acids undergo protonation or deprotonation when exposed to a switch in pH. This pH trigger provides a control over the self-assembling mechanism of the peptides and has been utilized in the generation of peptide-based materials.[42] One such example is the peptide-functionalized nanoparticle designed by Stevens et al. containing an acidic leucine zipper. The leucine zipper includes the repeated sequence of SGDLENEVAQL-EREVRSLEDEAAELEQKVSRLKNEIEDLKAE which is used in biosensing as it is pH sensitive. In the pH range of 7−8.5, the α-helix coiled structure is converted to a random coil.[43] Another study conducted in solution and on surfaces by Minelli and colleagues involved investigation of the conformational and self-assembly capabilities of de novo peptides generated from the structure of the yeast transcriptional activator GCN4. They demonstrated how peptide shape and assembly were controlled by the pH and polarity of the solvent.[44]

A self-assembling peptide sequence, Ac-$C(FKFE)_2CG\text{-}NH_2$ was cyclized by Bowerman et al. through disulfide bond formation between cysteine residues. Reduction of the disulfide bond provides conformational flexibility to the peptide, allowing it to self-assemble into a viscoelastic hydrogel.[45] Photo-responsive peptide systems are obtained by the introduction of a photochromic group leading to photomechanical changes on exposure to light. Photoregulated azobenzene cross-linked peptide EACAREAAAREAACRQ, which is used for probing protein function, is a light-sensitive peptide. Time-resolved IR spectroscopy was used to detect phototriggered helix formation in a 16-residue peptide with a built-in conformational photo-switch.[46] Some peptides have shown sensitivity toward heat, ultimately causing a sol−gel transformation. Pochan et al. designed a tiny de novo peptide, MAX3, that self-assembles with thermoreversibility into a hydrogel network. Importantly, the peptide has to first fold into a self-assembly friendly conformation before it can be hydrogelated. MAX3 unfolds at room temperature, resulting in a low viscosity aqueous solution. The peptide undergoes a unimolecular folding event as the temperature rises, resulting in an amphiphilic β-hairpin self-assembling into a hydrogel network.[47]

2.1. Peptide Hydrogel for Drug Delivery. Drug delivery is a prime approach accomplished for transport of the active pharmaceutical ingredient (API) or a formulation to a specific target site in order to achieve the desired therapeutic effect. Owing to poor absorption, low bioavailability, fluctuations in plasma concentration, frequent dosing, high first pass metabolism, lack of specificity, side effects, and toxicity, conventional drug delivery systems are frequently being replaced by newer drug delivery systems (DDSs) in the form of liposomes, niosomes, nanoparticles, implants, and hydrogels.[48] Biocompatible, biodegradable, stimuli-responsive, less toxic hydrogel-based drug delivery systems, aided to control rate of drug release, improve the efficiency, allow in situ gelation, and minimize side effects.[49]

Short peptide-based hydrogels with improved pharmacokinetic parameters and reduced side effects and toxicity have been developed for drug delivery.[50] Based on the self-assembling property of peptides, Li and co-workers fabricated a peptide supramolecular hydrogel (Nap-GFFY) for ocular drug delivery of diclofenac sodium. The formulation showed rapid release of diclofenac sodium over a period of 24 h, resulting in better corneal penetration, improved ophthalmic bioavailability, and

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

reduced frequency of drug administration. Nap-GFFY hydrogel was found to be nontoxic with no sign of eye irritation after a single instillation.[51] Liu and associates designed a self-assembling peptide (SAP)/heparin (SAP/Hep) hydrogel to codeliver TNF-α neutralizing antibody (anti-TNF-α) and hepatocyte growth factor (HGF) for treatment of ischemia−reperfusion (I/R)-induced organ injury. The SAP−drug hydrogel showed controlled and sequential drug release of the two drugs (anti-TNF-α/HGF) to gain anti-inflammatory and pro-proliferative effects in a single injection. It promoted cell proliferation and differentiation and is thus found to be a promising drug delivery platform for tissue repair in I/R-induced organ injury.[52] Liu and colleagues produced a SAP-based supramolecular hydrogel consisting of two drugs, viz. chlorambucil (CRB) and a peptide drug, YSV. The peptide−drug conjugate CRB-FFE-YSV monomer has the ability to form a hydrogel with a heating and cooling process. The resultant hydrogel has the ability to release the two drugs simultaneously along with improved drug stability, cellular uptake, and enhanced antitumor activity in vivo and in vitro.[53]

2.2. Peptide Hydrogel for Tissue Engineering. In many different approaches to tissue engineering, hydrogels made from natural polymers have been very successful and promising. However, the limitations associated with the hydrogels made from natural polymers lead to explorations of alternative biomaterials such as peptides and polypeptides which can mimic the extracellular enviornment.[54]

Peptides can form different well-ordered structures such as nanofibers, nanomicelles, and nanotubes through the interactions among the backbone, side chain, and the terminus (C and N). The hierarchal architecture is largely dependent on the secondary structure of the peptides. For example, Woolfson's team created a variety of α-helical coiled-coil systems, and they form "hydrogelating self-assembling fibers" (hSAFs) made up of two helical peptides (SAF-p1 and SAF-p2) that congregated into an offset helical heterodimer with complementary "sticky ends". hSAFs, similar to Matrigel, supported both growth and differentiation of rat adrenal pheochromocytoma cells for sustained periods in culture.[55] RADA16-I has been made commercially available as a product under the name PuraMatrix because of its exceptional biocompatibility for fostering the creation of new cells and the regeneration of damaged tissue in the case of nerve regeneration and spinal cord regeneration[56,57]

2.3. Peptide Hydrogel for Wound Healing. The process of wound healing may be broken down into four separate stages, all of which are deeply connected with one another and oftentimes overlap with one another. These phases include tissue remodeling and resolution, as well as hemostasis, inflammation, and proliferation.[58] Peptide-based hydrogel dressings give the benefit of absorbing wound exudates, which in turn stimulates fibroblast proliferation, keratinocyte migration, and eventually the re-epithelialization of the wound.[59]

Wu and co-workers prepared hydrogels composed of polyester amide (PEA). A peptide with the sequence of RRRFRGDK (P3) was attached to the hydrogel surface in order to increase the hemorrhage control and antibacterial capabilities of the material. Both the hydrogel based on PEA and the peptide-functionalized hydrogel (Gel-*g*-P3) have multiple functions, including the ability to absorb water, a high level of mechanical strength, enzymatic biodegradability, and good cytocompatibility and hemocompatibility. In the end, PEA-based hydrogels as well as Gel-*g*-P3 hydrogel were used to treat an infected full-thickness wound as well as a place where bleeding had occurred. The Gel-*g*-P3 hydrogel showed superior effectiveness in terms of both preventing bleeding and promoting wound healing. All of the data showed that the peptide-functionalized PEA-based hydrogel would be good candidates for controlling bleeding and mending wounds.[60] In a study performed by Deng and co-workers, it was shown that recombinant human collagen (RHC) that has been coupled with chitosan produces hydrogels with better rigidity. The analysis of the differences between hydrogels made with the inclusion of moderate levels of RHC was related to better gelation behaviors, mechanical strength, and stability, according to varied RHC ratios. Further research conducted in vitro demonstrated that these hydrogels were compatible with living organisms. According to the findings of the in vivo burn model, hydrogels with a higher level of RHC conjugation demonstrated increased cell infiltration, generated more vessel formation, and, as a result, sped up the process of wound healing. In conclusion, RHC−chitosan hydrogels have shown promise for application in the treatment of burn injuries.[61]

2.4. Peptide Hydrogel for 3D Cell Culture. Overcoming the limitations of 2D cell culture, 3D cell culture holds superiority as it not only favors cell−cell interactions and cell−extracellular matrix interactions but also preserves the cellular morphology and phenotype, thereby mimicking the in vivo conditions. Growing importance of 3D cell culture in basic biological sciences led to the development of cell culture matrices.[62] According to Nguyen and West, the hydrogel as a scaffold for cell culture provides a network of cross-linked polymer with high water content, allowing transport of oxygen, nutrients, and growth factors, simulating the in vivo extracellular matrix.[63]

A conventional hydrogel-based scaffold as a culture medium lacks a signaling mechanism and good mechanical properties, but peptide-based hydrogels consisting of short peptide sequences resembling ECM mimetic proteins can fine-tune mechanical properties of hydrogels and promote cell signaling.[64] Nie et al. synthesized collagen peptide-grafted sodium alginate (SA-COP) hydrogel as a medium for cell culture. They found that addition of the collagen peptide to sodium alginate improved the biological properties of the hydrogel. The SA-COP hydrogel showed good hydrogen peroxide scavenging activity, increased cell viability, and promoted cell growth.[65] Käpylä and associates developed photodegradable, poly(ethylene glycol) diacrylate-based hydrogels functionalized with a cell-adhesive RGD peptide as dynamically tunable, shape-changing scaffolds for culturing cells.[66] Tomasini and colleagues demonstrated that pseudopeptides containing the d-Oxd or the d-pGlu [Oxd = (4*R*,5*S*)-4-methyl-5-carboxyl-oxazolidin-2-one, pGlu, pyroglutamic acid] moiety and selected amino acids in the presence of calcium chloride have the ability to induce the formation of insoluble salts that get organized into fibers leading to hydrogels at physiological pH. This hydrogel has a thixotropic property and is injectable, which makes it suitable for cell culture and tissue regeneration.[67]

3. INJECTABLE HYDROGELS

A hydrogel's versatility originates from its adaptable structure, which has been enabled by substantial advances in materials engineering, polymer science, and chemistry.[68] These material systems are flowable aqueous solutions before administration, but once injected, they immediately solidify under physiological circumstances. These characteristic physiochemical properties of the injectable hydrogel are governed by factors such as

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

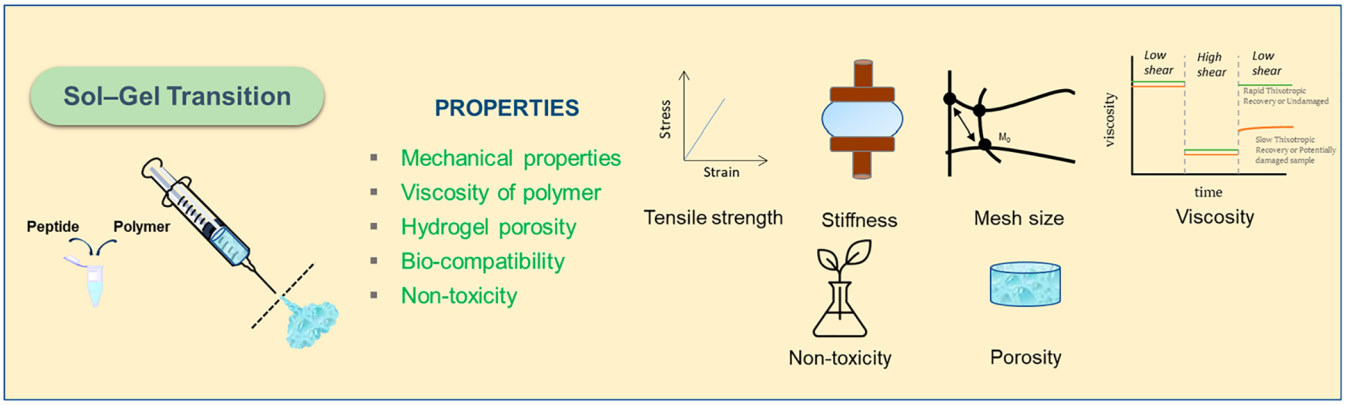


Figure 2. Preparation and properties of injectable hydrogels.

viscoelasticity. When a hydrogel comes in contact with any tissue, it experiences certain strain. Therefore, the hydrogel must show flexibility which can be determined by its viscoelasticity. The viscosity of the polymer regulates the syringeability of the hydrogel for its minimal invasiveness during surgical procedures.[69] Another factor is hydrogel porosity where interconnected networks are preferred for better assistance of nutrient movement to the tissues, attachment, proliferation, and attachment to the surrounding tissue. For the hydrogel to withstand the dynamic environment of the body, proper mechanical properties such as tensile strength, stiffness, mesh size, density, etc. are required.[70] The tensile strength can be assessed by compression and tension analysis using rheometry and dynamic mechanical analysis. Mesh size is the linear distance between two adjoining cross-links, and it influences the diffusion of nutrients along the matrix. Increase in cross-linking increases stiffness but reduces the elasticity of the hydrogel and increases the brittleness. Proper assessment of these properties is a requisite for the proper physiological functioning with respect to the biomedical applications (Figure 2).[71]

Injectable hydrogels can be used as biological transporters and injected into a lesion location after simply mixing with diverse biological products. This allows for the preservation of biological product characteristics and long-term drug release, as well as a decrease in early burst release and unwanted pharmaceutical effects.[72] Thermosensitive hydrogels are particularly intriguing as specific injectable biomaterials since they form gels spontaneously when exposed to body temperature and do not require extra chemical treatment. The demand for regulated medication or cell delivery is growing in tandem with the fast advancement of regenerative medicine. Biodegradable and injectable synthetic hydrogels provide an alternative to standard medication carriers and tissue engineering scaffolds.[73]

An upcoming advancement in injectable hydrogels is the Atrigel technology which releases the therapeutics in a controlled manner from days to a few months. This technology needs a single injection and consists of biodegradable polymers that are dissolved in biocompatible carriers. After the injection of a liquid polymer system into the body using normal needles and syringes, these polymers solidify upon contact with body fluid and produce a solid implant/depot.[74] For the first time, the Atrigel system was developed by Dunn and co-workers at Southern Research Institute in Birmingham, Alabama, in 1987, and the first license of the technology was given to Vipont Research Laboratories (which later became Atrix Laboratories) for the subgingival delivery of antimicrobials to treat periodontal disease.[75] The Atrigel drug delivery system is a patented drug delivery technology that can be utilized for the administration of drug parenterally as well as in the localized tissue. The technology has protection of 33 patents in the United States and 35 patents in the rest of the world. The patents cover from the basic technology in the Atrigel system to the advanced process improvements.[76] The Atrigel system offers biocompatibility and compatibility with wide range of compounds (peptides, proteins, vaccines and natural products) and offers advantages of sustained release. Direct delivery of the depot to the targeted area causes the minimum systemic side effects and helps to achieve higher drug concentration at the desired site of action.[77] For example, Eligard employed Atrigel as a drug carrier for the management of advanced prostate cancer; it contained an LHRH agonist leuprolide acetate and PLGA 75/25 dissolved in *N*-methyl-2-pyrrolidone (NMP).[78,79]

3.1. Peptide-Based Injectable Hydrogel. Commonly used polymers for injectable hydrogels fall into two broad categories: natural polymers (e.g., alginate, chitosan, collagen, etc.) and synthetic polymers (e.g., PEG, PVA, PLGA, PEO, etc.). However, the use of natural polymers is restricted due to the risk of unknown toxicity and lack of control of mechanical properties.[80] Synthetic polymers also possess disadvantages in certain applications as they lack cell adhesion, which is an important criterion in tissue engineering. Hence, bio-inspired peptide-based injectable hydrogels are rising in the current research scenario due their unique properties such as specialized biological activity, adjustable spatial organization biocompatibility, biodegradability, and ease of modification.[81] Unlike animal-derived matrices and synthetic polymer-based hydrogels, peptide-based hydrogels are more suitable for bioapplication.[82] These hydrogels have three-dimensional networks that can encapsulate large biologics and release them in a stimuli-responsive and drug-controlled manner. Peptides have a role in the circulation and metabolism of active chemicals, as well as biological identification, and can control physiological activities in vivo,[83] antigen–antibody reactions,[33] and signal transduction.[84] Furthermore, peptide-based hydrogels may be easily adjusted and functionalized by modifying the side chains and backbones to meet the needs of individual applications.[85] As a result, peptide-based injectable hydrogels have a lot of potential in biomedical applications such as tissue engineering,[86] drug/gene delivery,[87] antibacterial agents,[88] and wound healing.[89] A number of peptide sequences possessing the ability to form injectable hydrogels have been reported over the years.

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

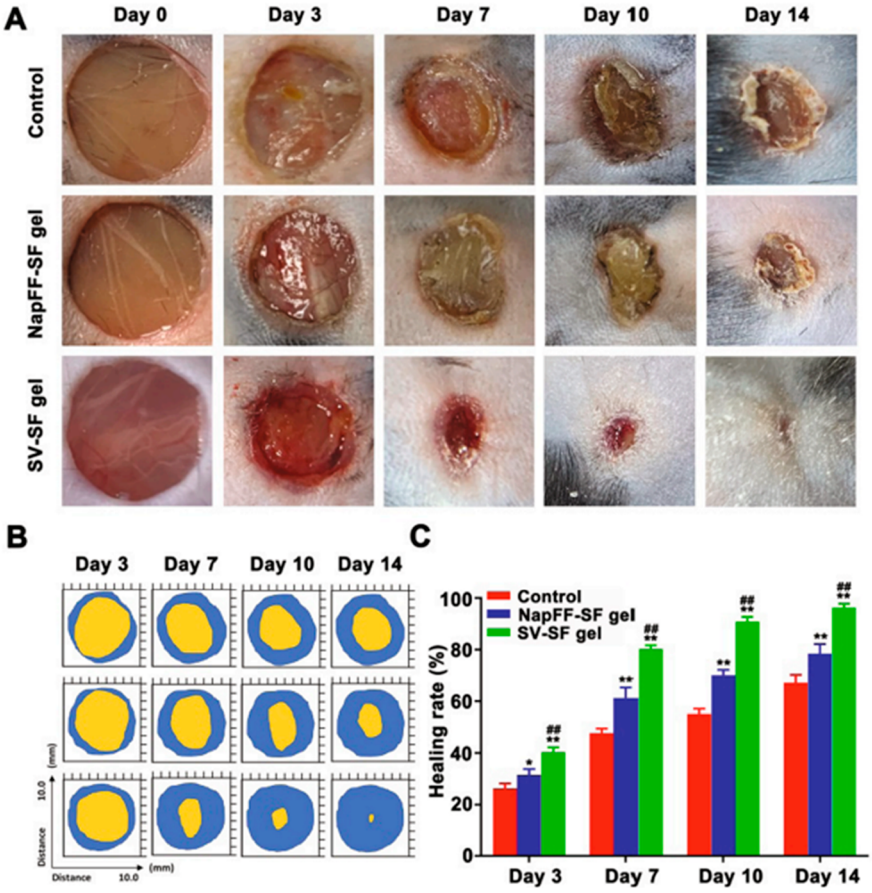


Figure 3. (A) Photographic images of wounds on the dorsal skin of mice treated by the SV-SF and NapFF SF hydrogels on days 0, 3, 7, 10, and 14. (B) Traces of wound boundaries on the dorsal skin of mice treated by the SV-SF and NapFF-SF hydrogels on days 0, 3, 7, 10, and 14. The blue area indicates the initial wound area (day 0), and the yellow area signifies the wound area on days 3, 7, 10, and 14. (C) Quantitative data about wound healing rates in different groups on days 3, 7, 10, and 14 (*P < 0.05, **P < 0.01, vs control group; ##P < 0.01, vs NapFF-SF group). Reprinted with permission from ref 97. Copyright 2021 Elsevier.

3.1.1. Peptide-Based Injectable Hydrogels for Drug Delivery. Injectable hydrogels are often produced by a rapid sol−gel phase shift or through chemical polymerization in situ. They may be injected directly into the target areas, allowing the targeted drug delivery. mPEG-*b*-PELG was documented by Wu's research group to form an in situ thermosensitive hydrogel that codelivered cisplatin and interleukin-15, ultimately increasing the overall efficacy in treating melanoma.[90] KRGDKK peptide containing the RGD sequence was coupled to PCLA-PEG-PCLA to yield a thermosensitive hydrogel in a study conducted by Xun et al. The hydrogel demonstrated potential for use as a drug delivery vehicle to provide a controlled or sustained release without high initial burst release.[91]

Chen et al. developed a new, improved, self-healing elastin mimic peptide hydrogel (EMH) for the purpose of local distribution of salvianolic acid B. The capacity of EMH to mend itself is improved by the incorporation of SaB into polydopamine nanoparticles (SaB-PDA). Because of its low viscosity and high biocompatibility, the prehydrogel (SaB-PDA/pre-EMH) in vitro was shown to be an ideal candidate for intramyocardial injection.[92] Li and colleagues reported the design, screening, and assessment of an injectable peptide hydrogel designed to act as a local losartan depot with the intention of inhibiting fibroblasts associated with cancer and potentiating chemotherapy. They synthesized a set of peptide derivatives and discovered that C16-GNNQQNYKDOH (C_{16}-N) was superior to the others in hydrogel formation and drug encapsulation. This was due to its flexible hydrocarbon tail and interpeptide hydrogen bonding, both of which allowed supramolecular self-assembly into long filaments with hydrophobic cores. Losartan was released in a controlled manner from a hydrogel that was formed by the coassembly of C16-N and losartan.[93] Schneider employed an orthogonally self-assembled, injectable peptide hydrogel that was designed to sequentially deliver erlotinib and doxorubicin after being implanted by syringe into a brain tumor. They used an amphiphilic β-hairpin peptide designed de novo that can self-assemble in the presence of doxorubicin-loaded liposomes and free erlotinib to form rigid sequogels under physiological conditions. This helps the coadministration of erlotinib and doxorubicin in a time-staggered manner that sensitizes glioblastoma cells to apoptosis.[94] Hartgerink and team designed "STINGel", a peptide-based hydrogel for controlled release delivery of cyclic dinucleotides (CDNs). CDNs are immunotherapeutic agents used in cancer treatment. They used a multidomain peptide (MDP) hydrogel with the sequence K2(SL)6K2. Being positively charged, the hydrogel favored self-assembly to form a nanofiber matrix which can be delivered via a syringe. Improved survival rate was observed in head and neck cancer.[95]

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

3.1.2. Peptide-Based Injectable Hydrogels for Tissue Engineering. Due to their desirable properties as aqueous environments that can protect cells and drugs from inactivation, promote nutritional transport to cells, be modified with cell adhesion ligands, be injected in vivo, and have excellent biocompatibility, self-assembled peptide hydrogels have been investigated as potential scaffolds for tissue engineering.[96] Wang and co-workers fabricated injectable hydrogels consisting of silk fibroin (SF) and a peptide (NapFFSVVYGLR) for tissue engineering. The cooperative assembly formed from silk fibroin and NapFFSVVYGLR, as well as the bioactive functional role of the SVVYGLR epitope from the osteopontin protein, results in a formation of bioactive hydrogel with good gel stabilities and strong capabilities to promote endothelial cell adhesion, growth, and migration. When a SV-SF hydrogel is implanted into the affected part of mouse skin, it can increase vascularization and epimerization for epidermal repair by increasing collagen deposition and angiogenesis-related genes/proteins. These data imply that the bioactive hydrogel can be used for vascular regeneration and wound repair[97] (Figure 3). Mehrban et al. developed a hydrogel which was derived from decellularized extracellular matrix. They investigated whether murine macrophages are activated in vitro by hydrogels and whether these gels create an M1-like or M2-like phenotype. This hydrogel was injected into rats having abdominal wall lesions of varying thicknesses. Over the course of 28 days, there was a steady rise in mononuclear cell infiltration without any signs of hydrogel encapsulation or the production of multinucleate large cells. All hydrogels also produce an environment that is consistently anti-inflammatory. After 28 days, there is no visible distinction between the injected site and healthy tissue, indicating complete integration and promising future application as part of regenerative therapy methods for unmet clinical requirements.[98]

The KYFIL pentapeptide reported by Tang et al. could form robust injectable hydrogels and was studied for the encapsulation of oligodendrocyte progenitor cells, showcasing potential for use in tissue engineering.[86] PEG-CMP and PLG-*g*-TA/PEG are some injectable peptide-based hydrogels serving the purpose of tissue engineering scaffolds. In the case of PEG-CMP, an elevated level of cell-secreted collagens is one way in which hydrogels containing CMPs boost the bioactivity of tissue engineering scaffolds.[99] CMPs have multiple applications, including improving the interaction between the scaffold and the collagen produced by cells and regulating the elasticity of hydrogels through the formation and disruption of triple-helical physical cross-links. CMPs maintain their triple-helical structure in physically cross-linked PEG-CMP9 and chemically cross-linked PEG-CMP9-K hydrogels. PEG-CMP hydrogels have temperature-dependent elasticity due to the instability of intermolecular triple-helix cross-links at high temperatures. Modulating the triple-helix mediates cross-links in hydrogels to enhance cell growth, differentiation, and migration. The gels provided here are stiff like in vitro reconstituted collagen and fibrin gels and other cross-linked materials utilized for endothelial cell growth and directed neuron proliferation.[100]

In the case of PLG-*g*-TA/PEG, in the presence of low concentrations of HRP and H_2O_2, hydrogels of poly(L-glutamic acid) grafted with tyramine and poly(ethylene glycol) (denoted as PLG-*g*-TA/PEG) were rapidly formed. Mechanical strength, swelling ratio, and porous structure of hydrogel depended on HRP and H_2O_2 concentrations. According to the results of the live−dead staining and cell counting kit-8, the PLG-*g*-TA/PEG hydrogels had significant in vitro cytocompatibility. This hydrogel persisted. The in situ-produced hydrogels in rats' subcutaneous layer lasted 10 weeks and were biocompatible in vivo. The PLG-*g*-TA/PEG hydrogels could thus be interesting candidates for biological applications such as carriers for long-term sustained delivery of drugs and scaffolds for tissue engineering.[101] Using magnetically responsive (MR) self-assembling peptide hydrogels, Tran and colleagues presented a method to produce patternable and injectable hydrogels, where the RADA-16I peptide is utilized, and then validated the efficacy of these hydrogels to encourage and align axon infiltration at the site of a spinal cord lesion. After conducting in vitro studies, measurements of metrics such as axon development, orientation, inflammation, and glial scar formation revealed that magnetically aligned human mesenchymal stem cell-seeded (hMSC) hydrogels were superior.[102]

3.1.3. Peptide-Based Injectable Hydrogels for Wound Healing. Injectable hydrogels exhibit excellent fluidity when compared to conventional types of hydrogels. After being injected as a solution into the wound, these substances can create a gel in place, which has the ability to fill the wound in all dimensions. Because of this, they are able to reach irregular and deep wounds. Injectable hydrogels are an excellent choice for the healing of chronic wounds. They significantly lessen the requirement for invasive surgery. For example, various biopolymer-based composite hydrogels such as thermosensitive hydrogels from chitin whiskers for treatment of chronic wounds have been proven to be beneficial over conventional treatment. It helped in the decrease of gelation temperature and increased the mechanical property. The composite hydrogel was helpful in the administration of the drug linezolid.[103]

Similar to these composite hydrogels of biopolymers, various peptide-based injectable hydrogels also have a wide range of applications in the case of either chronic or acute wound healing. In relation to this, Wu et al. developed an injectable hydrogel where the peptide Ala-Glu-Lys-Ala was inserted in quaternized carboxymethyl chitosan (HTCC) which when cross-linked with Schiff's base exhibited tissue reconstruction and self-healing properties.[104] A way to generate peptide-modified nanofiber-reinforced hydrogels (NFRHs) by using the Schiff base cross-linking method has been presented by Wu and colleagues. These hydrogels were shown to have injectable and self-healing capabilities, as well as the ability to adapt to the contour of the wound, particularly irregular wounds. RRRFRADA, a novel antimicrobial peptide, was used to modify the nanofibers, and hydrogels loaded with nanofibers showed effective hemostatic capabilities as well as a powerful killing impact for bacterial biofilm.[105]

In order to treat infected wounds, Deng and colleagues developed a polypeptide−protein hydrogel with a 3D sterile microenvironment. This was accomplished through the coordinative cross-linking of thiolated BSA protein and KK polypeptide with a silver ion. The dynamic interactions between silver and silver sulfide gave this hydrogel its remarkable properties of rapid breakdown, injectability, self-healing, antibacterial activity, and angiogenic growth.[106] An injectable HMSC hydrogel based on silk fibroin peptide-grafted hydroxypropyl chitosan (HPCS-*g*-SFP) and oxidized microcrystalline cellulose (OMCC) was produced by Liu. Schiff base bonds were used in the creation of the hydrogel. In order to produce TMP-loaded HMSC hydrogel, tetramethylpyrazine (TMP) was first encapsulated inside of the HMSC hydrogel. Experiments on animals demonstrated that the TMP-loaded HMSC hydrogel enhanced rapid wound healing while

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

simultaneously reducing the formation of scar tissue. The injectable HMSC hydrogel with TMP injected into it was designed, and it has the potential to promote scarless wound healing.[107]

Apart from above-mentioned applications, injectable peptide hydrogels have also found applications in the case of bacterial infections where self-assembling antibacterial peptides (AMPs) are used as a major defense mechanism against a broad spectrum of microorganisms, especially for antibiotic-resistant bacterial infections.[108,109] AMPs that can form injectable hydrogel are very effective to manage bacterial infection through disintegration of scaffold and membrane disruption of bacteria.[110] Local administration of injectable peptide hydrogel to the infected site delivers the antibiotics at high concentration with minimal side effects.[111,112]

4. DIPEPTIDE HYDROGELS

Dipeptides being the shortest possible self-assembling peptide motif has attracted even more attention as it acts as a cost-effective alternative to peptide-based hydrogel while simplifying the process of production. Owing to low cost, feasible synthesis, low molecular weight, high biocompatibility, biodegradability and self-assembling property, dipeptide have supremacy over other peptides.[113] In 1995 Vegners et al. for the first time illustrated Fmoc-Leu-Asp, Fmoc-Ala-Asp, and Fmoc-Ile-Asp dipeptides for the formation of viscoelastic hydrogels by boiling peptide solutions in alkaline buffer at 100 °C where it has been used as carrier for antigen representation.[114] Another report of self-assembly property shown by peptides was given by Gazit. The dipeptide FF forming the core recognition unit of Aβ42 was reported to self-assemble into a hydrogel.[115]

Dipeptides can self-assemble as a result of various non-covalent interactions such as hydrogen bonding, electrostatic, van der Waals, $\pi-\pi$, and hydrophobic interactions. Hydrogen bonding plays a role in the stabilization of secondary structures such as α-helix and β-sheets. Yang et al. reported hydrogen bond facilitated self-assembly of cyclic Leu-Phe dipeptide strong hydrogel formation in physiological as well as harsh conditions such as pH (3, 5, 9, and 11), water, PBS, and trypsin. The role of hydrogen bonding in self-assembly was confirmed by Fourier transform infrared (FTIR) and computational analysis.[116] The $\pi-\pi$ interactions are due to the aromatic moieties and have a great impact on the self-assembly of the peptide. There are some reports of stable hydrogel formation due to $\pi-\pi$ interactions. Chauhan et al. reported Phe-ΔPhe and Leu-ΔPhe dipeptide-based hydrogels. The gel matrix could encapsulate and release bioactive molecules in a sustained manner and also showed no cytotoxicity on HeLa and L929 cell lines[117] Dipeptide hydrogel has wide applications in various fields. Recognizing the importance of biocompatible, conductive and transparent dipeptide hydrogels in preparation of soft electronic devices and biosensors, Yafeng Jing and his team presented a solution to overcome the difficulties associated with hydrophobic features of additives during the doping process. Hydrophilic conductive polydopamine (PDA)-doped polypyrrole (PPy) nanoparticles (denoted as PPy@PDA) were introduced into dipeptide hydrogel networks to form conductive nanofibrils in situ to achieve a good level of hydrophilic templating of the hydrogel networks. They used thermolysin as a catalyst to initiate condensation reactions between Fmoc-Y, Fmoc-L, Fmoc-T, Fmoc-S, and L-NH_2 to gain dipeptides which could self-assemble into a fibrous network and form stable hydrogels. The four conductive hydrogels possessed high transmittance across the visible spectrum and provided a complete conduction network for signaling in biosensors.[118]

Synthesis of various novel 4-biphenylacetic acid (BPAA)−dipeptide aromatic compounds was carried out by Fan and co-workers. Among that, only 4-biphenylacetic acid−diphenylalanine (BPAA-FF-OH) possessed the ability to form homogeneous and transparent hydrogels via a supramolecular self-assembling process through a temperature switch or ion induction. Temperature and ion dual-responsive BPAA-FF-OH hydrogel-supported cell adhesion, growth, and proliferation of L929 cells provided data for its use as matrix for 2D and 3D cell culture.[119] Nilsson and associates synthesized Fmoc-3F-Phe-Asp-OH and Fmoc3F-Phe-Arg-NH_2 dipeptides and found that they could undergo coassembly mediated by aromatic, hydrophobic, and Coulombic interactions to form two-component nanofibrils that elicit gelation of water. They revealed that these hydrogels could support the viability and growth of NIH 3T3 fibroblast cells due to supramolecular display of Arg and Asp at the nanofibril surface, which effectively mimics the integrin-binding RGD peptide of fibronectin, without covalent connection between the Arg and Asp functionality mapping out the importance of dipeptides in 2D and 3D cell cultures.[120]

The relationship between peptide chemical structures and their associated hydrogel characteristics is still unknown. Machine learning has been effectively applied to medical applications with accurate prediction, such as pathology picture detection.[121] However, few studies have used them in organic substance creation, and forecast accuracy is often less than 50%. Machine learning is primarily used for energy materials design; however, biomaterial engineering studies are limited. Based on peptides' two-dimensional chemical structures and self-assembly abilities, machine learning and a computational approach can predict gel formation.[122] Li and co-workers used machine learning to study how chemical factors affect peptide-like molecule hydrogel production. They used 2000 peptides to build a structurally diverse hydrogel library and evaluated each molecule. Machine learning detected self-assembly and computed the chemical properties of the materials, exposing their topological and physicochemical properties. The Ghose-Crippen LogKow (ALogP), the number of basic groups (nBase), and the number of hydrogen bond acceptors and donors (nHBAcc, nHBDon) affected hydrogel properties. Machine learning showed that quantum chemistry structure descriptors strongly correlate with gelling behavior.[123]

5. DIPEPTIDE INJECTABLE HYDROGELS

Various sequences of dipeptides with different modifications have been studied over the years for their ability to form hydrogels. These studies have revealed that dipeptides in solution when exposed to a trigger such as a change in the solvent system or a change in the pH force them to aggregate in the solution and arrange themselves into ordered structures due to noncovalent interactions which ultimately contribute to the packing of the hydrogel.[17] Some of the studies have reported the dipeptide hydrogels to exhibit thixotropic nature. Their shear thinning property enables the formed hydrogels to undergo a gel−sol−gel transition on exposure to an external stimuli such as mechanical stress. These reversible dynamics of the dipeptide hydrogels make them suitable for injectable applications in biomedical sciences.[124]

Dipeptide-based injectable hydrogels are a recent advancement and of special importance as they are low molecular weight

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

hydrogelators that are endowed with several advantages such as tunable mechanical properties, lower cost of production and synthetic feasibility. Also due to minimal invasiveness and the ability to retain the mechanical properties under the shear stress of the injection these are favorable over preformed implants.[125] Dipeptide-based injectable hydrogels have found widespread applicability in various domains of biomedicine such as field of drug delivery, tissue engineering, wound healing, and some other applications such as biosensing, bone regeneration (Figure 4).

Figure 4. Different biomedical applications of injectable hydrogels.

5.1. Applications. *5.1.1. Dipeptide Injectable Hydrogels for Drug Delivery.* The biomolecular self-assembly shown by dipeptides and the various other advantages mentioned previously make dipeptides promising candidates for drug delivery. Several studies have been carried out where dipeptides as standalone moieties have been able to serve as a carrier for a number of drugs. One such study was conducted by Govindaraju and co-workers who synthesized a series of cyclic dipeptides and studied their propensity to form organogels and hydrogels. They further carried out in situ gelation studies by dissolving the cyclic dipeptides in a biocompatible solvent such as NMP and injecting them into PBS. It was found that cyclo-(L-Glu(O-tBu)-L-Phe, **13**) exhibited an excellent gelation ability for organic and aqueous solvents at a critical gelator concentration less than 1 wt % and could form stable in situ gels when injected into PBS. It was also able to form a gel in situ when loaded with curcumin (10 wt % of curcumin). Therefore, the ability of cyclo-(L-Glu(O-tBu)-L-Phe, **13**) to form in situ gels alone and in the presence of curcumin provided evidence for its potential application in drug delivery.[126] Najafi et al. developed a dipeptide Fmoc-FF (**1**) hydrogel for the delivery of s-nitroso-*n*-acetylpenicillamine (SNAP) in the treatment of renal ischemia/reperfusion injury. This hydrogel exhibited a nanofibrous structure, shear thinning, and biocompatibility while providing a sustained release of nitric oxide over 7 days. Histopathological scores and renal function indices of the hydrogel indicated that an intralesional injection of Fmoc-FF-SNAP facilitated enhanced recovery of renal I/R injury. A reduction in the levels of biomarkers of oxidative stress and iNOS expression in comparison to the I/R control group were observed which could be attributed to the sustained release of nitric oxide. Enhancement of the eNOS expression reflected the regeneration of the injured endothelial tissue.[127]

Dipeptides consisting of amino acids have been well researched for their ability to form hydrogels, but proteolytic instability still stands as a major limitation. Therefore, Reja et al. explored backbone-modified dipeptides comprising amino acids that contribute to conformational flexibility and proteolytic stability. Boc-β(O)-δ5-Phe-β(O)-δ5-Phe (**11**) dipeptide was found to form a hydrogel in PBS (pH 7.4) at a concentration of 10 mg/mL, which could undergo a thixotropic transition as observed in the rheological experiments. The proteolytically stable and injectable hydrogel was further studied as a drug delivery vehicle for release of proflavine where 35% of drug was released in PBS over 18 h as monitored by UV−vis spectroscopy.[128] In another study, Chauhan et al. constructed a mechanically stable hydrogel by the self-assembly of a conformationally restricted ultrashort peptide (Leu-α, -dehydroPhe, **12**) under mild physiological aqueous conditions. Syringeability and time-dependent step-strain rheological experiments on 1 wt % dipeptide gel demonstrated its thixotropic behavior. Efficient entrapment of various hydrophilic and hydrophobic drugs along with a continuous and slow release from the dipeptide gel highlighted the potential application of the dipeptide gel as a drug delivery vehicle. Treatment of mice xenograft tumor models with mitoxantrone entrapped in dipeptide gel exhibited a decrease in the tumor growth and tumor volume, thereby indicating the use of the dipeptide gel for in vivo drug delivery.[129] Fmoc-Ala-Val and Fmoc-Ala-Phe are some more dipeptides reported by Banerjee et al. to form stable hydrogels at physiological pH. These hydrogels could provide a sustained release of vitamin B2 and B12 over the duration of 2 days.[130]

Evidence suggests that dipeptides when used along with another peptide or a nonpeptide as a composite or hybrid injectable hydrogel have also proven to be excellent carriers for drug delivery. Xuehai Yan and co-workers used bola-type amphiphiles (source: archaebacteria) and synthesized bola-dipeptide hydrogels in aqueous solutions (Di-FF or DgvFF). Hydrogels are demonstrated to be efficient carriers for hydrophilic molecules which can be injected for targeted delivery. Additionally, they confirmed that these hydrogels loaded with a photodynamic prodrug (5-aminolevulinic acid hydrochloride, 5-ALA) were suitable to be used as injectables for targeted delivery, sustained release, and in situ conversion of prodrug, leading to enhanced photodynamic antitumor therapy.[131] Another example includes a chitosan−dipeptide hydrogel consisting of self-assembling Fmoc-FF (**1**) that interacts electrostatically with glycol chitosan. The hybrid hydrogel possessing thixotropic nature was evaluated as a drug delivery carrier for doxorubicin whereby incorporation of doxorubicin increased the stability of the complex hydrogel. An in vitro release study of DOX-loaded hybrid hydrogels showed a gradual release of doxorubicin for 4 days followed by a constant release indicating a sustained release of the drug. Cytotoxicity tests performed on blank hydrogels and DOX-loaded hydrogels clearly indicated that the hydrogel in itself is nontoxic to A549 human lung cancer cells, while the latter diminished the cell viability significantly. Therefore, the chitosan−dipeptide hybrid hydrogel bears potential for use as

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

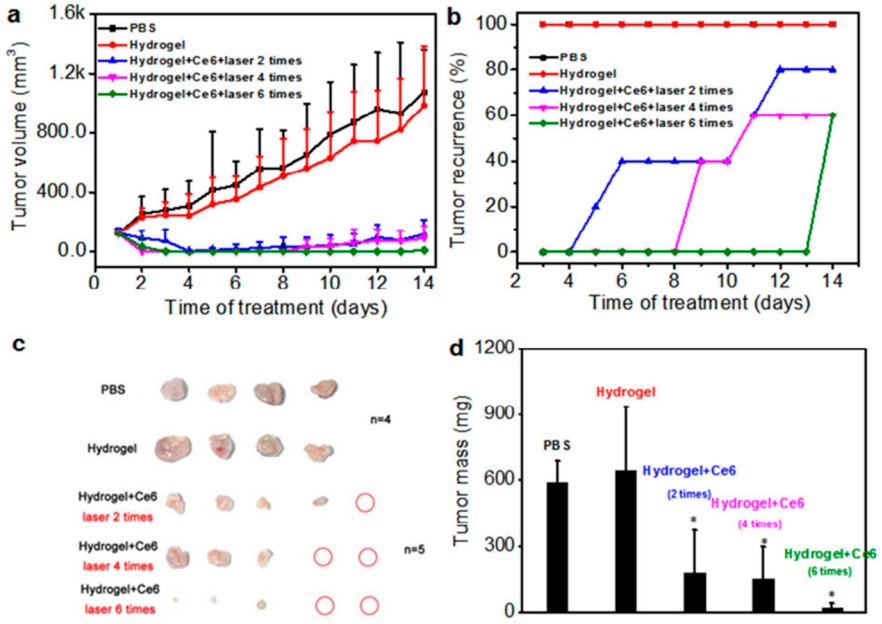


Figure 5. In vivo antitumor therapy. (a) Inhibition curves of tumors in different groups within 2 weeks period. (b) Tumor recurrence curves of the tumors in these groups. (c) Photographs of the resected tumors from each group obtained after 2 weeks. (d) Tumor mass cures of the resected tumors in these groups. All analyses were carried out in triplicate and the data were expressed as means and standard deviations (SD). Statistical analyses Analysis of variance (ANOVA) was performed in in vivo experiments. Values of $P < 0.05$ were considered statistically significant. Adapted from ref 133. Copyright 2017 American Chemical Society.

a drug delivery carrier for localized and controlled delivery of anticancer agents.[132]

Another hybrid injectable hydrogel was constructed by Yan et al. via the self-assembly of Fmoc-FF(1)/poly-L-lysine (PLL). The hydrogel showed properties ideal for drug loading and delivery such as self-healing, shear thinning, biodegradability, and excellent biocompatibility. The Fmoc-FF/PLL injectable hydrogel was studied for photodynamic antitumor therapy by encapsulating a photosensitive drug called chlorin e6, as shown in Figure 5. In vivo release studies of chlorin e6 from the said hydrogel indicated localized and sustained release of the drug, thereby reducing dose and toxicity of the photosensitive drug. In vivo photodynamic therapy studies revealed inhibition of tumor growth and reduction of the recurrence rate through the "once injection multiple treatments" method. The Fmoc-FF/PLL hydrogel with desirable mechanical properties providing a sustained and localized delivery of chlorin e6 along with facilitating an efficient and relatively **safe** photodynamic therapy presents significant promise for application in antitumor therapy.[133]

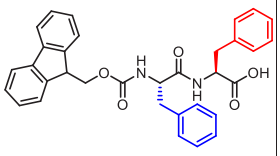
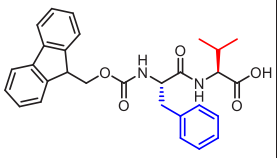
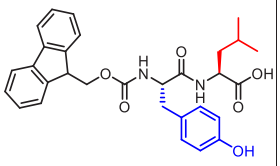
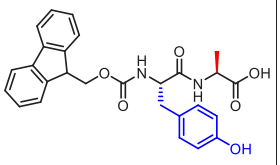
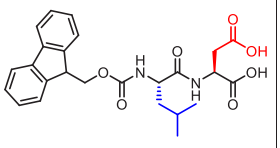
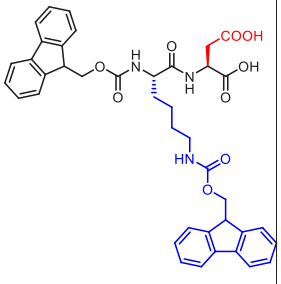
Photodynamic therapy (PDT) is an efficient alternative to antimicrobial drugs involving destruction of bacteria with the benefit of reduced frequency of antibiotic resistance. Due to high efficiency and superior chemistry fullerenes are effective photosensitizers for photodynamic therapy of bacteria albeit biomedical application of fullerenes faces the challenge of lower aqueous solubility and aggregation. This limitation was overcome by Zhang and co-workers where a hybrid hydrogel was fabricated from fullerene and self-assembling Fmoc-FF (**1**). Incorporation of fullerene in the hydrogel provided improved mechanical properties such as injectability and inhibition of aggregation of fullerene due to noncovalent interactions between the two. These properties eventually led to facilitation of a targeted and sustained release of fullerene, thereby improving its efficiency for photodynamic therapy. In vivo and in vitro antibacterial results displayed the effective inhibition of multiple-drug-resistant *Staphylococcus aureus* and could be used to promote wound healing.[134] Based on the current research, it is evident that dipeptides can act as excellent carriers for small molecules as well as large molecules while being able to enhance the bioavailability, provide targeted and controlled release, improve therapeutic action, and reduce toxicity.

5.1.2. Dipeptide Injectable Hydrogels for Tissue Engineering. The choice of scaffold is a crucial aspect in tissue engineering and is based on certain requirements such as adequate mechanical strength, biodegradability, biocompatibility, adaptable shape, and appropriate surface chemistry. Injectable dipeptide hydrogels fit perfectly into the prerequisites of a scaffold in tissue engineering, which will be seen in the studies listed below.[135]

Ghosh et al. incorporated Fmoc-FF (**1**) in alginate to yield a rigid yet injectable composite hydrogel which could serve as a potential scaffold for bone regeneration. SEM analysis revealed the resemblance of the composite hydrogel to the fibrillary nature of the bone extracellular matrix. High biocompatibility of the hydrogel as evaluated in the MTT assay along with its ability to induce osteoblast differentiation and mineralization of MC3T3-E1 preosteoblast cells provided further evidence for its use in bone regeneration.[136] In another study, Najafi et al. explored the ability of Fmoc-FV (**2**) to form a shear thinning and thermosensitive hydrogel via the pH titration method for 3D cell culture of various cells. Cellular experiments such as the 3D culture and live/dead assay indicated that the Fmoc-FV (**2**) hydrogel scaffold induced cellular proliferation, and this effect was observed to be more pronounced in the HUVEC and MDA-MB cell lines with fewer dead cells as compared to the WJMSC cell line. MTT assay and Alamar blue assay further confirmed that the Fmoc-FV (**2**) hydrogel scaffold did not show significant cytotoxicity.[137] Chakraborty et al. designed a minimalistic de novo dipeptide Fmoc-Lys (Fmoc)-Asp-OH (**6**)-based hydrogel

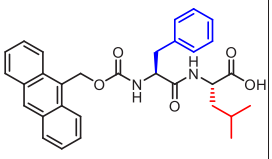
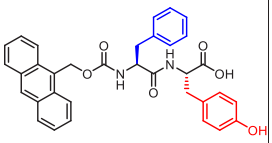
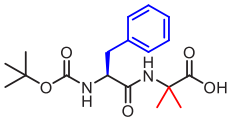
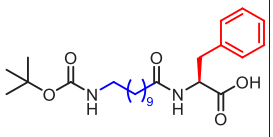
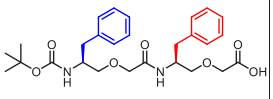
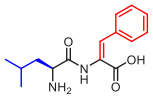
https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

Table 1. List of Injectable Dipeptide Hydrogels

Sr. No.	Dipeptide	Application	Structure	Reference
		Fmoc-protected Dipeptides		
1.	Fmoc-FF	Drug delivery, Tissue Engineering, Hybrid Hydrogels	 (1)	127,133 136 132
2.	Fmoc-FV	3D Culture of mesenchymal stem cells	 (2)	137
3.	Fmoc-YL	Endoscopic submucosal dissection filler	 (3)	146
4.	Fmoc-YA	Endoscopic submucosal dissection filler	 (4)	146
5.	Fmoc-LD	Drug Delivery	 (5)	114
6.	Fmoc-Lys(Fmoc)-Asp-OH	Tissue engineering	 (6)	138

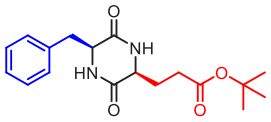
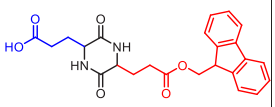
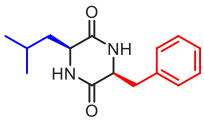
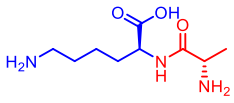
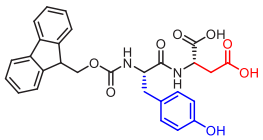
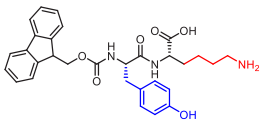
https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

Table 1. continued

Sr. No.	Dipeptide	Application	Structure	Reference
		N-Terminal modified Dipeptides		
7.	Amoc-FL	Tissue engineering	 (7)	140
8.	Amoc-FY	Tissue engineering	 (8)	140
		Dipeptides containing Unnatural Amino acids		
9.	Boc-Phe-Aib	Biosensing	 (9)	148
10.	Boc-AUDA-Phe-OH	Antibacterial	 (**10**)	147
		Backbone Modified Dipeptide		
11.	Boc-β(O)-δ5-Phe-β(O)-δ5-Phe	Drug Delivery Tissue Engineering	 (11)	128
12.	Leu-**α**,**β**-dehydroPhe	Drug Delivery, Tissue engineering Wound Healing	 (12)	129, 139 143

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

Table 1. continued

Sr. No.	Dipeptide	Application	Structure	Reference
		Cyclic Dipeptides		
13.	cyclo-(L-Glu(O-tBu)-L-Phe)	Drug Delivery	 (**13**)	126
14.	(D,L)-cyclo[Glu(OFm)-Glu]	Drug controlled release and Tissue engineering	 (14)	141
15.	Cyclo-(L-Leu-L-Phe)	Tissue engineering	 (15)	142
16	Ala-Lys	Carbohydrate based gel matrix	 (16)	149
17	Fmoc-YD	Bioink	 (17)	150
18	Fmoc-YK	Bioink	 (18)	150

with a critical gelator concentration as low as 0.002 wt % exhibiting thixotropic properties as assessed by the step strain experiment. The hydrogel was found to be cytocompatible in the XTT analysis and demonstrated potential for use as a 3D cell scaffolding material as it facilitated cell adherence followed by cell migration into the hydrogel bulk as observed in the live/dead assay carried out on fibroblast cells.[138] Yadav and team designed a self-assembling ultrashort peptide hydrogel containing a conformationally constrained dipeptide, namely, leucine-α,β-dehydrophenylalanine (Leu-ΔPhe) (**12**), having the ability to spontaneously self-assemble into a hydrogel. Such a hydrogel with solid-like porous structures, high water content, and biocompatible properties emerged as a good scaffold for tissue engineering applications. They evaluated that the mechanical strength of the hydrogel was in MPa range and thus was suitable for engineering highly stiff and complex tissues, such as bone, at relatively low concentrations. The hydrogel provided self-healing properties and supported growth and proliferation of three different mammalian cells including HEK293T, HeLa, and HepG2 in its matrix depicting its applications in cell culture as well. Stability studies showed that the Leu-ΔPhe hydrogel was stable against proteases, which drives it toward tissue engineering applications.[139]

Amoc (9-anthracenemethoxycarbonyl)-capped dipeptides such as Amoc-FL (**7**) and Amoc-FY (**8**) were reported by Gavel et al. to self-assemble into nanofibrous hydrogels via a pH

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

switch method. Rheological experiments revealed the excellent mechanical strength, thixotropic nature, and ease of injectability of the hydrogels. These mechanically robust, injectable, self-healing, and shape memory hydrogels could be a promising platform in the field of biomaterials and 3D bioprinting. MTT assay of the hydrogels on hWBCs was conducted to establish the biocompatibility and cellular proliferation. Furthermore, results from the hemolytic activity experiments and lipid peroxidation assay support the biocompatibility of these hydrogels which form a basic requirement in tissue engineering.[140] Racemates of chiral OFmoc monosubstituted cyclo(L-Glu-L-Glu) and cyclo-(D-Glu-D-Glu) (**14**) were designed by Wang et al. to yield a hydrogel at a minimum gelator concentration as low as 0.6 wt %. These hydrogels exhibit good thixotropic recyclability, as demonstrated in the dynamic rheological experiments. X-ray diffraction and differential scanning calorimetry studies carried out on the thixotropic hydrogels provide insight into the reason for its thixotropic profile, indicating that the random arrangement of the enantiomers leads to noncrystalline self-assemblies in the three-dimensional fibrous network of the hydrogel. The racemate hydrogels with a thixotropic recovery time range of 4−10 min at a MGC range of 0.6−1.2 wt % suggest its potential use as an injectable hydrogel scaffold for biomedical applications.[141]

Another cyclic dipeptide, cyclo-LF (**15**), was designed by Yang et al. for the formation of a hydrogel via a solvent switch method, which is stable under a variety of harsh conditions such as the presence of enzymes, salts, pH, or temperature. The cyclo-LF (**15**) hydrogel possesses an interconnected 3D nanofibrous structure due to the hydrophobic and intermolecular H-bonding interactions assisting aggregation of dipeptides in water followed by assembly into ordered fibers. The mechanical stability and shear thinning behavior determined by the strain-dependent oscillatory rheology experiment make it an ideal candidate for possible application in tissue engineering.[142] The use of dipeptide injectable hydrogels as scaffolds in tissue engineering is currently in progress and is attracting the attention of researchers in the biomedical field. Further development and work on these hydrogels will broaden their scope in tissue engineering. Some of the studies have reported the dipeptide hydrogels to exhibit thixotropic nature.

5.1.3. Dipeptide Injectable Hydrogels for Wound Healing. Thota et al. used a coassembly strategy to design a bioactive injectable ultrashort peptide hydrogel for use as a wound dressing material. The composite hydrogel comprising of Leu-α,-dehydroPhe (**12**) and a macrophage attracting short chemotactic factor (fMLF) facilitated wound healing via macrophage recruitment, as observed in the chemotactic migration assay. The continuous and slow release of ciprofloxacin provided by the hydrogel further aids in the process of wound healing.[143] Gavel et al. worked on the development of peptide-based coassembled thixotropic hydrogels in another study that showed excellent wound healing properties. They observed that although the Amoc-capped dipeptide FF self-assembled to form a hard and strong hydrogel owing to precipitation of noncovalent interactions, it lacked thixotropy and therefore could not serve as an injectable hydrogel. Addition of equimolar concentration of cyclodextrin (CD) to the self-assembling dipeptide-based hydrogel resulted in a coassembled hydrogel with good mechanical strength that was ideal for wound healing. The coassembled hydrogels were easy to inject with a syringe, thereby possessing thixotropy. Noncovalent interactions between cyclodextrin and peptide have been discovered using spectroscopic data, and these interactions could be the driving force behind systemic or organized nanostructures. The hydrogel was found to be effective against Gram-positive bacteria, and their biocompatibility was investigated in human embryonic kidneys (HEK293) and MCF-7 cell lines. In vivo wound healing activity of coconstructed hydrogels was explored by histopathological examination. Confocal laser scanning microscopic results demonstrate cellular uptake of coassembled hydrogel with blue fluorescence. Griess and hydroxyproline assays were used to examine biochemical investigations such as nitric oxide and collagen content. All of the foregoing evidence suggests wound healing efficacy as well as antibacterial activity.[144]

Table 1 summarizes the various dipeptide sequences that have been reported to for injectable hydrogels.

5.1.4. Miscellaneous Applications. Self-assembled peptide hydrogels that have been used for delivery of drugs, tissue regeneration or tissue engineering and oncology therapy have special features such as good biocompatibility and biodegradability as well as highly organized molecular structure and availability which highlight hydrogels. The only limiting factor is difficulty in injection due to poor rheological and mechanical properties of peptide hydrogels. Xing and co-workers overcame these drawbacks by forming a fibrous hydrogel from electrostatic coupling between Fmoc-FF (**1**) and poly-L-lysine (PLL). This fibrous hydrogel was self-healing and thixotropic leading to the formation of hydrogels that were suitable for injection. The nanofibers which are connected by disulfide bridge have been used for anticancer activity where the hydrogel has shown T cell response and suppression of tumor growth with increased half-life and safety. Hence these coassembled injectable hydrogels could be used for the development of vaccines for anticancer therapy.[145] Fmoc-FL, YL, LL, and YA dipeptides were designed by Ren et al. to form hydrogels possessing enhanced properties such as self-healing and tunable mechanical strength by altering or adjusting the hydrophobic and hydrogen bonding interactions of peptides through replacement of amino acids. Hydrophobic interactions are majorly responsible for the mechanical strength of hydrogels and strong hydrogen bonding between molecules contributes to self-healing properties. These dipeptide hydrogels had more durability, stiffness and lower induction of inflammatory response and acted as perfect endoscopic submucosal dissection (ESD) fillers for operations as shown in mice and mini pig experiments. In comparison to clinical ESD in normal saline, the peptide Fmoc-YL (**3**) hydrogel was discovered to be functional and utilized as the ESD filler to facilitate the operation and prevent perforation.[146]

Dipeptide (Boc-AUDA-Phe-COOH, **10**) containing an unnatural amino acid was found to form a hydrogel in the presence of phosphate buffer pH ranging from 6.0 to 8.8. Hydrogel which is formed at pH 7.46 was analyzed by wide-angle powder X-ray diffraction, small-angle X-ray scattering (SAXS), field emission scanning electron microscopy (FE-SEM), FTIR, high-resolution transmission electron microscopic (HR-TEM) imaging, and rheological analysis. Microscopic study revealed the formation of a nanofibrillar network of hydrogels which was observed visually, and rheological experiments at pH 7.46 showed that hydrogels exhibit thixotropy. The hydrogels showed excellent antibacterial activity toward Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* which are causative organisms of several infections. They were found to be compatible with human blood cells and fibroblast cells. Very interestingly, hydrogels were found to be resistant to enzyme degradation or proteolytic enzymes, and it was observed that any

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

change in the gelator peptide affects the bactericidal character.[147] N-terminal-modified dipeptides were explored for their hydrogelation by Gavel et al. where Amoc-protected dipeptides such as Amoc-FL-OH (**7**) and Amoc-FY-OH (**8**) self-assembled to form injectable, shape memory and self-healable hydrogels with antibacterial properties. Spectroscopic and microscopic study of the hydrogel revealed a nanofibrous network of the hydrogel. The inherent antibacterial properties of the hydrogel were tested against two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*). Potent antibacterial effects of the hydrogel were observed against Gram-positive and Gram- negative in the optical density method. Further cytotoxicity and biocompatibility of the hydrogels were established by MTT, lipid peroxidation (LPO) assay and hemolysis on human blood cells. Such mechanically robust, biocompatible and nontoxic hydrogels having inherent antibacterial properties could prove to be of great interest in treating localized bacterial infections and implantations of biomaterials.[140]

An interesting application of dipeptide-based injectable hydrogel was investigated by Nandi et al. In this study, a novel dipeptide containing unnatural amino acid (Boc-Phe-Aib-OH, **9**) was designed and used to construct a hydrogel via the addition of sodium hydroxide solution. The Boc-Phe-Aib−OH hydrogel displayed the property of thixotropicity as confirmed by rheological experiments. Selective gelation in the presence of sodium hydroxide solution was used to separate a mixture of diesel and water. Another unique feature that was observed with the injectable hydrogel was its ability to sense HCl vapor leading to deformation of the gel in the presence of HCl. It also shows phase selective gel formation in oil−water mixture. Therefore, the characteristic and unique features of Boc-Phe-Aib-OH injectable hydrogel makes it an interesting candidate for HCl sensors and conductive materials.[148]

A study conducted by Wu and associates presented a new opportunity for the peptides into carbohydrate-based gel matrices furnished with applications in the fields of food industry, tissue engineering and drug delivery. They fabricated Ala-Lys dipeptide (AK) (**16**) and the iota-carrageenan (*ι*-C)-based hydrogel and evaluated that addition of AK to *ι*-C promoted the gel strength of *ι*-C, leading to a sol−gel transition. Further, they proved that AK as a cationic dipeptide controlled the conversion of negatively charged *ι*-C from a random initial structure to a helical structure possibly due to hydrogen bonding and electrostatic interactions resulting in enhanced gel strength of hydrogel matrix.[149] Jian and associates developed two Fmoc-dipeptide bioinks viz. Fmoc-YD (**17**) and Fmoc-YK (**18**), oppositely charged that could self-assemble via electrostatic interactions into *β*-sheet amyloid structures for bioprinting applications. They evaluated the mechanical strength, degradation, and cell viability of the hydrogel to demonstrate its potential for bioprinting and 3D cell culture. In vitro fabrication of tumor spheroids by a "layer-by-layer" bioprinting strategy was further carried out.[150]

6. CHALLENGES WITH THE STATE-OF-ART RESEARCH OF INJECTABLE HYDROGELS

Although the demand of injectable hydrogels is growing on biomedical research,[151] it still accompanied with challenges that need the attentions. Mechanism of gelation, rate kinetics, viscosity during the injection time, mechanical strength after gelation, the duration of deterioration, and the release profile of bioactive factors are critical for sensitive biological therapeutics.[151] These injectable hydrogels, in order to be effective, targeted, and responsive to a broad range of medical diseases and pathogenesis, they require a set of design specifications linked to chemical-physical cross-linking and biological compatibility.[152] One of the challenge for the injectable hydrogel system is to maintain the mechanical robustness while maintaining low viscosity and sustainability of enough elasticity in situ.[153] The created system has to be stable, and it should be easily degraded and safely eliminated from the body.[5,154]

Therapeutic agents, such as small drug molecules, macromolecules, or cells, can be loaded and released into the environment via carriers with specific physicochemical properties. The size, affinity, and interactions of the cargo-gel are crucial to their efficient release. The prolonged release of proteins and medications from hydrogel compositions is essential for wound dressings.[155] To agitate the solute elution or improve its affinity to lengthen its retention period in such formulations, the hydrogel mesh size should be reduced via physical or chemical cross-linking.[156] For the objective of tissue regeneration, the hydrogel material must pierce, transform, and disintegrates to bulk hydrogel materials. The adhesion of cells, growth factors, cofactors to adhesive natural or synthetic materials such as hyaluronic acid, fibrin, or gelatin is crucial for hydrogel bioactivity.[157,158] It is crucial to reduce any immune reactions during in situ gel transitions in injectable hydrogel compositions. Immunological responses, such as fibrosis, inflammatory cascades, and hypersensitivity reactions, are regarded as the worst consequences of biomaterial implantation, injection, and insertion. These reactions are regarded as detrimental due to their potential consequences.[159] So, reducing injectable hydrogel related host immune reactions is a crucial biological design factor. Particle size plays important role in hydrogel development, particularly with micro/nanogel drug delivery systems, which is a challenging task to control. Size tends to play a role in the biocompatibility/biodegradability of a hydrogel.[160]

External factors such as temperature, pH or light may influence the use of injectable hydrogels. In case of temperature sensitive hydrogels, critical temperature at which gelation occurs may not be achieved whereas in case of photosensitive hydrogels lack of ability of UV light to penetrate the skin may interfere in the formation of hydrogel.[4] Another crucial factor to take into account is the stability of the formulation. Drug-loaded hydrogels should maintain the integrity of the matrix while being stable for the drug inside.[161]

7. CONCLUSIONS AND FUTURE PROSPECTS

Peptide-based injectable hydrogels have been the subject of much interest in biomedical research. They have proven to be of major utility in a variety of sectors, including drug delivery, tissue engineering, bioimaging, wound healing, bioprinting, antimicrobials, and controlling infectious diseases. The complexity, time-consuming, and costlier techniques involved in the synthesis of the large peptides is one of the most significant obstacles that must be overcome in order to successfully develop injectable hydrogels that are based on peptides. Due to the fact that dipeptides make up the shortest possible sequence of peptides, injectable hydrogels based on dipeptides have the potential to provide all of the benefits that are associated with peptide-based injectable hydrogels while simultaneously simplifying the production process. This gives dipeptide-based injectable hydrogels commercial value. Research on injectable hydrogels

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

based on dipeptides has shown that these materials have the potential to be employed as a drug delivery vehicle, biomaterial, and scaffold for cell growth, as well as to give therapeutic benefit. On the other hand, dipeptide-based injectable hydrogels have been shown to have considerably lower mechanical strength and lower stability in contrast to polymer-based hydrogels, which may present a challenge for their application in clinical settings. In addition, in comparison to peptide injectable hydrogels, dipeptide injectable hydrogels have a shorter sequence, which results in a lesser possibility of mechanical tunability. This is because mechanical tunability depends on the length of the sequence. As a result, there is a requirement for the rational design of a three-dimensional dipeptide structure. This can be accomplished by controlling the noncovalent interactions and coming up with strategies to develop dipeptide injectable hydrogels that are both more intelligent and more mechanically robust. Injectable hydrogels based on dipeptides, which we believe offer a significant amount of potential for study and could play a significant role in the field of biomedicine.

AUTHOR INFORMATION

Corresponding Author

Bichismita Sahu − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India;* orcid.org/0000-0002-0712-9494; Phone: +91 84240 22096; Email: bichismita@niperahm.res.in

Authors

Neeraj Kulkarni − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India*

Prajakta Rao − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India; Quality Operations, Novartis Healthcare Pvt. Ltd., Hyderabad 500081 Telangana, India*

Govinda Shivaji Jadhav − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India*

Bhakti Kulkarni − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India; Springer Nature Technology and Publishing Solutions, Pune 411013 Maharashtra, India*

Nagaraju Kanakavalli − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India; Aragen Life Sciences Pvt, Ltd., Hyderabad 500076 Telangana, India*

Shivani Kirad − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India*

Sujit Salunke − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India*

Vrushali Tanpure − *Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar 382355, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c05601

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the Department of Pharmaceuticals (DoP), Ministry of Chemicals and Fertilizers, India, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad and Director, NIPER-A for supporting our research in different forms at NIPER Ahmedabad.

REFERENCES

(1) Wichterle, O.; Lim, D. Hydrophilic gels for biological use. *Nature* **1960**, *185*, 117−118.

(2) Bharskar, G. J. A review on hydrogel. *World J. Pharm. Pharm. Sci.* **2020**, *9*, 1288−1298.

(3) Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. E. *Biomaterials Science: An Introduction to Materials in Medicine*; Academic Press, 1996.

(4) Vashist, A.; Walters, R.; Nair, M. Challenges and Future Prospects Associated with Smart Hydrogels for Drug Delivery and Imaging. In *Intelligent Hydrogels in Diagnostics and Therapeutics*, 1st ed.; Ghosal, A., Kaushik, A., Eds.; Taylor and Francis, 2020; pp 135−144.

(5) Vashist, A.; Kaushik, A.; Vashist, A.; Jayant, R. D.; Tomitaka, A.; Ahmad, S.; Gupta, Y. K.; Nair, M. Recent trends on hydrogel based drug delivery systems for infectious diseases. *Biomater. Sci.* **2016**, *4*, 1535−1553.

(6) El-Sherbiny, I. M.; Yacoub, M. H. Hydrogel scaffolds for tissue engineering: Progress and challenges. *Glob. Cardiol. Sci. Pract.* **2013**, *2013*, 38.

(7) Mondal, S.; Das, S.; Nandi, A. K. A review on recent advances in polymer and peptide hydrogels. *Soft Matter* **2020**, *16*, 1404−1454.

(8) Huynh, C. T.; Nguyen, M. K.; Lee, D. S. Injectable block copolymer hydrogels: achievements and future challenges for biomedical applications. *Macromolecules* **2011**, *44*, 6629−6636.

(9) Boonen, K.; Creemers, J. W.; Schoofs, L. Bioactive peptides, networks and systems biology. *Bioessays* **2009**, *31*, 300−314.

(10) Takahashi, K.; Ohba, K.; Kaneko, K. Ubiquitous expression and multiple functions of biologically active peptides. *Peptides* **2015**, *72*, 184−191.

(11) Collier, J. H.; Rudra, J. S.; Gasiorowski, J. Z.; Jung, J. P. Multi-component extracellular matrices based on peptide self-assembly. *Chem. Soc. Rev.* **2010**, *39*, 3413−3424.

(12) Liang, G.; Yang, Z.; Zhang, R.; Li, L.; Fan, Y.; Kuang, Y.; Gao, Y.; Wang, T.; Lu, W.; Xu, B. Supramolecular Hydrogel of a d-Amino Acid Dipeptide for Controlled Drug Release in Vivo. *Langmuir.* **2009**, *25*, 8419−8422.

(13) Yang, Z.; Xu, K.; Wang, L.; Gu, W.; Wei, H.; Zhang, M.; Xu, B. Self-assembly of small molecules affords multifunctional supramolecular hydrogels for topically treating simulated uranium wounds. *Chem. Commun.* **2005**, *35*, 4414−4416.

(14) Guimarães, C. F.; Gasperini, L.; Marques, A. P.; Reis, R. L. The stiffness of living tissues and its implications for tissue engineering. *Nat. Rev. Mater.* **2020**, *5*, 351−370.

(15) Misawa, H.; Kobayashi, N.; Soto-Gutierrez, A.; Chen, Y.; Yoshida, A.; Rivas-Carrillo, J. D.; Navarro-Alvarez, N.; Tanaka, K.; Miki, A.; Takei, J.; Ueda, T.; Tanaka, M.; Endo, H.; Tanaka, N.; Ozaki, T. PuraMatrix Facilitates Bone Regeneration in Bone Defects of Calvaria in Mice. *Cell Transplant* **2006**, *15*, 903−910.

(16) Kumar, D.; Workman, V. L.; O'Brien, M.; McLaren, J.; White, L.; Ragunath, K.; Rose, F.; Saiani, A.; Gough, J. E. Peptide hydrogels—a tissue engineering strategy for the prevention of oesophageal strictures. *Adv. Funct. Mater.* **2017**, *27*, 1702424.

(17) Das, R.; Gayakvad, B.; Shinde, S. D.; Rani, J.; Jain, A.; Sahu, B. Ultrashort Peptides—A Glimpse into the Structural Modifications and Their Applications as Biomaterials. *ACS Appl. Bio Mater.* **2020**, *3*, 5474−5499.

(18) Yadav, N.; Chauhan, M. K.; Chauhan, V. S. Short to ultrashort peptide-based hydrogels as a platform for biomedical applications. *Biomater. Sci.* **2020**, *8*, 84−100.

(19) Binaymotlagh, R.; Chronopoulou, L.; Haghighi, F. H.; Fratoddi, I.; Palocci, C. Peptide-based hydrogels: New materials for biosensing and biomedical applications. *Materials* **2022**, *15*, 5871.
(20) Kulkarni, N.; Shinde, S. D.; Jadhav, G. S.; Adsare, D. R.; Rao, K.; Kachhia, M.; Maingle, M.; Patil, S. P.; Arya, N.; Sahu, B. Peptide-chitosan engineered scaffolds for biomedical applications. *Bioconjug Chem.* **2021**, *32*, 448−465.
(21) Pramanik, B. Short Peptide-Based Smart Thixotropic Hydrogels. *Gels* **2022**, *8*, 569.
(22) Li, J.; Xing, R.; Bai, S.; Yan, X. Recent advances of self-assembling peptide-based hydrogels for biomedical applications. *Soft Matter* **2019**, *15*, 1704−1715.
(23) Yu, S.; Wang, C.; Yu, J.; Wang, J.; Lu, Y.; Zhang, Y.; Zhang, X.; Hu, Q.; Sun, W.; He, C.; Chen, X.; Gu, Z. Injectable bioresponsive gel depot for enhanced immune checkpoint blockade. *Adv. Mater.* **2018**, *30*, 1801527.
(24) Xing, B.; Yu, C. W.; Chow, K.-H.; Ho, P.-L.; Fu, D.; Xu, B. Hydrophobic interaction and hydrogen bonding cooperatively confer a vancomycin hydrogel: a potential candidate for biomaterials. *J. Am. Chem. Soc.* **2002**, *124*, 14846−14847.
(25) Li, Y.; Wang, F.; Cui, H. Peptide-based supramolecular hydrogels for delivery of biologics. *Bioeng. Transl. Med.* **2016**, *1*, 306−322.
(26) Mart, R. J.; Osborne, R. D.; Stevens, M. M.; Ulijn, R. V. Peptide-based stimuli-responsive biomaterials. *Soft Matter* **2006**, *2*, 822−835.
(27) Green, H.; Ochbaum, G.; Gitelman-Povimonsky, A.; Bitton, R.; Rapaport, H. RGD-presenting peptides in amphiphilic and anionic β-sheet hydrogels for improved interactions with cells. *RSC Adv.* **2018**, *8*, 10072−10080.
(28) Dasgupta, A.; Mondal, J. H.; Das, D. Peptide hydrogels. *RSC Adv.* **2013**, *3*, 9117−9149.
(29) Dasgupta, A.; Das, D. Designer peptide amphiphiles: self-assembly to applications. *Langmuir* **2019**, *35*, 10704−10724.
(30) Moreira Teixeira, L. S.; Feijen, J.; van Blitterswijk, C. A.; Dijkstra, P. J.; Karperien, M. Enzyme-catalyzed crosslinkable hydrogels: emerging strategies for tissue engineering. *Biomaterials* **2012**, *33*, 1281−1290.
(31) Wong, S. S.; Jameson, D. M.; Wong, S. S. *Chemistry of protein and nucleic acid cross-linking and conjugation*, 2nd ed.; CRC Press: Boca Raton, FL, 2012.
(32) Liu, C.; Zhang, Q.; Zhu, S.; Liu, H.; Chen, J. Preparation and applications of peptide-based injectable hydrogels. *RSC Adv.* **2019**, *9*, 28299−28311.
(33) Liyanage, W.; Ardona, H. A. M.; Mao, H.-Q.; Tovar, J. D. Cross-linking approaches to tuning the mechanical properties of peptide π-electron hydrogels. *Bioconjugate Chem.* **2017**, *28*, 751−759.
(34) Chronopoulou, L.; Daniele, M.; Perez, V.; Gentili, A.; Gasperi, T.; Lupi, S.; Palocci, C. A physico-chemical approach to the study of genipin crosslinking of biofabricated peptide hydrogels. *Process Biochem* **2018**, *70*, 110−116.
(35) Sivashanmugam, A.; Arun Kumar, R.; Vishnu Priya, M.; Nair, S. V.; Jayakumar, R. An overview of injectable polymeric hydrogels for tissue engineering. *Eur. Polym. J.* **2015**, *72*, 543−565.
(36) Abandansari, H. S.; Ghanian, M. H.; Varzideh, F.; Mahmoudi, E.; Rajabi, S.; Taheri, P.; Nabid, M. R.; Baharvand, H. In situ formation of interpenetrating polymer network using sequential thermal and click crosslinking for enhanced retention of transplanted cells. *Biomaterials* **2018**, *170*, 12−25.
(37) Moses, J. E.; Moorhouse, A. D. The growing applications of click chemistry. *Chem. Soc. Rev.* **2007**, *36*, 1249−1262.
(38) Bhattacharyya, T.; Panda, D.; Dash, J. Supramolecular Template-Directed In Situ Click Chemistry: A Bioinspired Approach to Synthesize G-Quadruplex DNA Ligands. *Org. Lett.* **2021**, *23*, 3004−3009.
(39) Wang, X.; Chen, S.; Wu, D.; Wu, Q.; Wei, Q.; He, B.; Lu, Q.; Wang, Q. Oxidoreductase-initiated radical polymerizations to design hydrogels and micro/nanogels: mechanism, molding, and applications. *Chem. Sci.* **2018**, *30*, 1705668.
(40) Hughes, M.; Debnath, S.; Knapp, C. W.; Ulijn, R. V. Antimicrobial properties of enzymatically triggered self-assembling aromatic peptide amphiphiles. *Biomater. Sci.* **2013**, *1*, 1138−1142.
(41) Shah, A.; Malik, M. S.; Khan, G. S.; Nosheen, E.; Iftikhar, F. J.; Khan, F. A.; Shukla, S. S.; Akhter, M. S.; Kraatz, H.-B.; Aminabhavi, T. M. Stimuli-responsive peptide-based biomaterials as drug delivery systems. *Chem. Eng. J.* **2018**, *353*, 559−583.
(42) Li, Z.; Zhu, Y.; Matson, J. B. pH-Responsive Self-Assembling Peptide-Based Biomaterials: Designs and Applications. *ACS Applied Bio Materials.* **2022**, DOI: 10.1021/acsabm.2c00188.
(43) Stevens, M. M.; Allen, S.; Sakata, J. K.; Davies, M. C.; Roberts, C. J.; Tendler, S. J.; Tirrell, D. A.; Williams, P. M. pH-dependent behavior of surface-immobilized artificial leucine zipper proteins. *Langmuir* **2004**, *20*, 7747−7752.
(44) Minelli, C.; Liew, J. X.; Muthu, M.; Andresen, H. Coiled coil peptide-functionalized surfaces for reversible molecular binding. *Soft Matter* **2013**, *9*, 5119−5124.
(45) Bowerman, C. J.; Nilsson, B. L. A reductive trigger for peptide self-assembly and hydrogelation. *J. Am. Chem. Soc.* **2010**, *132*, 9526−9527.
(46) Woolley, G. A. Photocontrolling peptide α helices. *Acc. Chem. Res.* **2005**, *38*, 486−493.
(47) Pochan, D. J.; Schneider, J. P.; Kretsinger, J.; Ozbas, B.; Rajagopal, K.; Haines, L. Thermally reversible hydrogels via intramolecular folding and consequent self-assembly of a de novo designed peptide. *J. Am. Chem. Soc.* **2003**, *125*, 11802−11803.
(48) Ghasemiyeh, P.; Mohammadi-Samani, S. Hydrogels as drug delivery systems; pros and cons. *Trends Pharmacol. Sci.* **2019**, *5*, 7−24.
(49) Rana, T.; Fatima, M.; Khan, A. Q.; Naeem, Z.; Javaid, S.; Sajid, N.; Habib, A. Hydrogels; A Novel Drug Delivery System. *Biomed. J. Sci. Tech. Res.* **2021**, *33*, 25486−25498.
(50) Wang, H.; Yang, Z. Short-peptide-based molecular hydrogels: novel gelation strategies and applications for tissue engineering and drug delivery. *Nanoscale* **2012**, *4*, 5259−5267.
(51) Liang, R.; Luo, Z.; Pu, G.; Wu, W.; Shi, S.; Yu, J.; Zhang, Z.; Chen, H.; Li, X. Self-assembled peptide-based supramolecular hydrogel for ophthalmic drug delivery. *RSC Adv.* **2016**, *6*, 76093−76098.
(52) Liu, S.; Zhang, L.; Cheng, J.; Lu, Y.; Liu, J. Sustained release of hepatocyte growth factor by cationic self-assembling peptide/heparin hybrid hydrogel improves β-cell survival and function through modulating inflammatory response. *Int. J. Nanomedicine* **2016**, *11*, 4875−4890.
(53) Yang, L.; Zhang, C.; Ren, C.; Liu, J.; Zhang, Y.; Wang, J.; Huang, F.; Zhang, L.; Liu, J. Supramolecular hydrogel based on chlorambucil and peptide drug for cancer combination therapy. *ACS Appl. Mater. Interfaces.* **2019**, *11*, 331−339.
(54) Lee, K. Y.; Mooney, D. J. Hydrogels for tissue engineering. *Chem. Rev.* **2001**, *101*, 1869−1880.
(55) Banwell, E. F.; Abelardo, E. S.; Adams, D. J.; Birchall, M. A.; Corrigan, A.; Donald, A. M.; Kirkland, M.; Serpell, L. C.; Butler, M. F.; Woolfson, D. N. Rational design and application of responsive α-helical peptide hydrogels. *Nat. Mater.* **2009**, *8*, 596−600.
(56) McGrath, A. M.; Novikova, L. N.; Novikov, L. N.; Wiberg, M. BD PuraMatrix peptide hydrogel seeded with Schwann cells for peripheral nerve regeneration. *Brain Res. Bull.* **2010**, *83*, 207−213.
(57) Moradi, F.; Bahktiari, M.; Joghataei, M. T.; Nobakht, M.; Soleimani, M.; Hasanzadeh, G.; Fallah, A.; Zarbakhsh, S.; Hejazian, L. B.; Shirmohammadi, M.; Maleki, F. BD PuraMatrix peptide hydrogel as a culture system for human fetal Schwann cells in spinal cord regeneration. *J. Neurosci. Res.* **2012**, *90*, 2335−2348.
(58) Guo, S. A.; DiPietro, L. A. Factors affecting wound healing. *J. Dent Res.* **2010**, *89*, 219−229.
(59) Stern, D.; Cui, H. Crafting polymeric and peptidic hydrogels for improved wound healing. *Adv. Healthc. Mater.* **2019**, *8*, 1900104.
(60) Zhu, J.; Han, H.; Li, F.; Wang, X.; Yu, J.; Qin, X.; Wu, D. Peptide-functionalized amino acid-derived pseudoprotein-based hydrogel with hemorrhage control and antibacterial activity for wound healing. *Chem. Mater.* **2019**, *31*, 4436−4450.

(61) Deng, A.; Yang, Y.; Du, S.; Yang, X.; Pang, S.; Wang, X.; Yang, S. Preparation of a recombinant collagen-peptide (RHC)-conjugated chitosan thermosensitive hydrogel for wound healing. *Mater. Sci. Eng. C* **2021**, *119*, 111555.
(62) Khurana, A.; Godugu, C. Alginate-based three-dimensional in vitro tumor models: A better alternative to current two-dimensional cell culture models. *Alginates and their biomedical applications*; Springer: Singapore, 2018; pp 157−183.
(63) Nguyen, K. T.; West, J. L. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* **2002**, *23*, 4307−4314.
(64) Bairagi, D.; Biswas, P.; Basu, K.; Hazra, S.; Hermida-Merino, D.; Sinha, D. K.; Hamley, I. W.; Banerjee, A. Self-assembling peptide-based hydrogel: regulation of mechanical stiffness and thermal stability and 3D cell culture of fibroblasts. *ACS Appl. Bio Mater.* **2019**, *2*, 5235−5244.
(65) Fan, L.; Cao, M.; Gao, S.; Wang, T.; Wu, H.; Peng, M.; Zhou, X.; Nie, M. Preparation and characterization of sodium alginate modified with collagen peptides. *Carbohydr. Polym.* **2013**, *93*, 380−385.
(66) Käpylä, E.; Delgado, S. M.; Kasko, A. M. Shape-changing photodegradable hydrogels for dynamic 3D cell culture. *ACS Appl. Mater. Interfaces.* **2016**, *8*, 17885−17893.
(67) Zanna, N.; Focaroli, S.; Merlettini, A.; Gentilucci, L.; Teti, G.; Falconi, M.; Tomasini, C. Thixotropic peptide-based physical hydrogels applied to three-dimensional cell culture. *ACS Omega* **2017**, *2*, 2374−2381.
(68) Mandal, A.; Clegg, J. R.; Anselmo, A. C.; Mitragotri, S. Hydrogels in the clinic. *Bioeng. Transl. Med,.* **2020**, *5*, 10158.
(69) Cao, H.; Duan, L.; Zhang, Y.; Cao, J.; Zhang, K. Current hydrogel advances in physicochemical and biological response-driven biomedical application diversity. *Signal Transduct. Target. Ther.* **2021**, *6*, 6.
(70) Collins, M. N.; Birkinshaw, C. Hyaluronic acid based scaffolds for tissue engineering—A review. *Carbohydr. Polym.* **2013**, *92*, 1262−1279.
(71) Alonso, J. M.; Andrade del Olmo, J.; Perez Gonzalez, R.; Saez-Martinez, V. Injectable hydrogels: From laboratory to industrialization. *Polymers.* **2021**, *13*, 650.
(72) Liu, C.; Zhang, Q.; Zhu, S.; Liu, H.; Chen, J. J. Preparation and applications of peptide-based injectable hydrogels. *RSC Adv.* **2019**, *9*, 28299−28311.
(73) Yu, L.; Ding, J. Injectable hydrogels as unique biomedical materials. *Chem. Soc. Rev.* **2008**, *37*, 1473−1481.
(74) Malik, K.; Singh, I.; Nagpal, M.; Arora, S. Atrigel: A potential parenteral controlled drug delivery system. *Der Pharmacia Sinica* **2010**, *1*, 74−81.
(75) Dunn, R. L.; English, J. P.; Cowsar, D. R.; Vanderbilt, D. P. Biodegradable in-situ forming implants and methods of producing the same. U.S. Patent Appl. 19904 938763, 1988.
(76) Patel, D. B. A review on atrigel drug delivery system. *J. Glob. Pharma Technol.* **2010**, *2*, 85−90.
(77) Thakare, E. B.; Malpure, P. S.; Maru, A. D.; Surana, S. S.; Chavan, B. R. Atrigel-Implants and Controlled Release Drug Delivery System: A Review. *Am. J. PharmTech Res.* **2019**, *9*, 134.
(78) Dunn, R. l.; Garett, J. S.; Rajavarapu, H.; Chandrashekhar, B. L. Polymeric delivery formulations of leuprolide with improved efficacy. U.S. Patent Appl. 20036565874, 2000.
(79) Sartor, O. Eligard: leuprolide acetate in a novel sustained-release delivery system. *Urology* **2003**, *61*, 25−31.
(80) Reddy, M.; Ponnamma, D.; Choudhary, R.; Sadasivuni, K. K. J. P. A comparative review of natural and synthetic biopolymer composite scaffolds. *Polymers.* **2021**, *13*, 1105.
(81) Altunbas, A.; Pochan, D. J. Peptide-based and polypeptide-based hydrogels for drug delivery and tissue engineering. *Top. Curr. Chem.* **2011**, *310*, 135−167.
(82) Seow, W. Y.; Hauser, C. A. Short to ultrashort peptide hydrogels for biomedical uses. *Mater. Today.* **2014**, *17*, 381−388.
(83) Serizawa, T.; Fukuta, H.; Date, T.; Sawada, T. Affinity-based release of polymer-binding peptides from hydrogels with the target segments of peptides. *Chem. Commun.* **2016**, *52*, 2241−2244.
(84) Kwon, M. Y.; Vega, S. L.; Gramlich, W. M.; Kim, M.; Mauck, R. L.; Burdick, J. A. Dose and Timing of N-Cadherin Mimetic Peptides Regulate MSC Chondrogenesis within Hydrogels. *Adv. Healthc. Mater.* **2018**, *7*, 1701199.
(85) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular hydrogelators and hydrogels: from soft matter to molecular biomaterials. *Chem. Rev.* **2015**, *115*, 13165−13307.
(86) Tang, J. D.; Mura, C.; Lampe, K. Stimuli-responsive, pentapeptide, nanofiber hydrogel for tissue engineering. *J. Am. Chem. Soc.* **2019**, *141*, 4886−4899.
(87) Liu, C.; Guo, X.; Ruan, C.; Hu, H.; Jiang, B.-P.; Liang, H.; Shen, X.-C. An injectable thermosensitive photothermal-network hydrogel for near-infrared-triggered drug delivery and synergistic photothermal-chemotherapy. *Acta. Biomater.* **2019**, *96*, 281−294.
(88) Sharma, A.; Gupta, A.; Khan, N.; DuttKonar, A. Can non-heterocyclic hydrophobic amino acids when tethered at the C-terminus of 12-hydroxy stearic acid-based amphiphilic derivatives drive hydrogelation propensity effectively. *New J. Chem.* **2020**, *44*, 9213−9222.
(89) Wang, L.; Li, J.; Zhang, D.; Ma, S.; Zhang, J.; Gao, F.; Guan, F.; Yao, M. Dual-enzymatically crosslinked and injectable hyaluronic acid hydrogels for potential application in tissue engineering. *RSC Adv.* **2020**, *10*, 2870−2876.
(90) Wu, X.; Wu, Y.; Ye, H.; Yu, S.; He, C.; Chen, X. J. Interleukin-15 and cisplatin co-encapsulated thermosensitive polypeptide hydrogels for combined immuno-chemotherapy. *J. Controlled Release* **2017**, *255*, 81−93.
(91) Xun, W.; Wu, D. Q.; Li, Z. Y.; Wang, H. Y.; Huang, F. W.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. Peptide-Functionalized Thermo-Sensitive Hydrogels for Sustained Drug Delivery. *Macromol. Biosci.* **2009**, *9*, 1219−1226.
(92) Chen, R.; Zhu, C.; Xu, L.; Gu, Y.; Ren, S.; Bai, H.; Zhou, Q.; Liu, X.; Lu, S.; Bi, X.; Li, W.; Jia, X.; Chen, Z. An injectable peptide hydrogel with excellent self-healing ability to continuously release salvianolic acid B for myocardial infarction. *Biomaterials* **2021**, *274*, 120855.
(93) Hu, C.; Liu, X.; Ran, W.; Meng, J.; Zhai, Y.; Zhang, P.; Yin, Q.; Yu, H.; Zhang, Z.; Li, Y. Regulating cancer associated fibroblasts with losartan-loaded injectable peptide hydrogel to potentiate chemotherapy in inhibiting growth and lung metastasis of triple negative breast cancer. *Biomaterials* **2017**, *144*, 60−72.
(94) Majumder, P.; Baxa, U.; Walsh, S. T.; Schneider, J. P. Design of a multicompartment hydrogel that facilitates time-resolved delivery of combination therapy and synergized killing of glioblastoma. *Angew. Chem. Int. Ed* **2018**, *57*, 15040−15044.
(95) Leach, D. G.; Dharmaraj, N.; Piotrowski, S. L.; Lopez-Silva, T. L.; Lei, Y. L.; Sikora, A. G.; Young, S.; Hartgerink, J. D. STINGel: Controlled release of a cyclic dinucleotide for enhanced cancer immunotherapy. *Biomaterials* **2018**, *163*, 67−75.
(96) Hoffman, A. S. Hydrogels for biomedical applications. *Adv. Drug Delivery Rev.* **2012**, *64*, 18−23.
(97) Wang, L.; Chen, Z.; Yan, Y.; He, C.; Li, X. Fabrication of injectable hydrogels from silk fibroin and angiogenic peptides for vascular growth and tissue regeneration. *Chem. Eng. J.* **2021**, *418*, 129308.
(98) Mehrban, N.; Pineda Molina, C.; Quijano, L. M.; Bowen, J.; Johnson, S. A.; Bartolacci, J.; Chang, J. T.; Scott, D. A.; Woolfson, D. N.; Birchall, M. A.; Badylak, S. F. Host macrophage response to injectable hydrogels derived from ECM and α-helical peptides. *Acta Biomater.* **2020**, *111*, 141−152.
(99) Lee, H. J.; Lee, J.-S.; Chansakul, T.; Yu, C.; Elisseeff, J. H.; Yu, S. M. Collagen mimetic peptide-conjugated photopolymerizable PEG hydrogel. *Biomaterials* **2006**, *27*, 5268−5276.
(100) Stahl, P. J.; Romano, N. H.; Wirtz, D.; Yu, S. M. PEG-based hydrogels with collagen mimetic peptide-mediated and tunable physical cross-links. *Biomacromolecules* **2010**, *11*, 2336−2344.
(101) Ren, K.; He, C.; Cheng, Y.; Li, G.; Chen, X. Injectable enzymatically crosslinked hydrogels based on a poly (l-glutamic acid) graft copolymer. *Polym. Chem.* **2014**, *5*, 5069−5076.
(102) Tran, K. A.; Jin, Y.; Bouyer, J.; DeOre, B. J.; Suprewicz, Ł.; Figel, A.; Walens, H.; Fischer, I.; Galie, P. A. Magnetic alignment of injectable hydrogel scaffolds for spinal cord injury repair. *Biomater. Sci.* **2022**, *10*, 2237−2247.

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

(103) Xia, G.; Liu, Y.; Tian, M.; Gao, P.; Bao, Z.; Bai, X.; Yu, X.; Lang, X.; Hu, S.; Chen, X. Nanoparticles/thermosensitive hydrogel reinforced with chitin whiskers as a wound dressing for treating chronic wounds. *J. Mater. Chem. B* **2017**, *5*, 3172−3185.
(104) Qiu, W.; Wang, Q.; Li, M.; Li, N.; Wang, X.; Yu, J.; Li, F.; Wu, D. Peptidoglycan-inspired peptide-modified injectable hydrogels with enhanced elimination capability of bacterial biofilm for chronic wound healing. *Compos. B. Eng.* **2021**, *227*, 109402.
(105) Qiu, W.; Han, H.; Li, M.; Li, N.; Wang, Q.; Qin, X.; Wang, X.; Yu, J.; Zhou, Y.; Li, Y.; Li, F.; Wu, D. Nanofibers reinforced injectable hydrogel with self-healing, antibacterial, and hemostatic properties for chronic wound healing. *J. Colloid Interface Sci.* **2021**, *596*, 312−323.
(106) Cheng, L.; Cai, Z.; Ye, T.; Yu, X.; Chen, Z.; Yan, Y.; Qi, J.; Wang, L.; Liu, Z.; Cui, W.; Deng, L. Injectable polypeptide-protein hydrogels for promoting infected wound healing. *Adv. Funct. Mater.* **2020**, *30*, 2001196.
(107) Liu, S.; Zhao, Y.; Wei, H.; Nie, L.; Ding, P.; Sun, H.; Guo, Y.; Chen, T.; Okoro, O. V.; Shavandi, A.; Fan, L. Injectable hydrogels based on silk fibroin peptide grafted hydroxypropyl chitosan and oxidized microcrystalline cellulose for scarless wound healing. *Colloids Surf. A Physicochem. Eng. Asp.* **2022**, *647*, 129062.
(108) Salick, D. A.; Kretsinger, J. K.; Pochan, D. J.; Schneider, J. P. Inherent antibacterial activity of a peptide-based β-hairpin hydrogel. *J. Am. Chem. Soc.* **2007**, *129*, 14793−14799.
(109) Veiga, A. S.; Sinthuvanich, C.; Gaspar, D.; Franquelim, H. G.; Castanho, M. A.; Schneider, J. P. Arginine-rich self-assembling peptides as potent antibacterial gels. *Biomaterials* **2012**, *33*, 8907−8916.
(110) Schmidt, N. W.; Mishra, A.; Lai, G. H.; Davis, M.; Sanders, L. K.; Tran, D.; Garcia, A.; Tai, K. P.; McCray, P. B.; Ouellette, A. J.; Selsted, M. E.; Wong, G. C. L. Criterion for amino acid composition of defensins and antimicrobial peptides based on geometry of membrane destabilization. *J. Am. Chem. Soc.* **2011**, *133*, 6720−6727.
(111) De Giglio, E.; Cometa, S.; Ricci, M. A.; Cafagna, D.; Savino, A. M.; Sabbatini, L.; Orciani, M.; Ceci, E.; Novello, L.; Tantillo, G. M.; Mattioli-Belmonte, M. Ciprofloxacin-modified electrosynthesized hydrogel coatings to prevent titanium-implant-associated infections. *Acta Biomater.* **2011**, *7*, 882−891.
(112) Marchesan, S.; Qu, Y.; Waddington, L. J.; Easton, C. D.; Glattauer, V.; Lithgow, T. J.; McLean, K. M.; Forsythe, J. S.; Hartley, P. G. Self-assembly of ciprofloxacin and a tripeptide into an antimicrobial nanostructured hydrogel. *Biomaterials* **2013**, *34*, 3678−3687.
(113) Baral, A.; Basak, S.; Basu, K.; Dehsorkhi, A.; Hamley, I. W.; Banerjee, A. Time-dependent gel to gel transformation of a peptide based supramolecular gelator. *Soft Matter* **2015**, *11*, 4944−4951.
(114) Vegners, R.; Shestakova, I.; Kalvinsh, I.; Ezzell, R. M.; Janmey, P. A. Use of a gel-forming dipeptide derivative as a carrier for antigen presentation. *Journal of peptide science: an official publication of the European Peptide Society* **1995**, *1*, 371−378.
(115) Reches, M.; Gazit, E. Casting metal nanowires within discrete self-assembled peptide nanotubes. *Science* **2003**, *300*, 625−627.
(116) Yang, M.; Xing, R.; Shen, G.; Yuan, C.; Yan, X. A versatile cyclic dipeptide hydrogelator: Self-assembly and rheology in various physiological conditions. *Colloids Surf. A Physicochem. Eng. Asp.* **2019**, *572*, 259−265.
(117) Panda, J. J.; Mishra, A.; Basu, A.; Chauhan, V. S. Stimuli responsive self-assembled hydrogel of a low molecular weight free dipeptide with potential for tunable drug delivery. *Biomacromolecules* **2008**, *9*, 2244−2250.
(118) Jing, Y.; Wang, A.; Li, J.; Li, Q.; Han, Q.; Zheng, X.; Cao, H.; Bai, S. 2022. Preparation of conductive and transparent dipeptide hydrogels for wearable biosensor. *Bio-Des. Manuf.* **2022**, *5*, 153−162.
(119) Bian, S.; Cai, H.; Cui, Y.; He, M.; Cao, W.; Chen, X.; Sun, Y.; Liang, J.; Fan, Y.; Zhang, X. Temperature and ion dual responsive biphenyl-dipeptide supramolecular hydrogels as extracellular matrix mimic-scaffolds for cell culture applications. *J. Mater. Chem. B* **2017**, *5*, 3667−3674.
(120) Liyanage, W.; Vats, K.; Rajbhandary, A.; Benoit, D. S.; Nilsson, B. L. Multicomponent dipeptide hydrogels as extracellular matrix-mimetic scaffolds for cell culture applications. *Chem. Commun.* **2015**, *51*, 11260−11263.
(121) Deo, R. C. Machine learning in medicine. *Circulation 132* **2015**, *132*, 1920−1930.
(122) Zhou, P.; Yuan, C.; Yan, X. Computational approaches for understanding and predicting the self-assembled peptide hydrogels. *Curr. Opin. Colloid Interface Sci.* **2022**, *62*, 101645.
(123) Li, F.; Han, J.; Cao, T.; Lam, W.; Fan, B.; Tang, W.; Chen, S.; Fok, K. L.; Li, L. 2019. Design of self-assembly dipeptide hydrogels and machine learning via their chemical features. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 11259−11264.
(124) Uman, S.; Dhand, A.; Burdick, J. A. Recent advances in shear-thinning and self-healing hydrogels for biomedical applications. *J. Appl. Polym. Sci.* **2020**, *137*, 48668.
(125) Kim, B. S.; Cho, C. S. Injectable hydrogels for regenerative medicine. *Tissue Eng. Regen. Med.* **2018**, *15*, 511−512.
(126) Manchineella, S.; Murugan, N. A.; Govindaraju, T. Cyclic dipeptide-based ambidextrous supergelators: minimalistic rational design, structure-gelation studies, and in situ hydrogelation. *Biomacromolecules* **2017**, *18*, 3581−3590.
(127) Najafi, H.; Abolmaali, S. S.; Heidari, R.; Valizadeh, H.; Jafari, M.; Tamaddon, A. M.; Azarpira, N. Nitric oxide releasing nanofibrous Fmoc-dipeptide hydrogels for amelioration of renal ischemia/reperfusion injury. *J. Controlled Release* **2021**, *337*, 1−13.
(128) Reja, R. M.; Patel, R.; Kumar, V.; Jha, A.; Gopi, H. N. Divergent supramolecular gelation of backbone modified short hybrid δ-peptides. *Biomacromolecules* **2019**, *20*, 1254−1262.
(129) Thota, C. K.; Yadav, N.; Chauhan, V. S. A novel highly stable and injectable hydrogel based on a conformationally restricted ultrashort peptide. *Sci. Rep.* **2016**, *6*, 31167.
(130) Nanda, J.; Banerjee, A. β-Amino acid containing proteolitically stable dipeptide-based hydrogels: encapsulation and sustained release of some important biomolecules at physiological pH and temperature. *Soft Matter* **2012**, *8*, 3380−3386.
(131) Zou, Q.; Chang, R.; Xing, R.; Yuan, C.; Yan, X. Injectable self-assembled bola-dipeptide hydrogels for sustained photodynamic prodrug delivery and enhanced tumor therapy. *J. Controlled Release* **2020**, *319*, 344−351.
(132) Shim, J.; Kang, J.; Yun, S. I. Chitosan-dipeptide hydrogels as potential anticancer drug delivery systems. *Int. J. Biol. Macromol.* **2021**, *187*, 399−408.
(133) Abbas, M.; Xing, R.; Zhang, N.; Zou, Q.; Yan, X. Antitumor photodynamic therapy based on dipeptide fibrous hydrogels with incorporation of photosensitive drugs. *ACS Biomater. Sci. Eng.* **2018**, *4*, 2046−2052.
(134) Zhang, Y.; Zhang, H.; Zou, Q.; Xing, R.; Jiao, T.; Yan, X. An injectable dipeptide-fullerene supramolecular hydrogel for photodynamic antibacterial therapy. *J. Mater. Chem. B* **2018**, *6*, 7335−7342.
(135) Sun, Y.; Nan, D.; Jin, H.; Qu, X. Recent advances of injectable hydrogels for drug delivery and tissue engineering applications. *Polym. Test.* **2020**, *81*, 106283.
(136) Ghosh, M.; Halperin-Sternfeld, M.; Grinberg, I.; Adler-Abramovich, L. Injectable alginate-peptide composite hydrogel as a scaffold for bone tissue regeneration. *Nanomaterials* **2019**, *9*, 497.
(137) Najafi, H.; Tamaddon, A. M.; Abolmaali, S.; Borandeh, S.; Azarpira, N. Structural, mechanical, and biological characterization of hierarchical nanofibrous Fmoc-phenylalanine-valine hydrogels for 3D culture of differentiated and mesenchymal stem cells. *Soft Matter* **2021**, *17*, 57−67.
(138) Chakraborty, P.; Tang, Y.; Yamamoto, T.; Yao, Y.; Guterman, T.; Zilberzwige-Tal, S.; Adadi, N.; Ji, W.; Dvir, T.; Ramamoorthy, A.; Wei, G.; Gazit, E. Unusual Two-Step Assembly of a Minimalistic Dipeptide-Based Functional Hypergelator. *Adv. Mater.* **2020**, *32*, 1906043.
(139) Yadav, N.; Chauhan, M. K.; Chauhan, V. S. Conformationally constrained dipeptide-based hydrogel as a platform for 3D cell growth and tissue engineering applications. *Appl. Nanosci.* **2021**, *11*, 2019−2031.

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570

(140) Gavel, P. K.; Dev, D.; Parmar, H. S.; Bhasin, S.; Das, A. K. Investigations of peptide-based biocompatible injectable shape-memory hydrogels: differential biological effects on bacterial and human blood cells. *Adv. Mater. Interfaces* **2018**, *10*, 10729−10740.
(141) Wang, L.; Jin, X.; Ye, L.; Zhang, A.-y.; Bezuidenhout, D.; Feng, Z. Rapidly Recoverable Thixotropic Hydrogels from the Racemate of Chiral OFm Monosubstituted Cyclo (Glu-Glu) Derivatives. *Langmuir* **2017**, *33*, 13821−13827.
(142) Yang, M.; Xing, R.; Shen, G.; Yuan, C.; Yan, X. A versatile cyclic dipeptide hydrogelator: Self-assembly and rheology in various physiological conditions. *Colloids Surf. A: Physicochem. Eng. Asp.* **2019**, *572*, 259−265.
(143) Thota, C. K.; Berger, A. A.; Elomaa, L.; Nie, C.; Böttcher, C.; Koksch, B. Coassembly generates peptide hydrogel with wound dressing material properties. *ACS Omega* **2020**, *5*, 8557−8563.
(144) Gavel, P. K.; Kumar, N.; Parmar, H. S.; Das, A. K. Evaluation of a peptide-based coassembled nanofibrous and thixotropic hydrogel for dermal wound healing. *ACS Appl. Bio Mater.* **2020**, *3*, 3326−3336.
(145) Xing, R.; Li, S.; Zhang, N.; Shen, G.; Mohwald, H.; Yan, X. Self-assembled injectable peptide hydrogels capable of triggering antitumor immune response. *Biomacromolecules* **2017**, *18*, 3514−3523.
(146) Ren, P.; Li, J.; Zhao, L.; Wang, A.; Wang, M.; Li, J.; Jian, H.; Li, X.; Yan, X.; Bai, S. Dipeptide self-assembled hydrogels with shear-thinning and instantaneous self-healing properties determined by peptide sequences. *ACS Appl. Mater. Interfaces* **2020**, *12*, 21433−21440.
(147) Baral, A.; Roy, S.; Ghosh, S.; Hermida-Merino, D.; Hamley, I. W.; Banerjee, A. A peptide-based mechano-sensitive, proteolytically stable hydrogel with remarkable antibacterial properties. *Langmuir* **2016**, *32*, 1836−1845.
(148) Nandi, S. K.; Maji, K.; Haldar, D. Self-healing hydrogel from a dipeptide and HCl sensing. *ACS Omega* **2018**, *3*, 3744−3751.
(149) Wang, Y. Q.; Han, Y. T.; Yan, J. N.; Du, Y. N.; Jiang, X. Y.; Wu, H. T. Gel properties and network structure of the hydrogel constructed by iota-carrageenan and Ala-Lys dipeptide. *International J. Biol. Macromol.* **2021**, *182*, 244−251.
(150) Jian, H.; Wang, M.; Dong, Q.; Li, J.; Wang, A.; Li, X.; Ren, P.; Bai, S. Dipeptide self-assembled hydrogels with tunable mechanical properties and degradability for 3D bioprinting. *ACS Appl. Mater. Interfaces* **2019**, *11*, 46419−46426.
(151) Jacob, S.; Nair, A. B.; Shah, J.; Sreeharsha, N.; Gupta, S.; Shinu, P. Emerging role of hydrogels in drug delivery systems, tissue engineering and wound management. *Pharmaceutics,* **2021**, *13*, 357.
(152) Almawash, S.; Osman, S. K.; Mustafa, G.; El Hamd, M. A. Current and Future Prospective of Injectable Hydrogels -Design Challenges and Limitations. *Pharmaceuticals* **2022**, *15*, 371.
(153) Guvendiren, M.; Lu, H. D.; Burdick, J. A. Shear-thinning hydrogels for biomedical applications. *Soft Matter* **2012**, *8*, 260−272.
(154) Vashist, A.; Kaushik, A.; Vashist, A.; Sagar, V.; Ghosal, A.; Gupta, Y. K.; Ahmad, S.; Nair, M. Advances in carbon nanotubes-hydrogel hybrids in nanomedicine for therapeutics. *Adv. Healthc. Mater.* **2018**, *7*, 1701213.
(155) Mandal, A.; Clegg, J. R.; Anselmo, A. C.; Mitragotri, S. Hydrogels in the clinic. *Bioeng Transl Med.* **2020**, *5*, No. e10158.
(156) Kong, L.; Wu, Z.; Zhao, H.; Cui, H.; Shen, J.; Chang, J.; Li, H.; He, Y. Bioactive injectable hydrogels containing desferrioxamine and bioglass for diabetic wound healing. *ACS Appl. Mater. Interfaces* **2018**, *10*, 30103−14.
(157) Lee, J. H. Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering. *Biomater. Res.* **2018**, *22*, 27.
(158) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular hydrogelators and hydrogels: from soft matter to molecular biomaterials. *Chem. Rev.* **2015**, *115*, 13165−307.
(159) Zhang, L.; Ren, X.; Zhang, Y.; Zhang, K. Step-growth polymerization method for ultrahigh molecular weight polymers. *ACS Macro Lett.* **2019**, *8*, 948−54.
(160) Vashist, A.; Kaushik, A.; Alexis, K.; Dev Jayant, R.; Sagar, V.; Vashist, A.; Nair, M. Bioresponsive injectable hydrogels for on-demand drug release and tissue engineering. *Curr. Pharm. Des.* **2017**, *23*, 3595−3602.
(161) Vashist, A.; Kaushik, A.; Vashist, A.; Bala, J.; Nikkhah-Moshaie, R.; Sagar, V.; Nair, M. Nanogels as potential drug nanocarriers for CNS drug delivery. *Drug Discovery Today* **2018**, *23*, 1436−1443.

https://doi.org/10.1021/acsomega.2c05601
ACS Omega 2023, 8, 3551−3570