



# Designing Multifunctional Carrier for Regenerative Medicines Based on Scaffolding Hydrogel System Loaded With Natural Antimicrobial Agents Extracted from Various Plant Sources

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## ABSTRACT:

Hydrogels have gained fabulous biochemical and mechanical property, which has made them highly attractive focal points within the field of wound dressings. However, an extensive review of the utility of hydrogel as a dressing for wounds is still pending. This chapter initially summarizes the skin wound healing process and then ties evaluation parameters and subsequently evaluates the advanced capabilities of hydrogel dressings, such as antimicrobial property, adhesion and hemostasis, anti-inflammatory and anti-oxidation, substance transport, self-healing, enhancement of response, conductivity, and the newly recognized wound monitoring include, and the processes taken to obtain these capabilities are all classified and discussed. In addition, applications of hydrogel wound dressing for treatment of various types of wounds, such as incisional wound and the excisional wound are summarised.

**Keywords:** Hydrogel, scaffolds, new drug delivery carrier, plant extraction, keratin, polymers

## 1. Introduction:

Scaffolds have three-dimensional cross-linked polymer network. They are hydrophilic in nature. They are unique material because they can absorb and retain nearly about 10-20 times water of its molecular weight without dissolving and hence become swollen. The potential of hydrogels of content water comes from hydrophilic functional groups which is attached to the polymer structure. These hydrogels are smart enough that they give response in the environmental changes such as ionic strength, pH, temperature, presence of enzyme, electric field and shrink or swell accordingly [1]. Hydrogel technology used in many different fields such as food additives, pharmaceuticals drug carrier, biomedical implant tissue engineering, etc. In pharmacy it is a revolutionary product which has been explored in many promising applications such as wound healing, reduce chances of infection, wound dressing, reduce pain and as the skin substitute. It can show a critical role in controlling the wound environment and providing a suitable medium for the delivery of biomolecules [2].

### Ideal characteristics of a hydrogel must be:

- Maintain a moist environment around the wound.
- Absorb the wound exudate.
- Prevent micro-organism to infect the wound.
- Allow the wound for the gaseous exchange.
- Should be non-toxic, non-allergic and biocompatible.
- It must be easy to remove without any trauma to the wound.

Due to its simplicity in manufacturing and self-application hydrogels have been conventionally used as a drug carrier. They are capable of delivering genetically engineered proteins and peptides. Hydrogels may be synthesized in many chemical ways. These include one-step procedures such as polymerization. The parallel cross-linking of multifunctional monomers, by reacting polymers with suitable crosslinking agents and as well as multiple-step procedures involving the synthesis of polymer molecules having reactive groups and their subsequent cross-linking agents [3].

### Advantages of Scaffolds:

- They are bio-compatible and also bio-degradable in nature.
- For preventing drug loss applied topically to bypass the first-pass metabolism.

- c) Sustained and prolonged action compared to conventional doses.
- d) Decreased side-effects and dose of administration.
- e) Improved drug utilization and patient compliance.
- f) Lower daily cost due to fewer dosage is required.
- g) They pose good transport properties and easy to modify.

#### Disadvantages of Scaffolds:

- a) It can cause a sensation due to the movement of maggots.
- b) Include thrombosis at the anastomosis site.
- c) Hydrogel in contact lenses can cause hypoxia, dehydration and eye reaction.
- d) They are non-adherent and must be secured by the secondary dressing.
- e) Have low mechanical strength and difficult to handle [4].

#### 1.1 Hydrogels and their properties:

Main properties of hydrogels are -

i) **Swelling** – Hydrogels are mainly hydrophilic in nature, so their most common property is swelling. A small environmental change like an electrical signal, pH, any kind of ionic changes and temperature leads to change in the physical structure of a hydrogel (Divya Juyal, et al. 2017). This is the fundamental property of a hydrogel. Some hydrogels change their volume by swelling but some undergo changes between gel phases and solution phases. (Lee and Park et al. 1996). When a hydrogel swells it becomes rubbery and more elastic. The degree of cross-linking decides the capacity of up taking the water [5].

The swelling ratio can be measured by –  $(M_{\text{hydrated}} - M_{\text{dehydrated}}) / M_{\text{dehydrated}}$

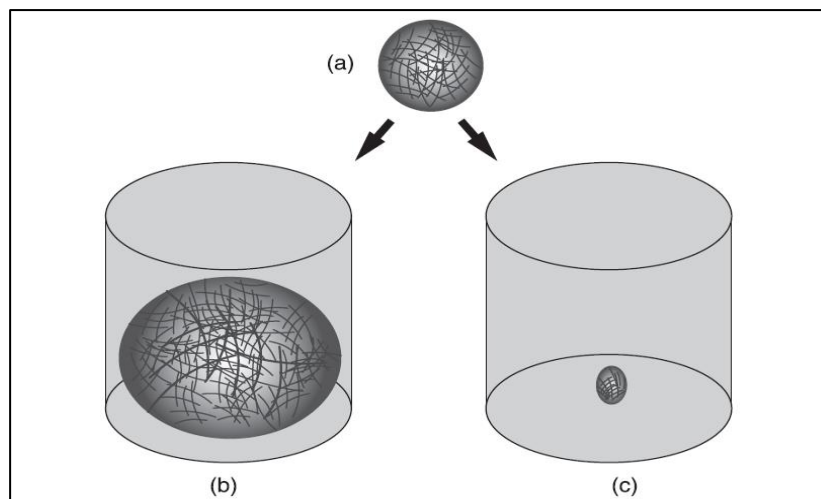


Fig. 1: a) dehydrated, b) swollen, c) shrunken hydrogels as the changes in the environment and influence the hydrophilicity.

Source: Hydrogel swelling behavior and its biomedical applications H. HOLBACK, Y. YEO and K. PARK, Purdue University, USA

Here M represents the mass of the Hydrogel. Interaction between water and hydrogel leads to swelling instead of dissolving in the water because of the network of cross-linked hydrophilic polymers [6]. There are two major theories to determine the water content in a hydrogel –

a) Equilibrium swelling theory – The Flory-Rehner equation describes that mixing of liquid and polymer molecules can be used to analyzed hydrogels without ionic domains. The status of the swelling hydrogel in a fluid is determined by two forces. One is thermodynamic force and another is the force that stretched polymer chains.

b) Rubber elastic theory – From a mechanical perspective, hydrogels act as a natural rubber that prevents change during stress. Flory and Treloar use the elastic properties to describe the structure of a hydrogel [7].

ii) **Mechanical properties**- Mechanical properties depends on the degree of crosslinking. Expected mechanical properties can be established by the increasing or decreasing of the crosslinking. If the crosslinking is increased, hydrogels become more brittle in structure. Mechanical strength can be increased by using different polymers or cross-linkers like glutaraldehyde, keratin, etc[8].

iii) **Biocompatibility**- It is very important if we are going to use any hydrogel products on humans. Biocompatibility mainly depends on the polymers, especially any natural polymer like chitosan, guar-gum etc. It should be nontoxic in nature if we want to use in biomedical fields. Cytotoxicity methods can be used in cell culture method to evaluate the toxicity of hydrogel [9].

According to the crosslinking hydrogels can be classified as –

### 1.1.1 Physical hydrogel:

Physically cross-linked hydrogels have weak junctions which may be vulnerable. This types of chains are prepared by physical interaction as hydrogen bonds, polymer chain entanglements and hydrophobic interaction. Physical hydrogels are in-able to completely reform and if dissociates it may be responsible for the increased rate of drug release. These types of hydrogels affect the pore size and may remain enlarged that delivers uncertain amounts of drug molecules. The flexibility of a hydrogel depends on the crosslinking of the chemical bonds [10].

In physical hydrogel, occurs weak cross-linking and provides high flexibility. Xanthan paint, polymer-polymer complexes, mature acacia gum, etc. used to prepare hydrogel but they form weak bonds. Elastomers, block co-polymers, gelatines are used for the strong bond formation in physical hydrogels. This produces glassy modules, lamellar microcrystals, double/ triple helices which give strength to bonds and produce a mechanically strong hydrogel. Physical hydrogels obtained self-recovery and shear-thinning characteristics which provides great potential in bio-medical injection and 3D printing [11].

### 1.1.2 Chemical hydrogel:

Hydrogels can be prepared in different ways but through the chemical crosslinking it develop strong bonds and create permanent junctions. In this process, two different polymers are linked together with the help of a crosslinking agent. These types of cross-linking can be achieved by the reaction of their functional groups such as COOH, OH and NH<sub>2</sub> with the cross-linker such as glutaraldehyde[12]. These chemical crosslinking can be classified as crystalline, semi-crystalline and amorphous [13]. Hydrophobic interactions are also happening in chemical hydrogels where it is used as a self-healing hydrogel. These types of hydrogels have a high degree of toughness due to the strong bond and long-time of hydrophobic interaction. Hydrogels can respond in different types of environmental changes like light, thermal, pH based on cross-linked network and chemical structure. This can be useful for many biomedical applications like drug delivery systems, targeted dry delivery and wound healing, etc [14].

Chemical hydrogels are mainly prepared by the covalently cross-linked bond which provides mechanical stability and elasticity. But it lacks self-recovery due to irreversible cross-linking methods. The bond strength of physical hydrogels can be converted into chemical hydrogels by condensation (Critical percolation), the addition of kinetic growth agents (Poly-divinylbenzene), grafting (CMC-g-acrylic acid) and cross-linkers (cis-polyisoprene, glutaraldehyde)[15].

### 1.1.3 Composite hydrogel:

Composite hydrogels are the mixtures of natural and synthetic polymers. Natural polymers have huge advantages in the biomedical application such as they are bio-degradable, bio-compatible, non-toxic for the human body. On the other hand, synthetic polymers are good for increased mechanical strength and give the hydrogel a desired characteristic by changes in the amount of specific synthetic polymers. These two types of polymers used to prepare composite hydrogels to achieve the goodness of natural polymer and the stability of the synthetic polymer. [16].

Composite hydrogels show good water retention properties, equilibrium-swelling ratio, excellent air permeability and desired mechanical properties which applies to the practical application like tissue engineering [17]. These types of hydrogels are more preferable for the drug delivery system as a hydrogel-based transdermal product, microspheres or matrices. Bio-active materials that have hydrophobic nature entrapped within called hydrophobic micro-domains to protect it from the environment as well as leaking. These composite materials had two phases – the continuous phase and the dispersed phase. Continuous phase may be hydrophilic such as hydrogel polymers which are cross-linked together and another one is dispersed phase which may be hydrophobic in nature such as fatty-acid, oil, wax, etc. Both phases are not miscible but absorbable [18].

Composite hydrogels also used for the environment such as hydrogel-silica composite. This composite is mainly used for the purification of water by absorption of the metal pollutants and other ionic impurities. Hydrogel-silica composite is stable in pH, temparaure and other environmental stimuli [19].

Polypeptide and heparin composite hydrogels are prepared for cell growth and wound healing properties. Poly (L-lysine) is chosen as a polymer because it contains only cationic charge and heparin contains anionic charge helps in the crosslinking process and also helps to tune the physical and mechanical properties very easily. It shows good cell compatibility, low hemolytic activity as well as antibacterial activity. The reason of using heparin is it induced heparin-binding FGF, EGF and VEGF which helps in cell growth [20].

## 1.2 Polymers used for hydrogel preparation:

### 1.2.1 Classification of polymers

We can classify the polymers according to their source-

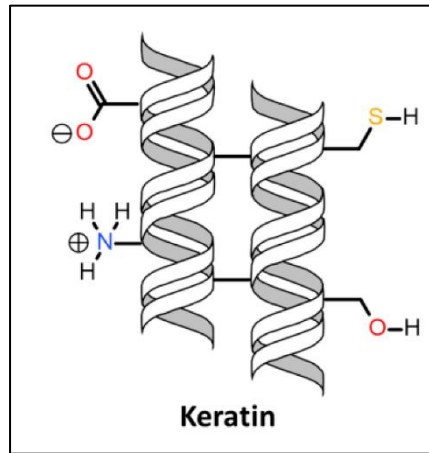
**a) Natural polymers-** Those polymers are found in nature like plants or animal sources.

#### Advantages of natural polymers:

1. Natural polymers can be effectively biodegradable and they don't produce hurt impacts on individuals.
2. Biocompatible-their rate of disgrace is commonly conversely relative to the degree of synthetic modification.
3. Condensation polymerization is the procedure by which normally regular polymers are synthesized.
4. Non-harmful/non-fiery – the entirety of this material are starch or protein in natures and made out of rehashing monosaccharide or amino corrosive units separately. Henceforth they are non-poisonous.
5. Highly permeable.
6. Easy and modest to planning and creation in correlation with manufactured polymers [21].

**Example:****i) Keratin-**

Keratins are the natural proteins as well as polymers mainly found in the epidermal layer of skin, hair, nails, etc. Humans also tried to extract keratin from animals like animal skin, horns, feathers, claws and from animal hair and wools. Natural polymers have highly reactive chemical groups (hydroxyl, carboxyl, amide, sulfhydryl) which make them more hydrophilic and helps to interact with bioactive molecules.

**Fig. 2: Structure of Keratin**

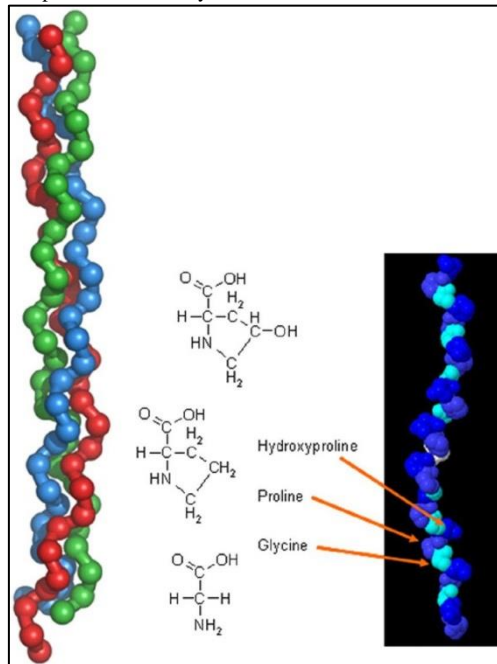
Source: Donato, R.K. and Mija, A., 2020. Keratin Associations with Synthetic, Biosynthetic and Natural Polymers: An Extensive Review. *Polymers*, 12(1), p.32.

**Use of keratin in Bio-medical application-**

- a) Use in the formation of film type materials.
- b) It is used as a coating material.
- c) Preparation of scaffolds
- d) Preparation of hydrogels [22].

**ii) Collagen-**

Collagen is a Greek word, where kola means “Glue” and gen means “Producing”. Collagen is an ideal matrix for tissue engineering because it is a protein-based natural polymer. As it is a polymeric protein so it mainly consists of an amino acid chain.

**Fig. 3: Structure of collagen.**

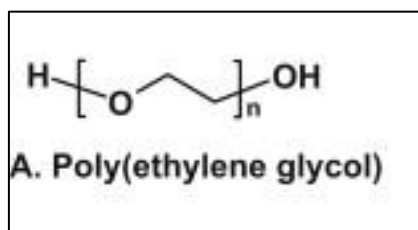
Source: Ileana, R. and Francois, K., 2014. Biopolymers for application in photonics. NBI-technologies, (4).

It is found in our bone, tissue, muscles, tendons, blood vessels, ligaments. More than 25 collagentypes are found till the date. Collagen typeI is the most abundant and most investigated biomedically approved protein used in medical research work. Procollagen is the first synthesized collagen which is a long-chained molecule and has numbers of physical and chemical properties that are suitable in pharmaceutical industries [23].

**b) Semi-synthetic polymer-** These are the natural polymers but they are chemically modified. They are more stable than any natural polymers.

E.g. – Cellulose nitrate, Cellulose acetate, Cellulose derivatives, Polyethylene glycol (PEG) etc.

Polyethylene glycol (PEG) – It is a poly-ether compound that is used as a polymer in medical industries. According to its molecular weight, it can be polyoxyethylene (POE) or polyethylene oxide (PEO). It is mainly used as excipients in medicines.



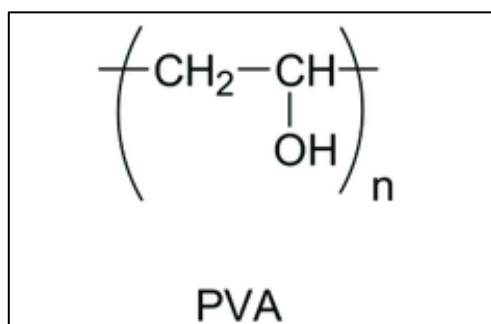
**Fig. 4: Structure of PEG**

Source: Zuckerkandl, E. and Pauling, L., 1965. Evolutionary divergence and convergence in proteins. In *Evolving genes and proteins* (pp. 97-166). Academic Press.

**c) Synthetic polymer-** They are mainly chemically based. Almost all pharmaceutical work we used this type of polymer because it is stable in nature and the properties are well known.

e.g. - Synthetic rubber, polyvinyl alcohol (PVA), etc.

Polyvinyl alcohol (PVA) – It is a synthetic polymer and good water-soluble. PVA has low toxicity level, good bio-compatibility and low propensity in protein adhesion. It is used in eye drops, contact lenses, hydrogel preparation and as an aid in suspension polymerization[24].



**Fig. 5: Structure of PVA**

Source: Higa, M., Kakihana, Y., Sugimoto, T. and Toyota, K., 2019. Preparation of PVA-Based Hollow Fiber Ion-Exchange Membranes and Their Performance for Donnan Dialysis. *Membranes*, 9(1), p.4.

### 1.3 Classification of hydrogels:

#### a) Based on polymeric composite:

**i) Homo-polymer** – These types of polymers derived from a single kind of monomer, which is a basic structural unit of any polymer network. It may have a crossed-linked structure which depends on the nature of the monomer and also in polymerization technique.

E.g. - These types of polymer can be synthesized by using polyethylene glycol dimethacrylate (as a cross-linker) and a mono polymer, poly HEMA (2-hydroxyethyl methacrylate). Benzoin isobutyl ether can be used as an ultra-violet sensitive agent.

It is mainly be used in contact lenses. Now a days, it is applied in artificial skin manufacturing, bone marrow and spinal cord cell regeneration, scaffolds for promotion of cell adherence and wound dressing. It always shows good wound healing properties.

**ii) Co-polymer** – These types of polymers are composed of two same or more different monomers with minimum one hydrophilic component. It can be arranged as a random or block manner.

E.g. - They can be prepared by polymerization of BLG N-carboxyanhydride conjugate with diamine group which is placed at the end of the polymer chains. These types of hydrogels were pH and temperature-sensitive. It is also used as a carrier for drug delivery systems.

**iii) Multi-polymer interpenetrating polymeric hydrogel (IPN)** - It is an important type of hydrogels which is prepared by two independent crossed-linked polymers. Polymers can be either synthetic or natural. After polymerization network like structure can be observed. In a semi IPN hydrogel, one

of the components is the cross-linked polymer and another is a non-cross-linked polymer. Many hydrogels faced thermodynamic incompatibility because of the permanently interlinked network segments and for this phase separation is limited. This IPN method can solve this problem. The temporary interlock structure of IPN hydrogel provides a huge amount of stability for the bulk and the surface morphology. These have some advantages such as:

- a) Provide tough mechanical properties.
- b) Controllable physical properties.
- c) Efficient drug loading with minimum loss.

**iv) Interpenetrating network** – There is a combination of two polymers where one is synthesized or cross-linked in the presence of others. A polymerization initiator and some suitable monomers are placed during the preparation to get the desired hydrogel. This method prepared stiffer hydrogels with tough mechanical properties and more efficient for drug loading than any other conventional method of hydrogel preparation [25].

#### **b) Based on the source:**

**i) Natural hydrogels** – Natural hydrogels are mainly harmless and have good biocompatibility and good cell adhesion properties. They are also biodegradable in nature. There are three types of natural polymers which are used to synthesize hydrogels such as protein (Gelatin, collagens, etc.), polysaccharides (Chitosan, alginate, etc.) and those which are derived from decellularized tissue. Natural hydrogels are mainly synthesized from proteins are very efficient because it makes the gel more bioactive and biocompatible. They are more suitable for biomedical applications for their ability to promote cell functions. These types of hydrogel's structure and properties are similar to the cells where it applies. The source of this type of component is mainly extracted from animal parts like bones, tails and also from some insects [26].

**ii) Synthetic hydrogel** – These types of hydrogels are more useful than natural ones because they can be engineered for achieving the verities of chemical and mechanical properties. It is very useful because these types of hydrogels lack any kind of toxicity, bio-incompatibility as well as immunogenicity problems. Polyethylene glycol (PEG) does not show these types of properties. So, it is widely used to prepare synthetic hydrogels. Poly (hydroxyethyl methacrylate) (HEMA), Polyvinyl alcohol (PVA), Polyacrylic acid (PAA), Polymethacrylic acid (PMMA) are some examples of polymers that are intended to synthesize hydrogels [27].

**iii) Hybrid hydrogel** – These types of hydrogels are prepared with both synthetic and natural polymers. For this combination, hydrogels get some advantages like an increase in mechanical strength, durability and improve stability. Natural polymers like chitosan, dextran and collagens combined with synthetic ones such as poly (N-isopropyl acrylamide) and polyvinyl alcohol (PVA) to achieve hybrid hydrogel [28].

#### **c) According to bio-degradability:**

**i) Bio-degradable hydrogel** – Mainly natural polymers such as fibrin, agar and chitosan are bio-degradable polymers that are used to prepare these types of hydrogels. Some synthetic polymers are also bio-degradable such as polyamides, N-isopropyl acrylamide, Polyaldehyde guluronate, etc. These types of hydrogels are used in different sectors like veterinary, environmental, agriculture and in pharmaceutical applications (for controlled release of drug delivery and targeted drug delivery). In the pharmacy field, it provides some interesting advantages such as improved flexibility, good diffusion properties of drugs like protein as well as synthetic, provides good stability and enhanced biocompatibility. These types of hydrogels degrade in a living body with different procedure – solubilization, chemical hydrolysis, enzymatic hydrolysis, Ionising hydrolysis.

**ii) Non-biodegradable hydrogel** – These types of hydrogels are mainly prepared from synthetic polymers such as 2- hydroxypropyl methacrylate (HPMA), 2- hydroxyethyl methacrylate (HEMA), acryl amide (AAM) and methoxyl poly (ethylene glycol) (MPEG). The problem of these types are, they are mainly used externally especially tropically. It has to remove physically after a certain period of time [29].

#### **d) Classification based on configuration:**

**i) Amorphous (Non-crystalline)** – These are the formulation of water, polymer and other ingredients, which do not have any shape. This can contain lots of water which helps to rehydrate tissue wounds and provide quick recovery. These types of hydrogels have some good properties like cooling actions which reduce pain, non-adherent, rehydrate the wound, easy application and removal. Amorphous hydrogels are mainly used for primary wound dressing in treatment and also can be used in minor burns, deep wounds, radiation dermatitis and wound with necrosis.

**ii) Semi-crystalline** – This type of hydrogels are mainly mixtures of the crystalline and amorphous phase. They are produced by photopolymerization. It has good mechanical properties. These hydrogels have shape memory function when heat applies.

**iii) Crystalline** – Crystalline hydrogels consist of a 3D colloidal crystalline structure. They are formed by the physical accumulation of polymer chains. This accumulation caused by hydrogen bonding. It also creates crystallization, helix-like structure and shows complexation. This type of hydrogels are prepared with poly(N-isopropylacrylamide) (PNIPAM) and N-hydroxymethylacrylamide (NMA) which are mixed together and allowed to dry in room temperature then it shows a crystalline form in the structure [30].

#### **e) According to physical appearance:**

Depending on the process of polymerization, hydrogels can appear as:

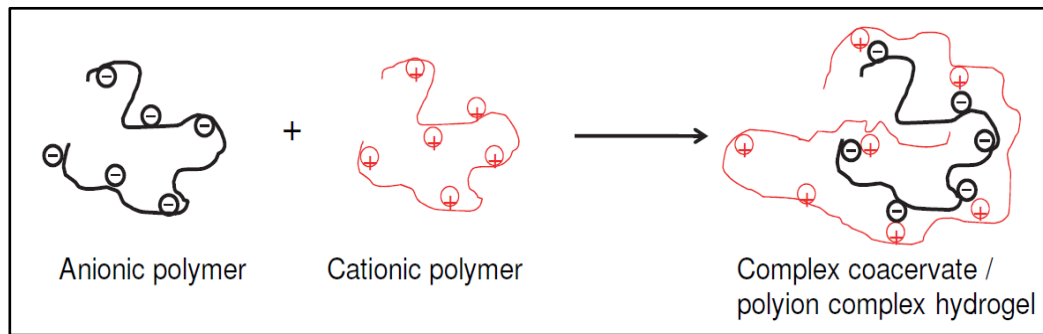
- I. Matrix
- II. ii) Film
- III. iii) Microsphere

#### **f) Classification according to the network electrical charge:**

They are classified in four groups on the basis of electrical charges on their cross-linked chains. They are:

- I. Non-ionic (Neutral)

## II. ii) Ionic (Include cation and anion)



**Fig. 6: Hydrogel formation with different ionic charge**

Source: Argin-Soysal, S., Kofinas, P. and Lo, Y.M., 2009. Effect of complexation conditions on xanthan–chitosan polyelectrolyte complex gels. Food hydrocolloids, 23(1).

iii) Amphoteric – containing both acid and basic groups.

iv) Zwitter ions – contain both anionic and cationic groups.

**g) According to the mechanism of controlled drug release system:**

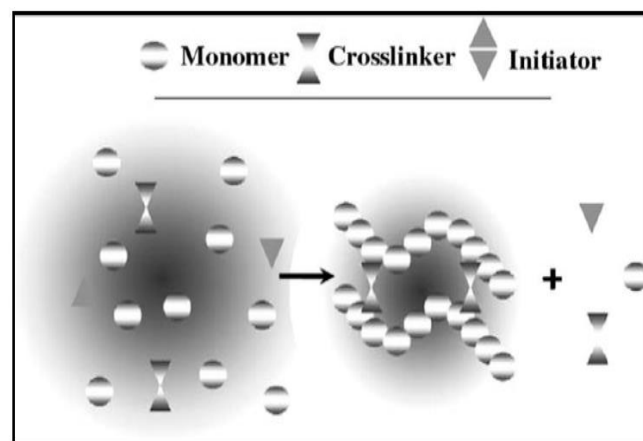
- i) Swelling controlled released system
- ii) Environment response system
- iii) Chemically controlled release system
- iv) Diffusion controlled released system
- v) The ionic control release system
- vi) The enzymatic controlled release system

**1.3.1 Different techniques for preparation of hydrogel:**

Hydrogels are formed with polymer networks that consist of hydrophilic properties. Sometimes it consists of both hydrophilic and hydrophobic monomers to regulate the properties as per desire. Multifunctional cross-linkers also used with polymers by using co-polymerization. There are three ways to form a hydrogel:

- i) Ionizing radiation
- ii) Linking polymer chains by chemical reactions
- iii) Physical interaction such as crystallization, entanglements and electrostatics.

There are three parts in a hydrogel are monomer, initiator and cross-linker.



**Fig. 7: Schematic diagram of hydrogel preparation**

Source: Jeong, B., Kim, S.W. and Bae, Y.H., 2012. Thermosensitive sol-gel reversible hydrogels. Advanced drug delivery reviews, 64.

These hydrogels can prepare in many ways like:

**i) Bulk polymerization:**

These are formed with one or different types of vinyl monomers. A small number of cross-linkers are included in the preparation. For the initiation of polymerization reaction radiation, ultra-violet or chemical catalysts are needed. They can be formed in different shapes and sizes include films, particles, emulsions, rods and membranes.

**ii) Free radical polymerization:**

Acrylates, amides and vinyl lactams are the monomers mainly used in this kind of preparation. These polymers have ideal functional groups which help to functionalization of the radical polymerization groups. These types of polymerization include chain transfer, propagation, initiation and termination like steps. Ultra-violet, thermal radiation and redox initiators are used to convert the monomers into active forms. These active monomers conjugate together and do the propagation, transfer of radicals and at last termination to stop the process when hydrogels are prepared and no monomers left for the reaction [31]

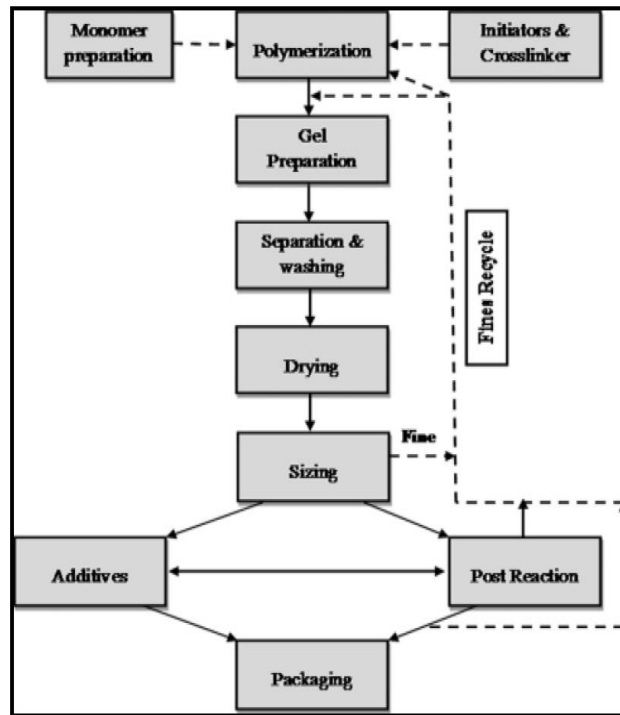


Fig. 8: Solution polymerization preparation diagram

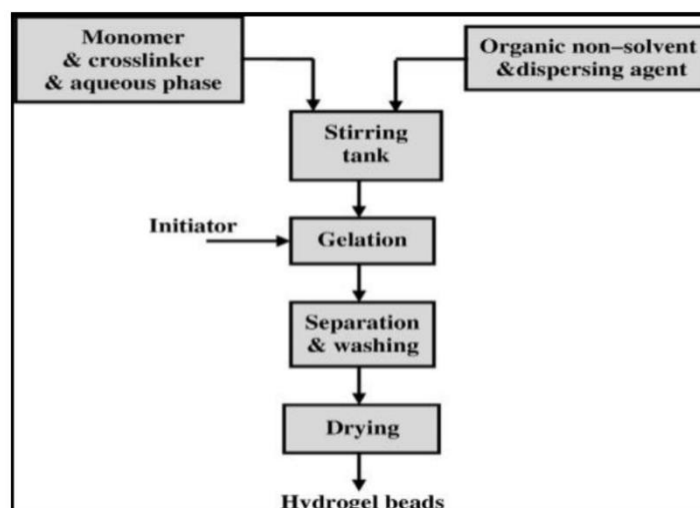
**iii) Solution polymerization or Cross-linking:**

Source: Chen, J., An, R., Han, L., Wang, X., Zhang, Y., Shi, L. and Ran, R., 2019. Tough hydrophobic association hydrogels with self-healing and reforming capabilities achieved by polymeric core-shell nanoparticles. *Materials Science and Engineering: C*, 99

Ionic or neutral monomers are used to prepare this type of hydrogel with the help of the multifunctional cross-linking agent. The initiators of this polymerization are uv-irradiation, thermal radiation or by redox initiator. This has a major advantage over bulk polymerization because the solvent used as a heat sink in this preparation. The prepared hydrogels are washed with distilled water throughout to remove extractable polymers, unused cross-linkers and impurities. The solvents are mainly used in these preparations are ethanol, water, benzyl alcohol or water-ethanol mixtures.

**iv) Suspension polymerization or inverse-suspension polymerization:**

Fig. 9: Preparation of suspension polymerization





Source: Pakdel, P.M. and Peighambardoust, S.J., 2018. Review on recent progress in chitosan-based hydrogels for wastewater treatment application. *Carbohydrate polymers*, 201, pp.264-279.

Grinding is not required because the product is already obtained as powder form or as a microsphere (Beads). For this preparation, if the water-in-oil (W/O) is chosen then it is referred as Inverse suspension and for Oil-in-water (O/W) it is suspension polymerization. In, O/W preparation initiators and monomers are dispersed in a hydrocarbon phase in a homogenous mixture. To maintain the viscosity, dispersant type and agitation speed of the hydrogels resin particle size and shape is considerable. The dispersion is thermodynamically unstable and to stabilize it needs the addition of low hydrophilic-lipophilic-balance (HLB) suspending agent with continuous agitation[32].

#### v) Grafting to support:

Bulk polymerizations have a weak structure. So, it is necessary to improve the mechanical properties by grafted a surface quoted into stronger support. It is achieved by doing polymerization of monomers with free radicals which formed a stronger chain of monomers that are covalently bonded and provide support.

#### vi) Polymerization by irradiation:

Unsaturated hydrogel compounds are prepared by the help of the initiators like gamma rays, electron beams and ionizing high energy radiation. Irradiation of aqueous polymer solution fabricate radicals on the polymeric chains. Covalent bonds can be achieved by the recombination of the micro-radicals which provides good stability. Polyethylene glycol (PEG), Polyacrylic acid, polyvinyl alcohol (PVA) are used for the polymerization by irradiation. By this method, we can get relatively pure and initiator free hydrogels.

#### vii) Physical crosslinking:

It is the most easiest and convenient route for the preparation of hydrogels. This has a simple mechanism of cross-linking of polymers by physical interaction. Physical cross-linking can be achieved by polyelectrolyte complexation, the interaction of ions like hydrogen bonding and hydrophobic association. Different methods are used for the physical cross-linking –

- Heating and cooling polymer solution - It is prepared by simultaneously heating and cooling of hydrogels which forms helix and junction zones that help the polymers like gelatin or chitosan to form physical cross-linking. It is also known as the freeze-thaw technique.
- Complex coacervation – Mixing of poly-anions and poly-cations can form complex coacervation. The mechanism of this preparation is that opposite charges adhered together and prepare soluble or insoluble complexes. This solubility mainly depends on the concentration and the pH of the solution. Coacervating poly-anionic xanthan with poly-cationic chitosan is the example of complex coacervation.
- Ionic interaction – The addition of opposite di or trivalent ionic polymers shows the cross-linking between polymers. These methods follow the principle of gelling poly-electrolyte solution. Some preparation through this method is chitosan-glycerol phosphate salt and chitosan-polylysine.
- Hydrogen bonding – A hydrogen bond can be formed between the electron-deficient

#### polymerization or inverse-suspension polymerization:

atom and a functional group which has a high electron density. For example, PA and PNVP formed hydrogen bonds between them. Polymer concentration, solution temperature, the molar ratio of each polymer, polymer structure and types of polymer can affect the synthesis of hydrogel[33].

Source: Hamdi, M., Feki, A., Bardaa, S., Li, S., Nagarajan, S., Mellouli, M., Boudawara, T., Sahnoun, Z., Nasri, M. and Nasri, R., 2020. A novel blue crab chitosan/protein composite hydrogel enriched with carotenoids endowed with distinguished wound healing capability: In vitro characterization and in vivo assessment. *Materials Science and Engineering*.

### 1.4 Application of hydrogel:

As soon as hydrogels discover it was used in anti-cancer and antibiotic drug delivery systems. It is also popular in bio-adhesive devices. They are very much attractive because it has both properties of control release and sustained release. They also have the ability to release the drug in a targeted location. It is used in many bio-medical sectors as –

#### 1.4.1 3D cell culture:

A 3D cell culture is a technique of creating an artificial environment where a cell can grow and interact with its surroundings without having any issue. 3D cell culture helps in vivo growth of a cell in any direction[34].

Hydrogels can mimic as the natural extracellular fluid which is important in the survival of cells and its proliferation. It has potential towards the in vivo like cell culture. Hydrogels have these types of ability because it can retain a high amount of water which enables the transport of gases and nutrients. Faster stress relaxation in polymers likes alginate-PEG helps to promote the cells osteogenesis, spreading and proliferation easily [35].

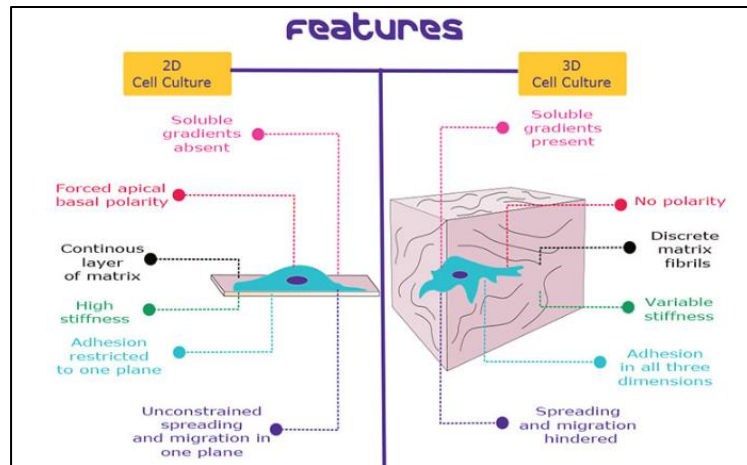


Fig. 10: Comparison between 2D cell culture and 3D cell culture

Source: Duval, K., Grover, H., Han, L.H., Mou, Y., Pegoraro, A.F., Fredberg, J. and Chen, Z., 2017. Modeling physiological events in 2D vs. 3D cell culture. *Physiology*, 32(4).

This picture shows the main differences between cells behavior and constraints when cultivated in a 2D environment, plated on a slide, even coated with hydrogels or any other ECM (Extracellular matrix) protein like collagen, compared to cells cultivated in a 3D environment, embedded in a hydrogel or any other ECM proteins[36].

#### 1.4.2 Skin regeneration:

Skin is a complex organ that helps to protect the internal tissues or organs from the external environment. Skin regeneration is also difficult because it requires a material that acts as an extracellular matrix, having growth factor, good mechanical properties and also having compatibility towards the cells without having any toxicity. Hydrogels have all of these properties because of polymers like chitosan, polyethylene glycol, etc [37]. Skin regeneration mainly occurs due to the migration of injured and inflammatory cells and the activation of fibroblasts by these polymers. Hydrogels are capable of creating fibronectin like structure which helps in blood clotting and induce cell proliferation for skin regeneration [38].

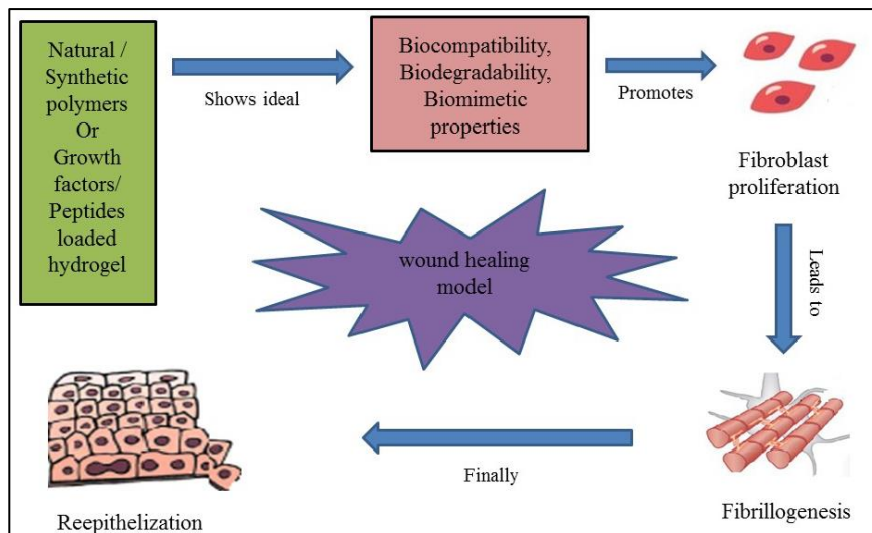


Fig. 11: hydrogel in skin regeneration (Wound healing)

Source: Liang, Y., Zhao, X., Hu, T., Han, Y. and Guo, B., 2019. Mussel-inspired, antibacterial, conductive, antioxidant, injectable composite hydrogel wound dressing to promote the regeneration of infected skin. *Journal of colloid and interface science*.

#### 1.4.3 Drug delivery:

Hydrogels can consist of both strong bonds and weak bonds separately which affect their crosslinking and influence the mechanical properties. Small changes in polymers can change the entire properties of hydrogels. Hydrogels can be used as a delayed-release, controlled release, or sustained release dosage form. They can deliver drugs in the different area of our body like –

Source: Sun, Y., Nan, D., Jin, H. and Qu, X., 2020. Recent advances of injectable hydrogels for drug delivery and tissue engineering applications. *Polymer Testing*, 81.

**i) Hydrogels for eyes** –According to the survey almost 70% to 80% ophthalmic solution lost due to acrimal drainage or blinking tear drainage and the bio-availability was decreased and did not give desired effect. In this situation, hydrogels are 100% safe and implanted under the conjunctiva. E.g. – Xylogiucan based gels are used for sustained delivery of timolol and pilocarpine in the eye.

**ii) Hydrogels for transdermal drug delivery** –The advantage of using hydrogels as a transdermal drug delivery system is that it can bypass the first-pass metabolism and increase the efficiency and bioavailability of drugs. It can give a constant and stable drug release. They act like living tissues and can be easily removed rather than other conventional topical products like ointments, patches, etc.

**iii) Vaginal route** – This route can bypass hepatic metabolism. Due to the large surface, thin mucous layer and lots of blood vessels drug absorption are increased. Drugs with higher molecular weight can pass the vaginal epithelium easily.

**iv) Oral route** – Oral routes are the most convenient and easily accessible route. Mucosal adhesive hydrogels are mainly used for local viral and fungal infections.

**v) Gastro-Intestinal track** –It is the most common and popular route of drug delivery. It has some major disadvantages like acidic or enzymatic degradation, effects on drug release due to pH, cannot avoid first-pass metabolism, etc. Sustained released gastro retentive hydrogels are made to increase the bioavailability, stability and effect of poorly absorbed oral drugs.

**vi) Hydrogels for brains** –Like other parts of the body, drug delivery in the brain is so much difficult because of the blood brain barrier (BBB). About 95% of the drugs are unable to cross blood brain barrier. For that reason, a very low amount of drugs are able to act in the Central Nervous System. Long term sustained effect of camptothecin loaded with PLGA microspheres was observed in rats. These microspheres increase the survival period in rats against malignant gliomas.

**vii) Soft contact lenses** – First commercially available silicone hydrogels have two different approaches. One is the development of silicon monomers with enhanced compatibility by Bausch and Lomb, another is the development of siloxy monomers which is hydrophilic and oxygen permeable due to hydrophilic polyethylene oxide and poly-siloxane unit.

**viii) Rectal delivery** – Hydrogels can be modified as bio-adhesive which helps in rectal drug delivery. Miyazaki et al explored the xyloglucan gel with a thermal gelling property for drug delivery [39].

**ix) Subcutaneous drugs** –PHEMA and cytarabine (Arc-c) cross-linked together to form hydrogel for the subcutaneous delivery as anti-cancer drug. Now a days, these hydrogels are developed as a fully bio-degradable system which does not need any kind of surgery for removal.

**x) Novel hydrogel for controlled drug delivery** – HYPAN is the novel hydrogel which has a good property of controlled drug delivery.

**xi) Hydrogels for gene delivery** -Proper modification can lead the hydrogels as a targeted drug delivery system. It has the ability to target specific cells for delivering nucleic acid for gene therapy. Hydrogels have the potential for the therapy of many genetic disorders.

**xii) Cosmetology** - Hydrogels can be infused in the breast for aesthetic reasons. These implants are filled with hydroxyl propyl cellulose polysaccharide gel and have a silicone elastomer shell [40].

#### **1.4.4 Tissue engineering:**

Tissue engineering is the combination of cell, engineering and materials. It is used to replace or remove the tissues which are damaged or infected. Natural and synthetic polymers are used to prepare hydrogels which are capable of tissue engineering. Polymers cross-linked with metal ions (Fe, Co, Na, Ca, K, etc.) shows tissue engineering properties. Macromolecules like phagosomes are delivered by the micronized hydrogels into the cytoplasm. This property is also used in cartilage repairing. Agarose, Methylcellulose is used in tissue engineering. Hydrogels itself has cell-like properties which also helps in bio-compatibility [41].

#### **1.4.5 Diagnostics:**

Continuous developments in microfluidics qualify the lab-on-a-chip-based molecular diagnosis. For the prevention and transmission of the disease, accurate and rapid diagnosis is required. This system for pathogen detection based on DNA hydrogel formation during rolling circle amplification (RCA). This is a success but too much expensive for the real world [42].

In the last few decades, many investigators attempt the development of such products that can be used in the diagnosis as well as treatment of cancer cells. Hydrogels have some unique properties by which they are able to diagnose, reconstruct and treatment of the tumors. These hydrogel-based systems can be configured to respond in cancer cell-specific. We can diagnose the cancer cell if we reconstruct the tumor micro-environment in a hydrogel which helps to bind with the cancer cell and deliver the drug in the specific site [43].

#### **1.5 Major advancements in composite hydrogels:**

Composite hydrogels are a mixture of two different materials such as natural and synthetic polymers. Composite hydrogels have both the properties of the natural and synthetic polymer. Physical hydrogels are flexible and had a good elasticity but they are bounded with covalent bonds (ionic bonds). That's why they are not stable and easily degrade. But with the chemical hydrogel, the results are different because they have strong bonds which provide good stability but lack of elasticity. These physical and chemical hydrogels properties are hard to change.

In the other hand, composite hydrogels have some really good advantages over the physical and chemical hydrogels-

- I. They are stable in nature.
- II. ii) Good properties in bio-degradability, bio-compatibility.
- III. iii) Easily terminate any kind of toxicity like cytotoxicity.
- IV. iv) Having a good mechanical property.
- V. v) Crosslinking can easily change by manipulation of the polymeric components and cross-linkers to get the desired product.

Composite hydrogels are much more effective in the bio-medical field than other types of hydrogels. They are used in different areas of our body such as – tissue engineering, wound healing, tissue regeneration, cardiovascular and also in different types of delivery systems such as – targeted drug delivery, controlled as well as sustained release. Every type of system depends on the polymeric bonds and the drug storing inside a hydrogel. Now a days it is used to diagnose the cancerous cells, any infection inside the body. Composite hydrogels can be turned into a smart polymeric hydrogel which shows any effect on the specific site by sensing the environment like – pH-sensitive, enzyme sensitive, specific antigen sensitive, temperature-sensitive, etc. In the specific environment, their bond structures change and pores are increased to deliver the drugs.

## 2. Applications of hydrogel: A review on commercial and patented products:

Hydrogels are very popular now a days due to some special ability such as flexibility, mechanical strength, stability, bio-compatibility and water up taking properties. Hydrogels are synthesized by physical or chemical cross-linking with synthetic or natural hydrophilic polymers. These hydrogels can be used in many areas such as – agriculture, water plants and bio-medical industries. In the bio-medical field, it is used as wound dressing, drug delivery system, tissue engineering, contact lenses, etc.

In 1960, Lim and Wichterle described a polymer named poly-2-hydroxy-ethylmethacrylate (PHEMA) which can be used to prepare for biocompatible hydrogels for contact lenses. This product gets patent in 1962 and sold to the Lomb and Bausch. They got the approval for the production of PHEMA lenses by the food and drug administration in 1971 [44].

Contact lenses are two types – hard and soft. Hard lenses are long-lasting but soft lenses are more patient compliance. Soft lenses are made of hydrogels but hard lenses are made up of polymers (PMMA). So, soft lenses are biocompatible in nature. There are many products in the market such as Biofinity (CooperVision), Acuvue Oasys (Vistakon), PureVision2 (Bausch + Lomb) and Air Optix Aqua (Alcon) etc.

Hydrogels can be used to improve the ophthalmic drug delivery by increasing the drug loading capacity as well as increasing the drug release time. There are many strategies to modify the conventional lenses such as – Controlled hydrophobic-hydrophilic co-polymer ratio, ligand containing hydrogel, molecularly imprinted polymeric hydrogel, multi-layered structure for prolonged drug release, etc [45].

In 1998, a Mexican company named Ciba Vision released silicone hydrogel contact lenses. This can permeate oxygen very easily so it can improve lens-related dryness symptoms [46].

A colloid-laden hydrogel helps to protect the drug from the degradation by the ocular enzymes. It consists of nanoparticles, nanosuspensions and liposomes. It also improves the targeting of drugs, drug release and permeation [47].

Surface modified hydrogels are introduced in the market to improve ophthalmic drug delivery. Danion et al. encapsulated drugs into lipid vessels like liposomes and the bound the posterior and anterior surfaces for the use in lenses. The result shows a successful site-specific drug delivery system. This method applied on a large scale because of its easy and simple manufacturing at room temperature. Besides manufacturing, there is a problem with this method that it cannot deliver small molecular drugs and the drug release time is short [48].

Toshizumi Tanabe in 2014 discussed in his paper about the improved keratin hydrogels, the drug release ability and their evolution. He used three chemicals acetamidation, carboxymethylation and aminoethylation to terminate free cysteine residue to get carboxymethylated keratin (CMK), acetamidated keratin (AAK) and amino-ethylated keratin (AEK). Hydrogels are created by these three chemically modified keratins. He found the series of lowest concentration for preparing hydrogel CMK < AEK < AAK. By comparing them each hydrogel shrank in acidic medium, AAK swelled in basic and neutral medium. It is found that AAK and AEK support cell proliferation. After the evaluation of those three hydrogels, it concludes that AAK hydrogels were perfect for sustained release drug carriers. It is observed that the drug release can go up to three days, while the release from CMK and AEK hydrogels completed in the same day. Thus, he found that keratin and chemically modified keratin hydrogels were promising biomaterials that can act as a cell-substrate and used as a sustained release drug carrier [49].

Patrícia B. Malafaya, et al. presented a paper in 2007 on the large scale of natural polymers with specific attention on polysaccharides and proteins that are utilized in research or useful as cell carriers which is useful in tissue engineering or field targeting several biological tissues or as carriers for proteins, vitamins, etc. And they give a general overview on how to decide to achieve drug carriers with biodegradable natural polymers [50].

Hydrogels used in contact lenses must pass some important properties like the refractive index which should be in the scale of 1372-1381. The refractive index of the human eye varies from person to person. Luminous transmittance is defined as the transparency of the lenses whose minimum value should be 95% and for evaluation, a slit-lamp microscopy is used to measure the transparency. To avoid anoxia permeability of oxygen is an important factor which depends inversely to the thickness of the lens surface. Oxygen permeability should be 35 Dk for closed eye and 125 Dk for open eye. The stability of the hydrogel affects the working factor of the lens and the self-life of the product. Mechanical properties like elastic modulus (E) has an important property for the adhesion of the lens with the corneal epithelium. These properties of a hydrogel lenses must be exact to get the proper results [51].

Introducing Lidocaine a local anesthetic drug incorporate with chitosan-pectin bio-polymeric hydrogel for wound dressing. This was prepared by the physical cross-linking of those two bio-polymers. 3D printed polymers shows a self-adhesion to skin and good dimensional integrity. High swelling capability and the absorption of transuding shows good bio-compatibility properties [52].

Usually natural or synthetic polymers are used to prepare a hydrogel but we never think of preparing with 'green chemicals'. Rapid development in enzyme technology (Chemical-based method) provides stability and alternative of 'green catalyst'. Enzymes shows good potential in the formation of hydrogel for the wound healing process. Enzymes like laccases, peroxidases, transglutaminase are used for the hydrogel preparation. In this preparation enzymes act as a catalyst and induce the cell proliferation, angiogenesis, collagen synthesis, cell migration, neovascularization. This hydrogels can deliver the bio-molecules such as epidermal growth factor (EPGF), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), etc. and can support enzyme-catalysed reaction. To make it more productive anti-bacterial and anti-oxidant enzymes are incorporated with the hydrogels. Synthesized cellobiose dehydrogenase (CDH) has the both properties. The clinical testing was limited because of high cost of the growth factors and requires more dedication was required [53].

Anti-biotic resistance pathogens made the treatment for the skin wound difficult so, phage therapy is a promising technique for the treatment of anti-biotic resistance pathogens. Researchers create a novel PVA-SA hydrogel for the wound dressing and healing. Hydrogel mimic the extra-cellular matrix which provides the wound healing environment and surface of the hydrogel absorb the bacteriophages and antibiotic takes care about the wound infection. This combination shows the highest gel-fraction, swelling index and hemo-compatibility. The dual coated hydrogel membrane provide better treatment strategy [54].

Shilei Haodemonstrated in 2018 that Insulin can heal thewounded skin by stimulating angiogenesis and cellular migration. Keratin-insulin conjugated hydrogel was manufactured to increase effective skin regeneration. By the EDC/NHS reaction insulin-conjugated keratin was synthesized. This hydrogel shows samerheology, porpsity and water absorption properties like other keratin hydrogels.

The results exhibit the increased property of the keratin which stimulates the haemostasis and wound healing, which is appeared to having the probability of tissue regenerationby keratin-insulin based conjugated materials [55].

Marketed available hydrogels for ActivHeal<sup>®</sup>, Kito-activator, Luofucon, debrid gel, chitoheal gel, etc. Granugel<sup>®</sup> is produced by the Conva Tec Company for the management of wounds and it can be filled in dry cavity wounds to mimic the healing environment. Purilon Gel<sup>®</sup> (Coloplast) is mainly used for the wound healing of second and third-degree of burning [56].

In 2017 a researcher Rishabha et al. presented through his work a composite of ionic and non-ionic polymers of chitosan and guar gum as an excipient and evaluate it. He prepared it as a fast disintegrating drug delivery system. Chitosan was prepared with acetic acid solution and guar gum with deionizing water. He mixed the solution in same ratio in a constant temperature and dried. He evaluated the dried product such as flow behavior, swelling, micromeritics properties and surface characterization. He had prepared tablets with different ratio of hydrogels like 5,10,15,20,30 using phenytoin sodium as a model drug. He characterized the tablets with official methods like disintegration time, stability study, friability, hardness and in-vitro study.

The results of his research showed that the tablets passed all the evaluation and characteristics of the tablets did not change after six months of storage. Thus he concludes that these composites can act as good excipients and helps in fast disintegration drug delivery system [57].

Hydrogels have a very important contribution to tissue engineering. In 1988 tissue engineering was described as the replacement or improvement of the tissues through engineering and synthetic strategies. The properties of the hydrogels are so similar that they can easily and harmlessly blend with cells and tissues. In a European patent EP 1 664 168 B1, manufactured a hydrogel-based composition with polypropylene fumarate a self-cross-linkable polymer to produce porous scaffolds for tissue engineering and to treat skeletal defects. They are able to swell in water but did not flow as a liquid [58]. Chemically crosslinked PHEMA (Poly 2-hydroxyethyl methacrylate) tubes are manufactured with a diameter of 2.4 mm and the thickness of the wall is 40-400mm. This tube is used for the targeted regeneration of the nervous system [59].

Ellenburget al. were done correlating of physical and chemical properties of chitosan/PVA/Genipin and chitosan/PVA/ glutaraldehyde with their mechanical responses and their structure. Their proper structure was determined by FTIR spectroscopy.

The results showed the similarity between Genipin and glutaraldehyde of thermal properties, structural parameters and crystallinity. Elastic moduli of those two are slightly different, for glutaraldehyde  $2.82 \pm 0.33$  and Genipin  $2.08 \pm 0.11$  Genipin is slightly cytotoxic than glutaraldehyde. That means the preparation of hydrogels with Genipin was much more effective and had potential applications in regenerative medicine, tissue engineering, environmental industries [60].

Freezing-thawing method is used for the preparation of hydrogels. It has an advantage that it does not need any crosslinking agent. In this study, chitosan and polyvinyl alcohol were used in different freezing conditions to observe the different characteristics of the prepared product. After the preparation, many characterizations (morphology, swelling, porosity, etc.) was to done to ensure the product quality. Drug loading and drug release profiles were also evaluated.

In the result, the swelling ratio was affected by the chitosan content, temperature and the number of freezing cycles. With the help of the SEM micrographs, it shows that the size of the pores increases with the increase of chitosan content. Lower temperature or longer freezing time results in high porosity and small pores. The drug release time was found 30 hours [61].

Almost thirty years ago, superabsorbent polymers or SAPs are discovered. Polymers water retention property helped to developed SAPs which is used in diaper and agriculture industries now a days. In 1978 SAPs are first introduced in the market as a feminine napkin product. First-generation SAPs are prepared with acrylamide salts which are highly hydrophilic in nature and after the 90s it is prepared with composite hydrogels (Synthetic and natural). These hybrid hydrogels have good stability, strength good mechanical properties and elasticity[62].

We know about the advantages of hydrogels and their applications in different fields. Now in the year, 2020 hydrogels may be used with the bio-sensors. Hydrogels have extensive and wide sensing mechanism which can be used as biological or chemical sensors. These biosensors are prepared with the polymers, carbohydrate, peptides and DNA with the conjugation of conductive materials such as conducting polymers, nano-crystals, graphene, etc. Sensors are mainly worked by the voltage difference, conductance or resistance. Signals transferred through sensor geometry and electrodes which creates chemical changes in the bonds of hydrogel and provides a signal to the bio-sensors for the release of the bio-molecules. This can be used for the targeted delivery of proteins, DNA, etc. for a longer period [63].

A microRNA or miRNA is a micro non-coding RNA that contains 21 nucleotides found in some viruses, animals and also in plants. For the recognition of miRNA large instruments are used which is more costly and it is difficult to identify. Some hydrogels are used for the detection such as label-free polygonal plate fluorescent hydrogel (PPFH) biosensors are developed for the onsite identification. The handling of this bio-sensor is easy to handle. Rolling circle amplification method is used to increase DNA chains by targeting the miR-21. This provides a new kind of DNA hydrogel which is called polygonal plate hydrogel. This biosensor is affordable and easy to use from conventional methods. These biosensor hydrogels are able to identify the virus by detecting their micro-RNA [64].

Hydrogels can be used as a carrier for targeted drug delivery. For the brain targeted drug delivery, a hydrogel is prepared with the sterculia gum, PVP, alginate and polysaccharide. After the preparation, it was evaluated with XRD, <sup>13</sup>C NMR, AFM and FTIR. The results are impressive. The drug released slowly and follow first-order kinetics. These polymers were mucoadhesive, bio-compatible and anti-oxidant properties [65].

## Conclusion And Summary :

In the last few years, the use of hydrogels has been increased in different fields. Chitosan hydrogels have gained special attention in the field of biomedical applications due to certain properties such as bio-degradability, bio-compatibility and low toxicity. Chitosan hydrogels however suffer from problems such as low mechanical properties and low stability. To fix these problems other synthetic polymers are incorporated. The preparation method of hydrogels plays an important role in its structure, durability and use.

Natural and synthetic hydrogels are good in properties but composite hydrogels are better because they contain both the properties of natural and synthetic polymers. Synthetic polymers such as polyvinyl alcohol generally form the base for the incorporation of bioactive and other polymers. Natural polymers like keratin used as they have wound healing properties by creating fibronectin like structures around the damaged tissues and enhance the healing ability of the cell. Keratin used in this work was extracted from human hair. Keratin's introduction into the blend was intended for the enhancement of the mechanical strength and better stability of the hydrogel. This composite hydrogel can be considered as a novel device for potential applications in the biomedical field.

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