IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
July 2021 Vol.:21, Issue:4

Injectable Hydrogels, a New Drug Delivery System: Mechanism, Application and Future Perspectives

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Chaitanya A. Gulhane[1], Ashvini Y. Parbat[1], Gokul P. Tayade[1], Shivam A. Bartare[2], Jagdish V. Manwar[2*], Ravindra L. Bakal[1]

[1] Dr. Rajendra Gode Institute of Pharmacy, Mardi road, Amravati- 444 602, MS, India

[2] Dr. Rajendra Gode College of Pharmacy, Mardi road, Amravati- 444 602, MS, India

Submitted: 23 June 2021
Accepted: 30 June 2021
Published: 30 July 2021

Keywords: Injectable hydrogel; Cross-linking mechanisms; Advantages; Application

ABSTRACT

Injectable hydrogels are mainly used as carriers of therapeutic agents. They are prepared by chemical or physical gelation method. This system offers sustained release of drugs from several days to a months. Thus, it reduces the need of frequent dosing to the patient and increases patient compliance. Injectable hydrogels shows shear thinning property when injected into the body. Hydrogels with injectability under mild conditions are preferred within the field of biomedicine, especially for drug delivery, tissue engineering, etc. In the present article, we have covered various aspects regarding injectable hydrogel such as formulation development, types, mechanisms and applications.

HUMAN JOURNALS
www.ijppr.humanjournals.com

1. INTRODUCTION:

Injectable hydrogels are mainly used as carriers of therapeutic agents. Hydrogels are promising for a variety of medical applications because of their high water content and mechanical similarity with natural tissues [1]. When injectable hydrogels are made, they reduce the invasiveness of application, which in turn reduces surgical and recovery costs [2]. They are three-dimensional networks of cross-linked hydrophilic polymers. They possess several advantages over conventional sustained released systems [3].

In-situ forming injectable hydrogels can overcome the problems that occur with conventional systems [4]. Injectable hydrogel is one of the types in situ gelling systems which needs better control of gelation kinetics. Hydrogels with injectability under mild conditions are preferred in the field of biomedicine, especially for drug delivery and tissue engineering. Because of the favorable carrier property in three-dimension, it has biocompatibility, low invasiveness, and adaptable shape for administration. Despite the benefits, injectable hydrogels may also face some challenges to satisfy the varied clinical requirements. Biomaterials encompass an outsized and diverse array of materials that range from metallic orthopedic implants to polymeric constructs aimed towards replacing, restoring, or regenerating lost tissue structure and function. An ever-growing class of biomaterials is polymeric hydrogels, classically defined as three-dimensional (3D), water-swollen polymer networks formed as a result of physical or chemical cross-linking [5]. With plenty of water, hydrogels can have good biocompatibility, desirable biodegradability, and various kinds of payloads, e.g. drugs, peptides, proteins, and genes, can be loaded into a hydrogel matrix, by absorption and/or encapsulation method. Because of their high water content and mechanical resemblance to natural tissues, hydrogels show promising biocompatibility and potential for medical/biological applications. Injectable hydrogel formulations are especially attractive due to their minimally invasive delivery procedure, providing reduced healing time, reduced scarring, decreased risk of infection, and simple delivery compared with surgically implanted materials[6].

Besides, an injectable gel is a kind of in situ forming hydrogel, so that it simplifies the incorporation ofhence is recognized as a preferred delivery vehicle. However, except for the in situ gelling characteristics, the hydrogel is prepared in such a way that it will transport the sol or the pregel to a targeting site for gelation through an injection device[7]. Injectable hydrogel formulations are especially attractive due to their minimally invasive delivery

procedure, providing reduced healing time, reduced scarring, decreased risk of infection, and ease of delivery compared with surgically implanted materials[8,9]. Hydrogels also can be rendered injectable by preforming the gels into microparticles or nanoparticles. However, particulate systems (e.g., micelles, liposomes, polymer-drug conjugates, microparticles, and nanoparticles) that are injectable by their small size constitute a vast field of research. [10]. Faster or slower gelation kinetics would affect the injectability/mass transfer or the molding of bulk gel. The objectives of this review are to provide a summary of injectable hydrogel systems, describing their use in drug delivery, TE/regenerative medicine, and space-filling applications, also a mechanism for in situ gelations**(Fig.1).**

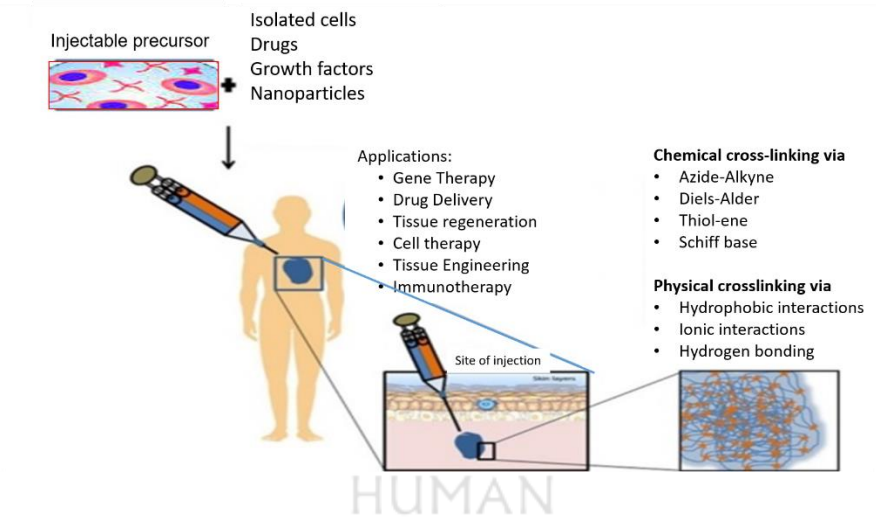

Figure No. 1: Mechanism of injectable hydrogels [11]

2.1. Physical crosslinking mechanisms

2.1.1 Ionic crosslinking

One of the principles of making ionic cross-linked hydrogels is to mix ionizable polymers with counter-ions. By changing the temperature, pH, or concentration of ions, the dynamics of sol-gel transition makes the injectability of the hydrogel [11]. The mechanical properties of the hydrogels are often further controlled by the relative molecular mass of the polymers and by the crosslinking density of the hydrogel, for example, by tuning the concentration of the polymer or the counter ions [12]. Another kind of ionic crosslinking hydrogel combines two oppositely charged polyelectrolytes. Here some typical polyelectrolyte includes polyline, poly (glutamic acid), mucopolysaccharides(HA), and sodium alginate. The gelation process is additionally sensitive to the concentration of gelatos, environmental pH, electric density, and temperature [13]. An immediate mixing of two polyelectrolytes with opposite charge

properties in solution will face some interactions which frequently led to a homogeneous mixture with large aggregates.

2.1.2. Hydrogen bonding

As a secondary force, hydrogen bonding (H-bonding) has dynamic nature and breaks at elevated temperatures. Therefore H-bonding may be a suitable crosslinking strategy for the preparation of injectable hydrogel. Besides it could endow the hydrogels with self-healing properties, thermoplasticity, and reprocessability [14]. A disadvantage associated with H-bonding cross-linked hydrogels is their poor resistance in the water, because hydration may cause the dissociation of H-bonding between polymer segments.

2.1.3. Hydrophobic interactions

Amphiphilic polymers forma hydrogel by the association of the hydrophobic moieties as physical crosslink points [15]. For an injection process, the polymeric amphiphiles may have either lower critical solution temperature (LCST) or upper critical solution temperature (UCST), which ensures a sol-gel transition by the change of environmental temperature. A familiar example is Pluronics®.

2.1.4. Host-guest interaction

The complex structures formed by host-guest chemistry are also considered as a kind of in situ forming physical crosslink that is helpful for the design of injectable hydrogel [16,17]. The host-guest interaction is reversible so that it has provided a wide use of the hydrogels in drug release and tissue engineering [18]. One advantage is that drug molecules are often seized by the host moieties to avoid a burst release.

2.1.5. π-π stacking interaction

The π-π stacking interaction is a special spatial arrangement of aromatic compounds, which usually occurs between relatively π electron-rich groups and π electron-deficient groups. As a result, the electron clouds flow from the electron-rich side to increase the electron density on the electron-poor side [19].

2.2. Chemical crosslinking mechanisms

2.2.1. Michael addition reaction

Among the in situ reactions for preparing injectable gels, Michael addition, referring to the conjugate addition reactions of an electrophilic conjugated system (electron acceptor) with a nucleophilic negative carbon ion (electron donor), attracts increasing attention in recent years mainly due to the high selectivity of the reaction under mild conditions [20,21].

2.2.2. Click chemistry

Click chemistry is additionally a sort of reaction with relatively rapid kinetics, proceeded by the connection of particular small units, with high yield and high selectivity. Click chemistry mainly concludes four sorts of reactions, i.e. cycloaddition reaction, nucleophilic ring-opening reaction, non-alkali carbonyl chemistry, and carbon-carbon multi-bond chemical reaction. Usually, the reactions need an initiator and/or catalyst but this hinders their bioactive application [22, 23]. Therefore, it is important to develop non-catalyst/initiator reaction systems for the preparation of environmentally friendly biomaterials like injectable hydrogels [24].

2.2.3. Enzymatic reaction

Enzymatic crosslinking is another choice to get injectable hydrogels, especially for facilitating protein-based gels. Various enzymes extracted from both plant and animal sources, like horseradish peroxidase (HRP), glucose oxidase (GOx), and laccase, are utilized to catalyze the formation of covalent crosslinks in gelling systems [25]. For instance, HRP mediated crosslinking by the use of hydrogen peroxide as the substrate is reported for getting injectable hydrogels through the conjugation of phenol and aniline derivatives [26].

2.3. Dynamic covalent bonding mechanisms

Different classes of dynamic covalent bonding including disulfide, Schiff base, oxime, hydrazine, and borate are applied for building injectable hydrogels [27]. In most cases, these bonds can be formed under physiological conditions, or triggered by internal or external stimuli like temperature, pH, and redox condition [28]. And the chemical equilibrium of the dynamic covalent bonding mediates the sol-gel transition or the degradability of the formed hydrogel [29].

3.1 Applications of Injectable Hydrogels

Tissue Regeneration Applications

Currently, the main target injectable hydrogels are on cartilage and bone, whereas other uses are intended for tissue repairs like eye, liver, and heart, also as drug release and delivery.

A] Angiogenesis

Angiogenesis, the formation of novel blood vessels, maybe a critical process in tissue regeneration. However, inadequate vascularization of the injectable compound has long been a barrier, resulting in necrosis or volume reduction after implantation. To resolve this problem, sustained release of certain growth factors such as vascular endothelial growth factor and basic fibroblast growth factor can be employed. Injectable scaffolds are studied as an appropriate delivery vehicle because of their easy preparation and handling[30,31].

B] Bone repairing

Injectable scaffolds have been extensively investigated for applications in bone tissue regeneration. Several factors, including macromonomer concentration, pre-treatment before injection, incorporation of cell adhesive peptide sequences, and controlled, localized release of growth factors within the injectable scaffolds, play an important role in bone formation[32,33]. Vishnu Priya *et al* developed an injectable hydrogel system consisting of chitin and poly (butylene succinate) loaded with fibrin nanoparticles and magnesium-doped bioglass. The gelatin microparticles incorporated in the hydrogel enhance bony bridging and mineralization within bone implant and defect interface area[34]. Huang *et al* have fabricated an injectable nanohydroxyapatite/glycol chitosan/hyaluronic acid composite hydrogel. All of these outcomes suggest that hydrogel could be a potential candidate for irregular bone regeneration (**Fig.2**).

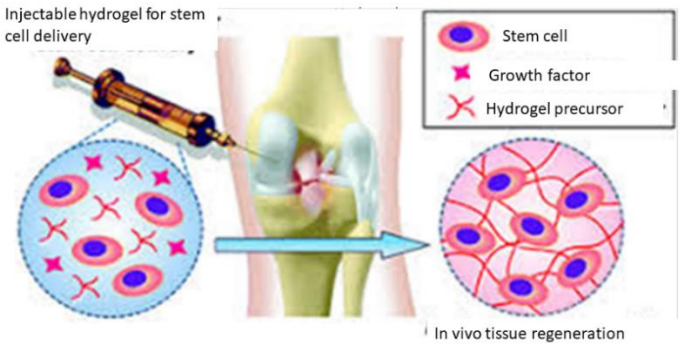

Figure No. 2: Delivery of drug in bone repairmen using hydrogel [37]

C] Cartilage regeneration

The limitation of cartilage tissue for self-repair and regeneration restricts the clinical application of TE cartilage. Kinard *et al* used the oligomer oligo(poly(ethylene glycol)fumarate) as a backbone to supply 3D injectable hydrogel networks to deliver cells and growth factors for cartilage reconstruction**(Fig. 3).**Glycosaminoglycan (GAG) content indicated that the hydrogel composite could also be a unique strategy for cartilage TE. Photo-initiating composite hydrogel, methacrylate glycol chitosan/hyaluronic acid, was shown to be cyto compatible with significantly increased cell proliferation and cartilaginous tissue[35,36].

The biomaterial has the potential to be used as a carrier of cells and bioactive molecules for treating cartilage damage. Therefore, careful control over the crosslinking density and structure of the macromonomers is vital to understand increased type II collagen synthesis and homogeneous distribution of GAG within the engineered cartilage[37,38].

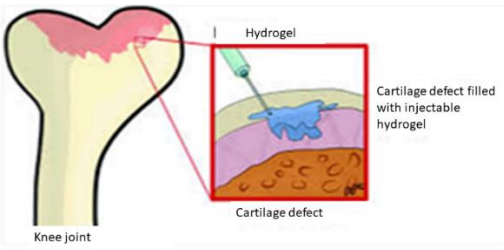

Figure No. 3: Hydrogels for cartilages regeneration

3.2. Delivery of therapeutic agents

Another extensive application of injectable hydrogels is their role as a carrier for the delivery of medicine, bioactive molecules, cells, and other therapeutic agents. (**Fig. 4)** Hydrogels are good Candidates for delivery because of their water-swollen porous structure, which provides

an appropriate environment for bioactive molecules and cells, allowing their controlled release [39,40]. Hydrogels also can be designed for the targeted delivery of therapeutic agents. These conventional hydrogels may contain toxic crosslinkers and catalysts, whereas some injectable hydrogels are often physically cross-linked to render them more biocompatible. Moreover, better homogenous encapsulation and minimal invasive administration are additional advantages of injectable hydrogels for this application [41, 42].

Among these, stimuli-responsive hydrogels can eject therapeutic drugs by changing their physical or chemical conditions (e.g. shrinkage) in response to external changes to their environment [43]. Double-walled microparticles loaded with anticancer drugs and embedded in an injectable alginate hydrogel showed superior results compared with free drugs for the treatment of breast carcinoma [44]. An insulin-loaded injectable gel composed of carboxymethyl-hexanoyl chitosan and integrated lysozyme nanoparticles was used for the management of problems associated with diabetes [45].

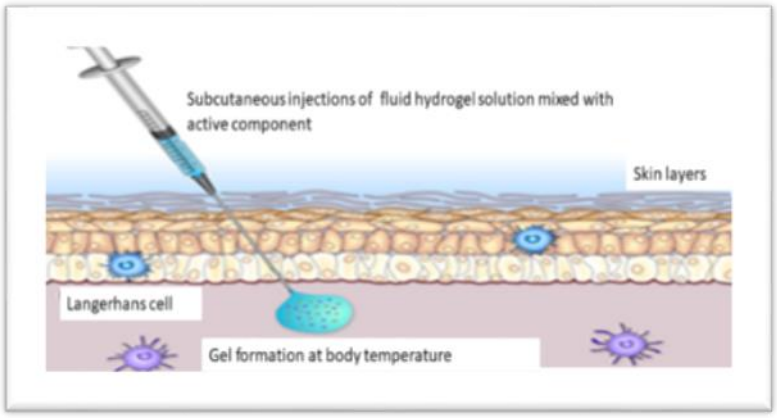

Figure No. 4: Delivery of therapeutic agents by Injectable hydrogels

3.3. For Minimally Invasive Surgery

The use of injectable hydrogels is again for endoscopic submucosal dissection and vascular embolization. Endoscopic submucosal dissection (ESD) may be a minimally **invasive** surgical procedure for the removal of early-stage tumors located in the alimentary canal. ESD separates the tumor from the muscular layer and helps to facilitate tumor removal[46].

3.4. Micro- And Nanocomposite Injectable Hydrogels

The use of micro-and Nanofillers has emerged as a strategy for generating hydrogel systems equipped with sets of advanced properties. It should be noted that the integration of nanomaterials into the structural network not only provides the hydrogel with properties of the fillers but also contributes to determining the bulk mechanical and biological behavior. For instance, mechanical reinforcement of hydrogels, measured as the increase of the elastic modulus, can be obtained following this strategy[47,48].

4. Advantages

- It is easy in handling.
- It can reach very deep tissue defects.
- It is minimally invasive.
- It has excellent defect margin adaptation.
- It offers to sustain the release of medicament from several days to a month and reduces the need for frequent dosing, thus increase patient compliance.
- An injectable hydrogel is clinically more convenient and simple to be used in traditional hydrogels.
- Multi-functional injectable hydrogels are capable of entrapping and delivering multiple therapeutic agents.
- They are porous in nature and allow nutrient transport.
- They have an aqueous environment for cells.
- They are easily biocompatible.

5. Disadvantage

- It’s more expensive.
- They are physically weak.

- It is difficult to sterilize it.

CONCLUSION:

Injectable hydrogel is often used for a controlled release of protein drugs like insulin by subcutaneous injection. There are various advantages including direct injection without any surgical procedure, no clogging during injection, straightforward drug loading to the polymer solution. Also dry powder form, easy to dissolve, easy sterilization by UV, simple dose adjustment, systemic biocompatibility with no inflammatory reaction, as well as less requirement of organic solvents during fabric. It has minimally invasive biomedical procedures, including endoscopic submucosal dissection, vascular chemoembolization, tissue engineering, and neural and cardiac tissue repair.

The clinical application of hydrogels remains limited by their mechanical properties and difficulties in sterilization. Commercially available examples of hydrogels are Granugel (ConvaTec) and Aquaflo (Covidien) used in wound healing. To the best of our knowledge, no injectable hydrogel within the surgical application has yet reached the market. Despite the pathway toward materials that can be clinically applied being studied with hurdles, researchers have identified chemistries, polymer chain lengths, and module concentrations that allow for tuning the mechanical behavior of the material and introducing advanced properties, for example, antimicrobial activity, self-healing, adhesiveness, and conductivity.

FUTURE PROSPECTIVE:

A wide range of injectable hydrogels is prepared and evaluated for various biomedical applications. Design approaches for the development of injectable hydrogel are based on *in situ* gelations and the shear-thinning behavior of hydrogels. While *in situ* gelations supports effective circumferential coverage of defects and host integration, the use of shear-thinning hydrogels avoids the involvement of any external problem. Gelling time and the nature of triggers are critical parameters for *in situ* gelling systems, whereas self-assembling processes flow under moderate pressure, and self-healing after injection dictates the success of shear-thinning hydrogels.

Attempts have been made to improve the mechanical properties of injectable systems by employing multitriggers and multi-crosslinking methods. The concept of shear-thinning hydrogel is comparatively new within the field of injectable hydrogels and studies are mostly

restricted to *in vitro*. Very few systems are reported to be used*in vivo*, having satisfied all the physical, structural, and mechanical properties. So far, the majority of studies conducted on injectable hydrogels have been short-term and conducted on animal models.

Decellularized extracellular matrix is another exciting strategy that has been used for making shear-thinning hydrogels and has demonstrated tissue regeneration potential. Safety concerns and sterility issues related to decellularized ECM must be addressed before the wide use of such systems is possible. Tracking the material upon injection and erosion by imaging approaches will prove beneficial. There are only a couple of products supported by injectable hydrogels currently available on the market, apart from those used as soft tissue augmenting agents and drug delivery depots. The designing of biomimetic hydrogels having tunable mechanical, gelation (for *in situ* gelling systems), self-healing (for shear-thinning systems), and degradation properties is essential for successful clinical translation of injectable hydrogels for various biomedical application.

REFERENCES

1. Van Vlierberghe S, Dubruel P, Schacht E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. Biomacromolecules, 2011;12(5):1387-408.
2. Mehrali M, Thakur A, Pennisi CP, Talebian S, Arpanaei A, Nikkhah M, Dolatshahi-Pirouz A. Nanoreinforced Hydrogels for Tissue Engineering: Biomaterials that are Compatible with Load-Bearing and Electroactive Tissues. Advanced Material, 2017; 29(8):1-26.
3. Tsou YH, Khoneisser J, Huang PC, Xu X. Hydrogel as a bioactive material to regulate stem cell fate. Bioactive Mater, 2016; 1(1):39-55.
4. Singh NK, Lee DS. In situ gelling pH- and temperature-sensitive biodegradable block copolymer hydrogels for drug delivery. J Control Release, 2014;193:214-27.
5. Dimatteo R, Darling NJ, Segura T. In situ forming injectable hydrogels for drug delivery and wound repair. Adv Drug Deliv Rev., 2018;127:167-184.
6. Sontyana AG, Mathew AP, Cho KH, Uthaman S, Park IK. Biopolymeric In Situ Hydrogels for Tissue Engineering and Bioimaging Applications. Tissue Eng Regen Med. 2018 ;15(5):575-590.
7. 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.,2017;23(24):3595-3602.
8. Wang K, Han Z. Injectable hydrogels for ophthalmic applications. J Control Release.,2017;268:212-224.
9. Cascone S, Lamberti G. Hydrogel-based commercial products for biomedical applications: A review. Int J Pharm, 2020;573:118803.
10. Malam Y,Loizidou M.Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer.Trends in pharmacological sciences,2009;30(11):592-599
11. Cirillo G, Spizzirri UG, Curcio M, Nicoletta FP, Iemma F. Injectable Hydrogels for Cancer Therapy over the Last Decade. Pharmaceutics,2019 ;11(9):486.
12. Bala I, Hariharan S, Kumar MN. PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst., 2004;21(5):387-422.
13. Duncan R. Development of HPMA copolymer-anticancer conjugates: clinical experience and lessons learnt. Adv Drug Deliv Rev., 2009;61(13):1131-48.
14. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. Adv Drug Deliv Rev., 2008;60(15):1638-49.

15. Park MR, Seo BB, Song SC. Dual ionic interaction system based on polyelectrolyte complex and ionic, injectable, and thermosensitive hydrogel for sustained release of human growth hormone. Biomaterials, 2013;34(4):1327-36.
16. Zhang X, Zhu Y, Cao L, Wang X, Zheng A, Chang, J. Alginate-aker injectable composite hydrogels promoted irregular bone regeneration through stem cell recruitment and osteogenic differentiation. Journal of Materials Chemistry B., 2018; 6(13), 1951–1964.
17. Bidarra SJ, Barrias CC, Granja PL. Injectable alginate hydrogels for cell delivery in tissue engineering. Acta Biomater, 2014;10(4):1646-62.
18. KudaibergenovS. &NurajeN. Intra- and Interpolyelectrolyte Complexes of Polyampholytes. Polymers, 2018; 10(10), 1146.
19. Tsitsilianis C, Serras G, Ko, Jung F, Papadakis, C. M, Rikkou-Kalourkoti M, Chassenieux C. Thermoresponsive Hydrogels Based on Telechelic Polyelectrolytes: From Dynamic to "Frozen" Networks. Macromolecule, 2018; 51(6), 2169–2179.
20. Lv X, Liu Y, Song S, Tong C, Shi X, Zhao Y, Zhang J, Hou M. Influence of chitosan oligosaccharide on the gelling and wound healing properties of injectable hydrogels based on carboxymethyl chitosan/alginate polyelectrolyte complexes. CarbohydrPolym, 2019;205:312-321.
21. Khunmanee S, Jeong Y, Park H. Crosslinking method of hyaluronic-based hydrogel for biomedical applications. J Tissue Eng, 2017;8:2041731417726464.
22. Dai X, Zhang Y, Gao L, Bai T, Wang W, Cui Y, Liu W. A Mechanically Strong, Highly Stable, Thermoplastic, and Self-Healable Supramolecular Polymer Hydrogel. Adv Mater, 2015;27(23):3566-71.
23. Ruel-Gariépy E, Leroux JC. In situ-forming hydrogels--review of temperature-sensitive systems. Eur J Pharm Biopharm, 2004;58(2):409-26.
24. Le T. M. D, Duong H. T. T, Thambi T, Giang Phan V. H, Jeong J. H, & Lee D. S. Bioinspired pH- and Temperature-Responsive Injectable Adhesive Hydrogels with Polyplexes Promotes Skin Wound Healing. Biomacromolecules, 2018 19(8), 3536–3548.
25. Mantha S., Pillai S., Khayambashi P., Upadhyay A., Zhang Y., Tao O., Tran S. D. Smart Hydrogels in Tissue Engineering and Regenerative Medicine. Materials, 2019;12(20), 3323.
26. Alexander A, Ajazuddin, Khan J, Saraf S, Saraf S. Poly(ethylene glycol)-poly(lactic-co-glycolic acid) based thermosensitive injectable hydrogels for biomedical applications. J Control Release, 2013;172(3):715-29.
27. Deng Z., Guo Y., Zhao X., Ma P. X., & Guo B. Multifunctional Stimuli-Responsive Hydrogels with Self-Healing, High Conductivity, and Rapid Recovery through Host–Guest Interactions. Chemistry of Materials. 2018; 30(5), 1729–1742.
28. Liu G., Yuan Q., Hollett G., Zhao W., Kang Y., & Wu, J. Cyclodextrin-based host–guest supramolecular hydrogel and its application in biomedical fields. Polymer Chemistry, 2018; 9(25), 3436–3449.
29. Constantin M., Bucatariu S., Ascenzi P., Simionescu B. C., &Fundueanu G. Poly(NIPAAm-co-β-cyclodextrin) microgels with drug hosting and temperature-dependent delivery properties. Reactive and Functional Polymers, 2014;84, 1–9.
30. Chang Y.-C., Chen Y.-D., Chen C.-H., Wen Y.-S., Lin J. T., Chen H.-Y.,Chao I. Crystal Engineering for π–π Stacking via Interaction between Electron-Rich and Electron-Deficient Heteroaromatics. The Journal of Organic Chemistry, 2008; 73(12), 4608–4614.
31. Sivashanmugam, A.; Arun Kumar, R.; Vishnu Priya, M.; Shantikumar V Nair; Dr. Jayakumar Rangasamy. An overview of injectable polymeric hydrogels for tissue engineering. Eur. Polym. J., 2015;72: 543–565.
32. Dong Y, Saeed AO, Hassan W, et al. "One-step" preparation of thiol-ene clickable PEG-based thermoresponsive hyperbranched copolymer for in situ crosslinking hybrid hydrogel. Macromolecular Rapid Communications, 2012;33(2):120-126.
33. Abandansari HS, Ghanian MH, Varzideh F, Mahmoudi E, Rajabi S, Taheri P, Nabid MR, 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.
34. Yang N., Wang Y., Zhang Q., Chen L., & Zhao Y.. In situ formation of poly (thiolated chitosan-co-alkylated β-cyclodextrin) hydrogels using click cross-linking for sustained drug release. Journal of Materials Science, 2018; 54 (2):1677-1691.
35. DeForest C. A., Polizzotti B. D., &Anseth K. S. Sequential click reactions for synthesizing and patterning

three-dimensional cell microenvironments. Nature Materials, 2009;8(8): 659–664.
36. Zheng J., Smith Callahan L. A., Hao J., Guo K., Wesdemiotis C., Weiss R. A., & Becker M. L. Strain-Promoted Cross-Linking of PEG-Based Hydrogels via Copper-Free Cycloaddition. ACS Macro Letters, 2012; 1(8): 1071–1073.
37. Bae K.H; and Kurisawa M;Recent advances in the design of injectable hydrogels for stem cell-based therapyJournal of materials chemistry B 7, 2019: 3775-3791.
38. Yan LP, Silva-Correia J, Ribeiro VP, Miranda-Gonçalves V, Correia C, da Silva Morais A, Sousa RA, Reis RM, Oliveira AL, Oliveira JM, Reis RL. Tumor Growth Suppression Induced by Biomimetic Silk Fibroin Hydrogels. Sci Rep, 2016;6:31037.
39. Huiyuan Wang, Danqing Zhu, Alexandra Paul, Lei Cai, Annika Enejder, Fan Yang, Sarah C. Heilshorn. Covalently adaptable elastin-like protein-hyaluronic acid (ELP-HA) hybrid hydrogels with secondary thermoresponsive crosslinking for injectable stem cell delivery. Adv. Funct. Mater, 2017;27(28): 1605609.
40. Qu J, Zhao X, Ma PX, Guo B. pH-responsive self-healing injectable hydrogel based on N-carboxyethyl chitosan for hepatocellular carcinoma therapy. Acta Biomater, 2017;58:168-180.
41. Shen Y., Li X., Huang Y., Chang G., Cao K., Yang, J. pH and redox dual stimuli-responsive injectable hydrogels based on carboxymethyl cellulose derivatives. Macromolecular Research. 2016; 24(7): 602–608.
42. Xu J, Liu Y, Hsu SH. Hydrogels Based on Schiff Base Linkages for Biomedical Applications. Molecules, 2019;24(16):3005.
43. Quang Vinh Nguyen, Dai Phu Huynh, Jae Hyung Park, Doo Sung Lee,Injectable polymeric hydrogels for the delivery of therapeutic agents: A review. Euro Polyp J, 2015; 72:602-19.
44. Mathew AP, Uthaman S, Cho KH, Cho CS, Park IK. Injectable hydrogels for delivering biotherapeutic molecules. Int J Biol Macromol, 2018;110:17-29.
45. Kiamahalleh MV, Mellati A, Madani SA, Pendleton P, Zhang H, Madani SH. Smart Carriers for Controlled Drug Delivery: Thermosensitive Polymers Embedded in Ordered Mesoporous Carbon. J Pharm Sci, 2017;106(6):1545-1552.
46. Davoodi P., Ng W. C., Yan W. C., Srinivasan M. P., & Wang, C.-H.. Double-Walled Microparticles-Embedded Self-Cross-Linked, Injectable, and Antibacterial Hydrogel for Controlled and Sustained Release of Chemotherapeutic Agents. ACS Applied Materials & Interfaces, 2016; 8(35): 22785–22800.
47. Chou HS, Larsson M, Hsiao MH, Chen YC, Röding M, Nydén M, Liu DM. Injectable insulin-lysozyme-loaded nanogels with enzymatically-controlled degradation and release for basal insulin treatment: In vitro characterization and in vivo observation. J Control Release, 2016;224:33-42.
48. Jalili NA, Jaiswal MK, Peak CW, Cross LM, Gaharwar AK. Injectable nanoengineered stimuli-responsive hydrogels for on-demand and localized therapeutic delivery. Nanoscale, 2017;9(40):15379-15389.

Image Author -1	Chaitanya. A Gulhane Assistant Professor, Dr.Rajendra Gode Institute of pharmacy,Amravati Mardi Road, Amravati, Dist. Amravati, MS, India Pin 444 602
Image Author -2	Gokul P. Tayade Student of B.pharm final year Dr.Rajendra Gode Institute of pharmacy,Amravati
Image Author -3	Ashvini Y. Parbat Assistant Professor, Dr.Rajendra Gode Institute of pharmacy,Amravati
Author-4	Shivam A. Bartare Lecturer, Dr.Rajendra Gode Institute of pharmacy,Amravati
Image Author -5	Jagdish V. Manwar Principal, Dr.Rajendra Gode College of pharmacy,Amravati
Image Author -6	Ravindra L. Bakal. Principal, Dr.Rajendra Gode Institute of pharmacy,Amravati