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Polymeric Hydrogels: Characterization and Biomedical Applications –A mini review

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Abstract

Hydrogels are crosslinked polymeric networks, which have the ability to hold water within the spaces available among the polymeric chains. The hydrogels have been used extensively in various biomedical applications, viz. drug delivery, cell carriers and/or entrapment, wound management and tissue engineering. Though far from extensive, this article has been devoted to study the common methods used for the characterization of the hydrogels and to review the range of applications of the same in health care.

Keywords: Hydrogels; Drug delivery; Water; Pores; Wound healing, Tissue engineering

Introduction

The word polymer has been derived from the Greek words polys (meaning many) and meros (part or unit) [1]. The polymers (e.g. proteins and celluloses) form the basic building block of life. For the development of modern health care products, there has been an increased interest for polymers among the researchers, which can be attributed to the easy availability of the wide range of polymers with desirable properties. Some of the key applications include reconstruction of load bearing hip and knee joints, tendons replacement, development of matrix for tissue engineering and drug delivery systems [2-9]. In this review article we will discuss about some of the characterization methods and applications of a special class of polymers known as hydrogels. The first thing which strikes in our mind when we hear about hydrogels is “What is hydrogel?”. The hydrogel can be defined as a crosslinked polymeric network which has the capacity to hold

water within its porous structure. The water holding capacity of the hydrogels arise mainly due to the presence of hydrophilic groups, viz. amino, carboxyl and hydroxyl groups, in the polymer chains. According to Hoffmann, the amount of water present in a hydrogel may vary from 10% to thousands of times of the weight of the xerogel [10]. A xerogel may be defined as a polymeric network devoid of water. The water holding capacity of a xerogel is dependent on the number of the hydrophilic groups and crosslinking density. Higher the number of the hydrophilic groups, higher is the water holding capacity while with an increase in the crosslinking density there is a decrease in the equilibrium swelling due to the decrease in the hydrophilic groups. As the crosslinking density increases, there is a subsequent increase in the hydrophobicity and a corresponding decrease in the stretchability of the polymer network. As mentioned above, hydrogels are crosslinked polymeric networks and hence provide the hydrogel with a 3-dimensional polymeric network structure. The use of hydrogel for biomedical applications dates back to 1960 when Wichterle and Lim developed crosslinked poly (hydroxyethyl methacrylate) (pHEMA) [11]. Apart from the synthetic polymers, viz. pHEMA and poly (methyl methacrylate) (pMMA), the use of natural polymers, often termed as biopolymers, for the development of hydrogels have gained a substantial importance over the years. Alginate and chitosan are the two biopolymers which have been extensively studied in the recent past. The use of hydrogels is not only limited to pharmaceutical and nutraceutical delivery but of late has also been extended to regenerative medicine.

Hydrogels can be classified into two groups depending on the nature of the crosslinking reaction. If the crosslinking reaction involves formation of covalent bonds, then the hydrogels are termed as permanent hydrogel. The examples of permanent hydrogels include pMMA and pHEMA. If the hydrogels are formed due to the physical interactions, viz. molecular entanglement, ionic interaction and hydrogen bonding, among the polymeric chains then the hydrogels are termed as physical hydrogels [10, 12]. The examples of physical hydrogels include polyvinyl alcohol-glycine hydrogels, gelatin gels and agar-agar gels. Hydrogels can also be categorized as conventional and stimuli responsive hydrogels [2]. Conventional hydrogels are the crosslinked polymer chains which absorb water when put in an aqueous media and there is no change in the equilibrium swelling with the change in the pH, temperature or electric field of the surrounding environment while the stimuli responsive hydrogels are the polymeric networks which change their equilibrium swelling with the change of the surrounding environment. pH sensitive

hydrogels have been used since long in the pharmaceutical industry as an enteric polymer. The enteric polymers/ hydrogels generally are used to either protect the stomach mucosa from the gastric irritant drugs (e.g. aspirin) or to protect the acid-labile drugs (e.g. penicillin G, erythromycin) from the harsh environment of the stomach [13]. pH sensitive hydrogels have also been used for the development of blood-glucose detection kit and insulin delivery [14]. Temperature sensitive hydrogels are being used in tissue culture. Electric field sensitive hydrogels have been used in artificial muscles, and controlled drug delivery systems [15].

As stated above, the xerogel starts to imbibe water when it is put in an aqueous media. Hence, determination of the amount of water imbibed within the hydrogel is an important criterion for characterizing the hydrogel for biomedical applications and is often represented in terms of % age swelling (%S) [2]. The % age swelling of the hydrogel is directly proportional to the amount of water imbibed within the hydrogel. Amount of water imbibed within the hydrogel influences the diffusional properties of a solute through the hydrogel. In general, the higher the % age swelling, the higher is the amount of water imbibed and the higher is the diffusion rate of the solute, though other factors, viz. micro-architecture of the polymer chain, may also play an important role. Experimentally, % age swelling can be determined by weight difference method and is expressed by the following equation [16]:

$$\%S = \frac{W_s - W_d}{W_d} x100 \qquad (1)$$

where, Ws= weight of swollen gel and Wd= weight of dry gel

Fick's equation is an important tool to explain the diffusion of solutes through polymer matrices and hence is used to model such systems [17]. The diffusion coefficients of solutes through a diffusible polymer matrix are experimentally determined in double cell diffusion apparatus. A typical double cell diffusion apparatus consists of a donor, where a concentrated solute solution is kept, and is separated from a receptor containing a pure solvent or simulated physiological fluid by the diffusible matrix. The double cell diffusion apparatus can be either vertical or horizontal (Figure 1 & 2). At any time, t, the concentration values in the two chambers can be used to calculate the diffusion coefficient, D, of the drug in the hydrogel from the following equation [16, 18]:

$$D = \frac{1}{\beta t} x \ln \frac{C_D(t) - C_R(t)}{C_D(0) - C_R(0)} \qquad (2)$$

with $$\beta = \frac{A_H}{W_H} x \left[\frac{1}{V_D} + \frac{1}{V_R} \right] \qquad (3)$$

where: $C_D(0)$ =initial concentration of drug in donor; $C_R(0)$=initial concentration of drug in receptor; $C_D(t)$=concentration of drug in donor after time t; $C_R(t)$=concentration of drug in receptor after time t; A_H=effective cross-sectional area of diffusion in the hydrogel sample; W_H=width of the hydrogel sample; V_D=Volume of drug solution in donor; and V_R=Volume of receptor fluid.

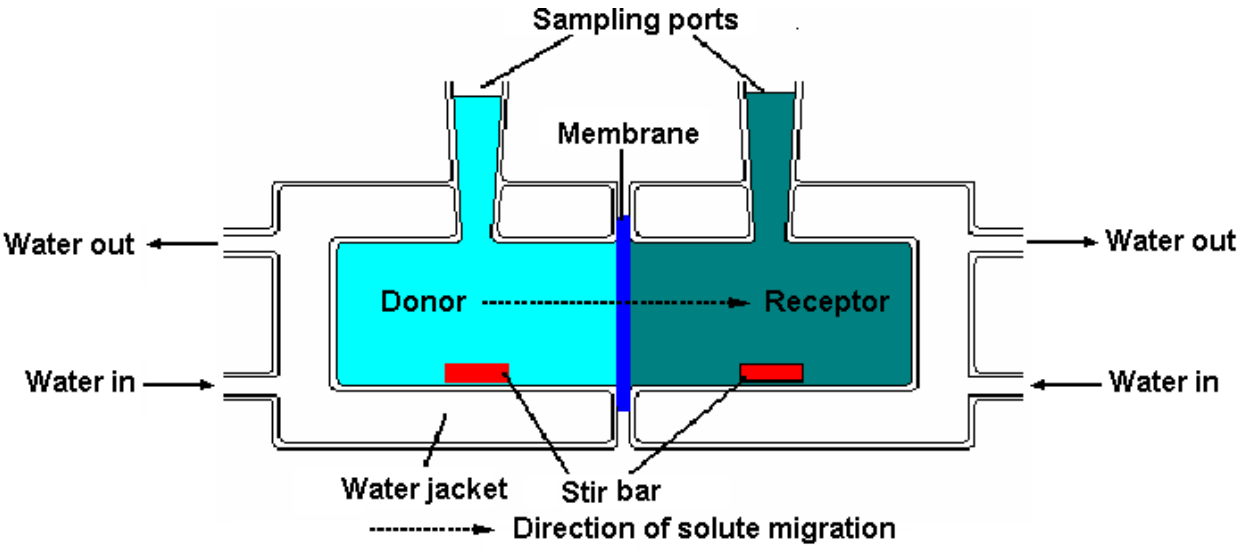


Figure 1. Schematic diagram of horizontal diffusion cell

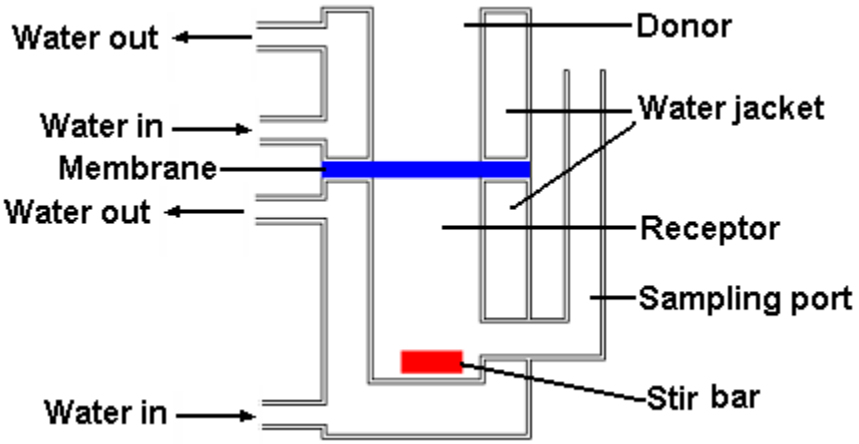


Figure 2. Schematic diagram of Vertical diffusion cell

For determining the diffusion coefficient of a solute, samples are withdrawn from the donor and receptor at regular intervals of time and analyzed for the solute concentration. Subsequently a graph of $-\ln\frac{C_D(t)-C_R(t)}{C_D(0)-C_R(0)}$ vs. time is plotted, which gives a straight line. The slope of the line is used for calculation and is used to determine the diffusion coefficient from equation (2). The structure, pore size and polymer composition of gel are taken into account by the diffusion coefficient [10]. Since the porous channels within the hydrogel are not straight and the sizes of the pores are not uniform, these factors are generally included in a factor usually known as tortuosity [10, 19]. Diffusion of solutes is also dependent on the nature of the crosslinker. As for example, by tailoring the chain length of polyethyleneglycol (PEG) chains of PEG-diacrylates, the hydrophilicity of the hydrogel could be tailored as per the requirements [20]. In ionically crosslinked hydrogels the charged polymers, for example chitosan and alginates, react with counter charged ions and/or polymers to form a networked structure. The crosslinking reaction of the ionically crosslinked hydrogels is dependent on the size of the crosslinker and the overall charges of the polymer and crosslinker [21]. The smaller the molecular size of the crosslinker, the easier is the diffusion of the same which results in a faster crosslinking reaction. Any drastic change of pH, may lead to the dissociation of ionic linkages which in turn may lead to the dissolution of the network and faster release of the solute. Diffusion and more specifically the release of the solute from the hydrogel is also dependent on the ionic or physical interaction of

the solute particles with the polymeric chains of the hydrogels. For example, release of cationic drugs will be affected in the presence of anionic alginates while the release of the anionic drugs will be affected in the presence of cationic chitosan, due to cation-anion interaction. It has been mentioned earlier that, with the increase in crosslinking density there is an increase in the hydrophobicity and decrease in the stretchability of the polymer network structure. Thus increase in crosslinking density results in decreased swelling of the hydrogel and reduced diffusion of the solute. The other factors which affect the diffusion of a solute include polymer molecular weight and solubility and molecular weight of the solute itself. In general, the lower the molecular weight of the polymer chain the higher is the diffusion of solute. Polar solutes of low molecular weight show higher diffusion coefficients.

Biocompatibility tests

Generally hydrogels are biocompatible and non-irritant in nature [22]. The biocompatibility of the hydrogels is generally associated with the hydrophilic nature of the same, which helps in washing off the toxic and un-reacted chemicals during synthesis. The presence of water in the system makes it soft and rubbery which offers least frictional irritation and provides a soothing effect when in contact with the physiological system. *In vitro* tests are not so expensive and hence are being preferred by the researchers to carry out the initial biocompatibility test.

In vitro tests for biocompatibility generally looks into the cyto-toxicity aspect of the material in the presence of live host cells and can usually be done in two ways. In the first method, the material whose biocompatibility has to be determined is placed in direct contact with the host environmental cells and is subsequently incubated for a specific period of time at 37^0 C. In the second method, the material is placed in a suitable physiological solution and is incubated for a specified period of time at 37^0 C to allow any leaching from the material. The leachates, so obtained, are used to carry out the biocompatibility tests in the presence of the cells. The researchers usually determine the cell viability and cell proliferation from the cyto-toxicity test. In a typical experiment, the hydrogels are usually sterilized (by immersing in ethanol and drying under Laminar air flow) followed by seeding with the host cells. After the seeding of the cells, the system is incubated for 1 h to allow cell attachment on the hydrogels which is followed by the addition of culture media and subsequent incubation at 37^0 C for allowing cell growth and proliferation. The cell proliferation can either be visualized by microscopy or by carrying out

MTT (tetrazolium salt, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay. MTT assay is a colorimetric method which allows quantification of cell growth and proliferation. The assay is based on the principle of reduction of MTT into purple colored formazan crystals in the presence of mitochondrial dehydrogenase. These purple colored formazan crystals are dissolved in dimethyl sulphoxide (DMSO) and are analyzed by measuring absorbance at 570 nm in a colorimeter. The quantity of formazan crystals is directly proportional to the number of the live cells [23]. A schematic representation of the method has been described in Figure 3.

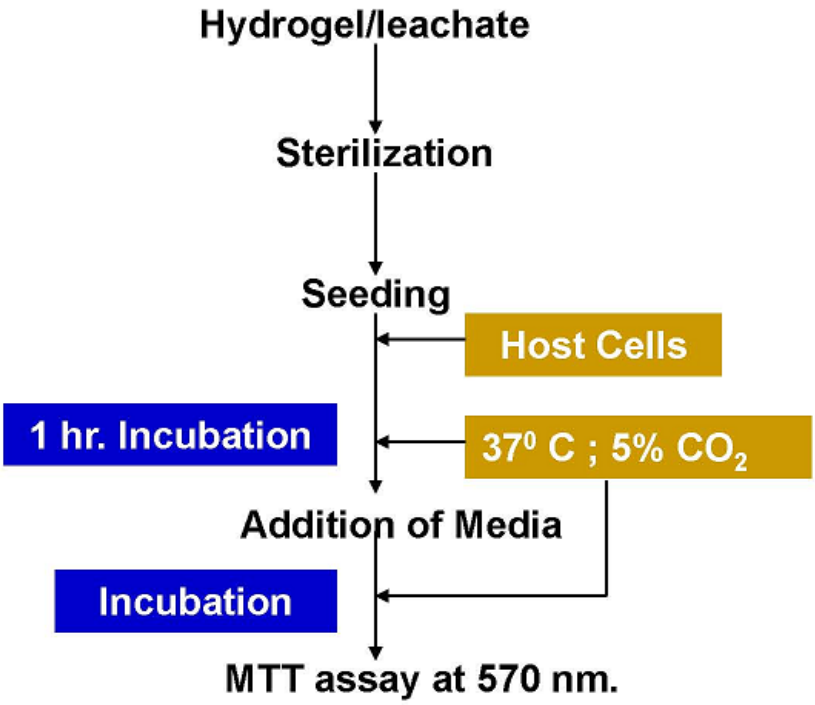


Figure 3. Schematic diagram of MTT assay

There appears to be no relationship between wettability and thromboresistance of a material but most materials scientists use contact angle for the characterization of the non-thrombogenic surfaces. Materials producing small contact angles with blood are considered to be compatible with blood, while materials producing large contact angles have poor blood compatibility [24]. Hydrophilicity has got an inverse relationship with the contact angle, i.e. lower contact angle indicates higher hydrophilicity of the material, and a proportional relationship with cell attachment, i.e. the higher the hydrophilicity, the greater is the cell attachment [25].

Hydrophilicity of the material influences the adsorption of blood proteins, which may promote cellular attachment, onto the material surface. The most common method for measuring the contact angle in a localized region of material surface is the sessile drop method, which employs an optical arrangement. In this method, the angle between the baseline of the drop and the tangent from the intersection of drop boundary and baseline is measured (Figure 4) and can be measured using a goniometer (Figure 5).

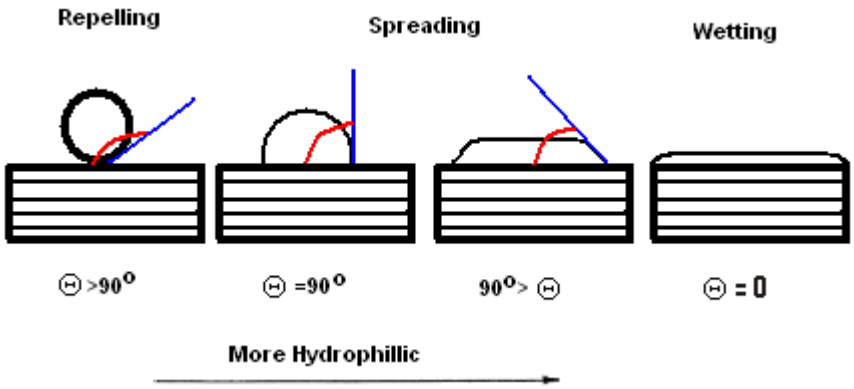


Figure 4. Effect of contact angle on the hydrophilicity of the solid surface.

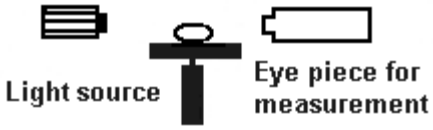


Figure5. Schematic diagram of a goniometer

Contact angle is also related with the interfacial free energy and can be best expressed by Young's relationship. The Young's relationship is given by:

$$\gamma_{SL} = \gamma_{SV} - \gamma\, Cos\,\Theta \qquad (4)$$

where: γ_{SL} denotes the solid-liquid interfacial energy, γ_{SV} denotes the solid-vapor interfacial energy, γ denotes the liquid-vapor energy and Θ denotes the contact angle.

From the Young's relationship it can be observed that contact angle is directly proportional to the interfacial free energy, i.e. as the contact angle increases, the interfacial free energy also increases. Findings from the researches indicate smooth hydrophilic surfaced biomaterials

generally possess good blood compatibility [24]. No single in vitro or in vivo experiments give a complete idea of biocompatibility but can reveal useful information. American Standard for Testing of Materials (ASTM) provides an in vitro method for the primary evaluation of biomaterials for blood compatibility [26]. The method deals with determination of % hemolysis of citrated goat blood in the presence of biomaterials. Calves are generally used to evaluate cardiovascular products because of relative ease of handling, convenience and less complex hematological profile. However, the evaluation of these products should be done in baboons and/or rhesus monkeys whose hematological profiles closely resemble to the hematological profile of humans.

Though *in vitro* tests provide useful information regarding the biocompatibility of the materials but for getting regulatory approval for application on human subjects, rigorous *in vivo* tests in animals are necessary.

Water vapor transmission rate

Water vapor transmission rate (WVTR) is defined as the quantity of the water vapor under specified temperature and humidity conditions, which passes through unit area of film material in fixed time. WVTR is measured in grams per square meter (g/m^2) over a 24 hours period according to the US standard ASTM E96-95 [27]. It is inversely proportional to the moisture retentive nature of a wound dressing i.e. the wound dressing with lower WVTR will be able to retain wound surface moisture. Typically, a wound dressing material showing WVTR less than 35 $g/m^2/hr$ is defined as moisture retentive and helps in a rapid healing [28].

Mechanical properties

It is important to characterize the hydrogels for their mechanical properties. This is because the hydrogels could be used in various biomedical applications, viz. ligament and tendon repair, wound dressing material, matrix for drug delivery and tissue engineering, and as cartilage replacement, which requires hydrogels with different properties. FDA also provides strict guidelines for the same depending upon the type of applications. As for example, a drug delivery device should maintain its integrity during the lifetime of the application, unless it has been designed to degrade. A common method of increasing the mechanical strength is by increasing the crosslinking density, resulting in the formation of stronger gels, but with the increase in

crosslinking density there is also a decrease in the % elongation of the hydrogels i.e. the hydrogels become brittle in nature. Hence, depending on the desired properties of the final products an optimum degree of crosslinking should be used. Copolymerization with a co-monomer, which may increase the H-bonding within the hydrogel, has also been utilized by many researchers to achieve desired mechanical properties [29].

Chemical/Physical analysis

Since presence of different functional groups play an important role in the water holding capacity of the hydrogel, hence, it becomes necessary to analyze the presence of different functional groups in a newly synthesized hydrogel. Also, determination of the functional group can provide some information on the composition of the polymeric network. The various techniques which are used for the purpose include infrared (IR) spectroscopy, UV-visible spectroscopy, nuclear magnetic resonance (NMR) and mass spectrometry [30]. The chemical bonds in a molecule are always either in stretching or in bending motion. The IR spectroscopy involves excitation of the functional groups with IR irradiation of a particular wavelength which results in the increase in the amplitude of the vibrations (bond stretching and bending) of the functional groups. The stretching vibrations can either be symmetric or asymmetric, while the bending vibrations can either be in-plane or out-of-plane. The change in the amplitude of the vibrations of the bonds is recorded by the IR instrument. For example, free O-H bond demonstrates stretching at 3650-3590 cm^{-1}. The position of the bond vibration of a functional group is dependent on the chemical environment around itself. Presence of functional groups which has the ability to give rise to H-bonding with the O-H group lowers the frequency of the vibration. Typically, an intermolecularly H-bonded hydroxyl group shows vibration at 3550-3450 cm^{-1}. Acidic carboxyl group shows C=O stretching at 1700 cm^{-1} and amines show N-H stretching at 3500-3300 cm^{-1}.Chemical bonds showing IR vibrations include Hydrocarbon chromophore (C-H, C-C,C=C, C=C), Carbonyl chromophore (C=O; ketone, aldehyde, ester, carboxylic acids, acid anhydride, acyl halide, amides), alcohols and phenols (O-H), amines (N-H, C-N), unsaturated nitrogen compounds (C=N, C=N, N=N,N=C=N), halogen compounds (C-F, C-Cl, C-Br, C-I) and sulphur compounds (S-H, C=S,S=O). The samples for IR analysis are generally analyzed as mulls in mineral oil or as potassium bromide (KBr) pellets in the range of 4000-400 cm^{-1}. IR spectra in

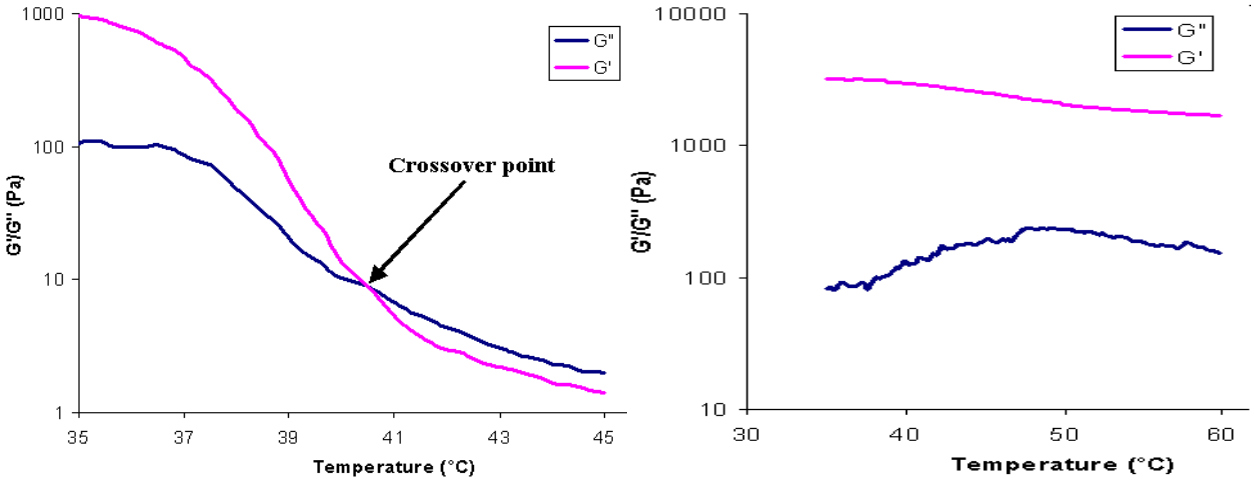
mineral oil show CH stretching of the hydrocarbon skeleton of mineral oil which may interfere with the analysis. The samples which cannot be analyzed by transmit infrared light mode are generally analyzed by attenuated total reflectance (ATR) mode, developed by Harrick and his co-workers, in which the IR light is reflected from the surface of the sample. This reflected light is collected by the instrumental setup which reveals the presence of the functional groups. The limitation of this technique is that only the surface groups can be analyzed while the functional groups in the core of the sample cannot be analyzed. This technique can be used to analyze samples up to the depth of ~ 5 microns [30-31].

The use of X-ray diffraction (XRD) for determining the crystalline nature of a substance has been used since long. XRD throws light on the properties of the different phases (for example structural make-up, % crystallinity and crystallite dimension, orientation and strains) present in the polymer/ polymer blend matrix. The XRD is based on the principle of diffraction of the X-rays from the atomic lattice of the specimen when the specimen is being irradiated with a beam of monochromatic x-rays. These diffracted rays are analyzed carefully to find out the angles of diffraction and the intensity of the diffracted rays. The angle of diffraction is useful in calculating interplanar atomic spacing (d-spacing). The d-spacing can reveal information, viz. unit cell size or lattice parameter, on the arrangement of atoms in a compound. The d-spacing and intensity information are unique for a particular material and act as a unique fingerprint for the material. Hence, the information can be used to characterize materials, both quantitatively and qualitatively, even in a mutli-component mixture. The width of the diffracted peaks can reveal information on the crystallite size and the micro-strain developed within a specimen [32].

Polymers are mainly amorphous in nature but some of them are semi-crystalline in nature, i.e. they have both amorphous and crystalline parts. The semi-crystalline polymers can be identified and characterized by XRD [33]. The crystalline parts in the polymers (also known as crystallite sites) are the regions where there is a presence of a highly ordered structure which act as crosslinked sites. The properties, viz. mechanical and water uptake, of a polymer may vary with the change in “degree of crystallinity” and is often represented as % crystallinity or crystalline/amorphous ratio. The degree of crystallinity is of immense importance in polymer industry and can be determined easily with the help of XRD [34].

Rheological analysis

The characterization of food materials using rheological properties have been done since long. The various food materials (e.g. yoghurt) can be classified as hydrogels and has been well classified by rheological techniques. Taking a lesson from the food industries, scientists are trying to use this powerful technique for the characterization of the polymers and hydrogels. As the molecular weight (MW) of a polymer is increased there is a corresponding increase in the zero shear viscosity. The MW of an unknown polymer can also be determined using rheology. In this method, the rheological properties of the polymer solution at different dilutions are determined and a plot of ratio of specific viscosity and concentration of the polymer solution ($\eta_{specific}$/ c) vs. concentration of the polymer solutions is made. Then the plot is extrapolated to the zero concentration of the polymer which corresponds to the MW of the polymer. This type of plot is named as Huggin's plot. The storage modulus (G') and loss modulus (G") of polymer solutions have been used to differentiate an uncrosslinked polymer from a crosslinked polymer. The G" is much higher than the G' for the uncrosslinked polymer solutions and is mainly attributed to the higher viscous property as compared to elastic property over the entire frequency range. While the G' is higher than the G" for the partially crosslinked polymer solutions. In addition to this, the slope of the G' also increases. For hydrogels, which are highly crosslinked polymer networks, both G' and G" are very high and are nearly parallel to each other (Figure 6 b) [35]. The G' and G" values of a hydrogel is measured in the linear viscosity range. In the case of uncrosslinked gel, the point where the G' and G" intersects each other is known as cross-over point and denotes the gel-sol transition temperature (Figure 6 a).



(a) **(b)**

Figure 6. Typical rheological behavior of gelatin hydrogels. (a) Uncrosslinked gel; (b) Gel crosslinked with genipin

The rheological studies can also be used to study the physical interactions within physical hydrogels. The addition of glycine in polyvinyl alcohol (PVA) leads to the formation of physical hydrogel due to the formation of intermolecular hydrogen bonding among the molecules. In a recent study, Pal *et al* studied the viscosity profile of PVA solution upon addition of glycine solution of different concentrations. The authors reported that PVA showed Newtonian flow behavior. As the amount of glycine was increased in the mixture, the viscosity was increased but the mixture showed Newtonian behavior up to a PVA: glycine ratio of 1:0.6 (w/w). With the subsequent increase in the glycine content, the flow behavior changed to non-Newtonian and was attributed to the strong hydrogen bonding (Figure 7) [36].

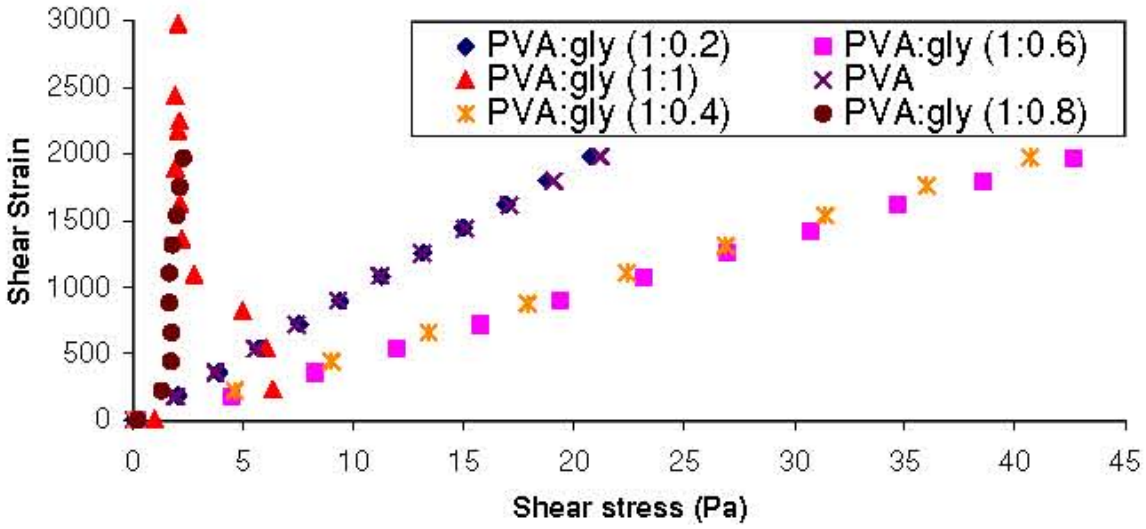


Figure 7. Stress-strain analysis of PVA and glycine mixtures [36]

Surface Topography

The surface morphology of a hydrogel can reveal minute important details. The monitoring of the surface properties of materials can either be done by contact profilometers or by non-contact profilometers. Atomic forced microscopy (AFM) is commonly used for determining the surface properties of the hydrogel and xero gels (Figure 8). AFM is a contact profilometer and can be operated either in contact mode (also known as static mode) or in tapping mode. In the static mode, the tip of the AFM instrument drags over the sample surface. The surface properties are measured as a function of the tip deflection as it moves over the surface. The deflection of the tip is generally measured using a laser beam detector which detects the reflected laser beam from the upper surface of the cantilever holding the tip. While in the tapping mode, the tip of the cantilever has a piezoelectric element (PZTe). The cantilever is oscillated in the resonance frequency of the PZTe. As the tip approaches the surface of the material, there is an increased interaction between the surface and the tip thereby resulting in the decrease in the amplitude of the oscillation. The decrease in the amplitude of oscillation is then recorded and compared with an external reference, which provides information on the surface characteristics of the material. The chances of sample damage are minimal in tapping mode as compared to contact mode [37].

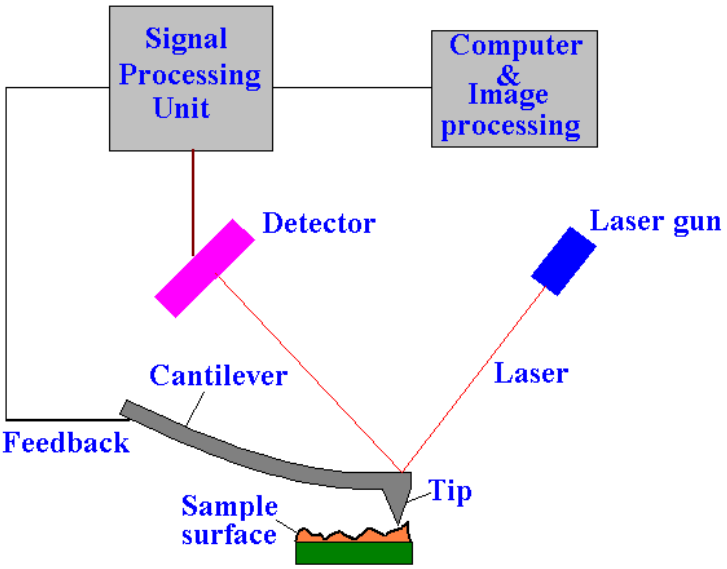
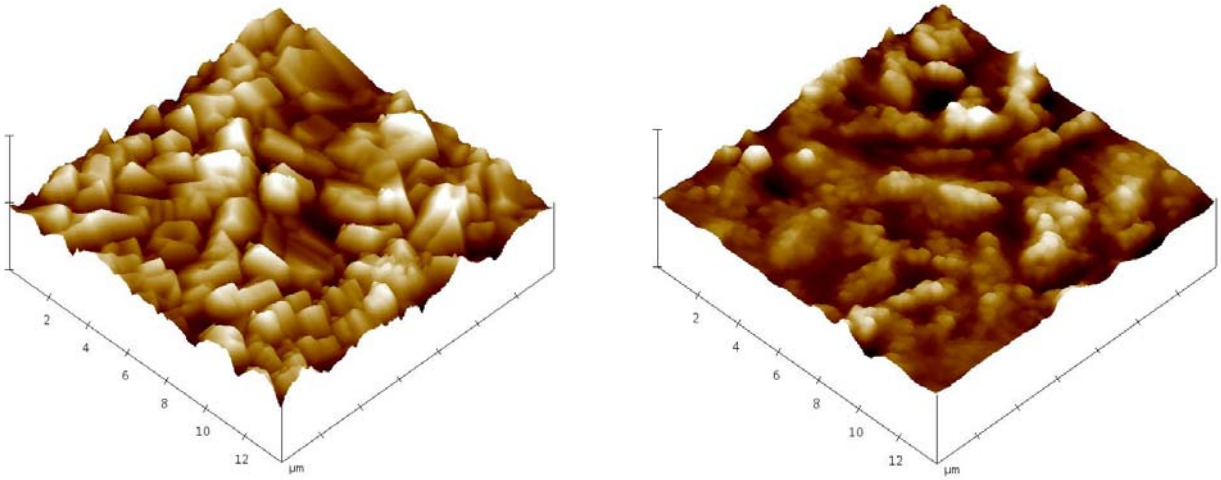


Figure 8. Schematic representation of the static AFM unit



(a) **(b)**

Figure 9. AFM image of carboxymethyl cellulose acrylate in Tapping mode (a) xero gel (b) Hydrated gel

Figure 9 shows the AFM image (in tapping mode) of carboxymethyl cellulose acrylate (SCMCAA) hydrogel prepared in our laboratory. The surface of the dry SCMCAA is showing

the presence of regular building blocks (BB) of sizes ~1-2 µm. The shapes of the building blocks are irregular with no visible pores. The surface morphology of the hydrated SCMCAA reveals the presence of sub-micron building blocks (SBB) with visible pores on the surface. This can be attributed to the lack of moisture in the xerogel, which results in the collapsing of the SBB thereby forming BB.

The surface characteristics can also be obtained by an optical profiler. In general, Vertical Scanning Interferometry (VSI) mode is used for scanning the surface of the samples. The method can measure the surface characteristics with nanometric resolution. In the method, a white light of wide spectra bandwidth is focused on the sample surface with the subsequent determination of high contrast-fringes on the sample's surface. As the fringes are adjusted in the field of view, the scanning of the surface is carried out. The method can also be used to analyze sample surfaces with regions of different materials or surfaces [38]. Figure 10 represents a surface of gelatin-sunflower oil solid emulsion crosslinked with genipin. The surface indicates that the surface of the sample is uneven with some protruding peaks of ~1 µm.

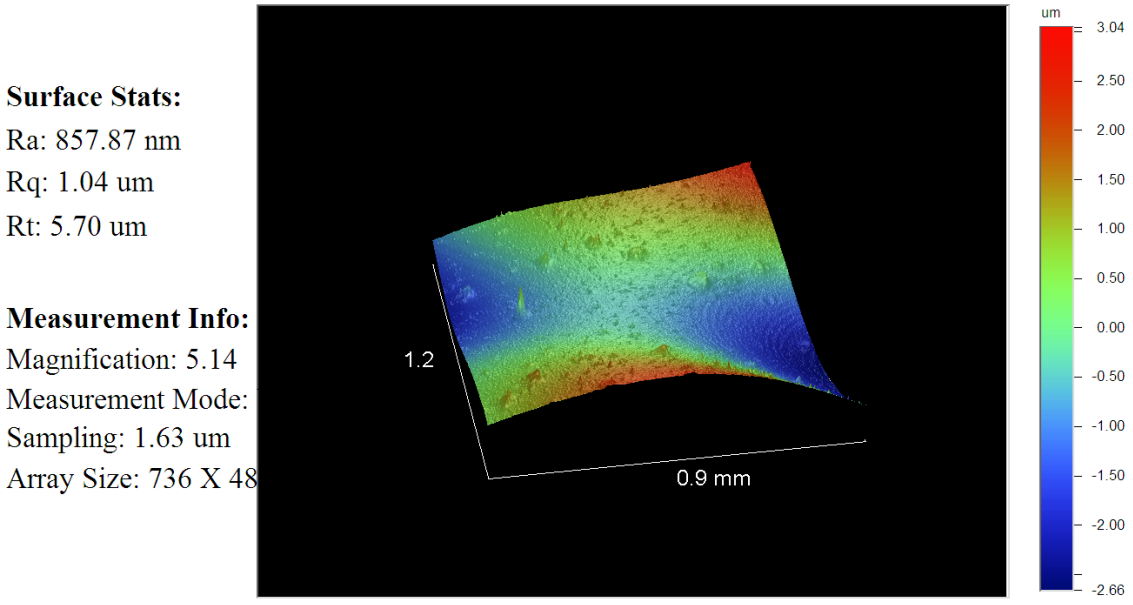


Figure 10. Optical profilometry image of gelatin-sunflower oil solid emulsion crosslinked with genipin.

Biomedical applications of hydrogels

A. Applications of hydrogels in drug delivery

Hydrogels have been used for the development of controlled delivery systems for a long time. When the drug bearing hydrogel comes in contact with aqueous medium, water penetrates into the system and dissolves the drug. Diffusion is the main phenomena by which the dissolved drug diffuses out of the delivery systems to the surrounding aqueous medium. Diffusion is defined as the movement of the individual molecules from the region of high solute concentration to a region of low concentration when the systems are separated by a polymeric membrane. This phenomenon of diffusion is mainly attributed to the Brownian motion. The delivery systems employing hydrogels for controlled release can be categorized into reservoir and matrix devices. As mentioned earlier, hydrogels are 3-dimensionally cross-linked polymer networks and hence act as a permeable matrix/membrane for the drug thereby governing the release rate of the drug. The diffusion of the drug through the hydrogels may be affected by the property (viz. pH sensitivity, light sensitivity, pressure sensitivity) of the hydrogel depending on the chemistry of the hydrogels and has been used successfully to design delivery systems which may release drug at a suitable environment. The drug transport mechanisms can be determined by fitting the early time release data to the following empirical relationship [39-40]:

$$\frac{M_t}{M_\infty} = kt^n \qquad (5)$$

Where M_t is amount of drug released at a given time t, M_∞ is amount of drug released at infinite time and k and n are the constants (characteristics of drug-polymer system). The diffusional exponent, n, is dependent on the geometry of the device as well as the physical mechanism of release

Lowman reported that the diffusional exponent (n) can give relative information about the release behavior of the bioactive agent from the hydrogel systems. He classified delivery systems based on the release profile of the bioactive agent from the system which include Fickian system (n=0.5), anomalous transport system (n=0.5-1), case II transport system (n=1) and super case II transport system (n>1) (Table 1) [41].

Table 1. Release mechanism from hydrogel based delivery systems [41]

Delivery Systems	Mechanism of release
Fickian System	Fickian diffusion
Anomalous transport	Fickian diffusion and polymer relaxation
Case II transport	Polymer relaxation
Super case II transport	Plasticization at gel layer

a. Reservoir system:

In reservoir drug delivery system, a drug-enriched core (often termed as reservoir) is encapsulated within a uniform polymeric membrane of hydrogel which allows the diffusion of drug through it (Figure 11) [42-44]. As the system comes in contact with water, water diffuses into the system and dissolves the drug and provides a concentration equivalent to the saturation solubility of the drug (Cs). The drug diffuses through the membrane to the external environment and the concentration falls below Cs.The solid drug present in the core dissolves and restores the concentration back to Cs.Thus the release of the drug from a reservoir system remains constant and follows zero order kinetics so long solid drug is present in the core. Once the solid drug is exhausted, the release becomes concentration dependent following first order kinetics. These kinds of drug delivery systems are mainly used to deliver the active agent by oral, ocular, uterine, or transdermal routes.

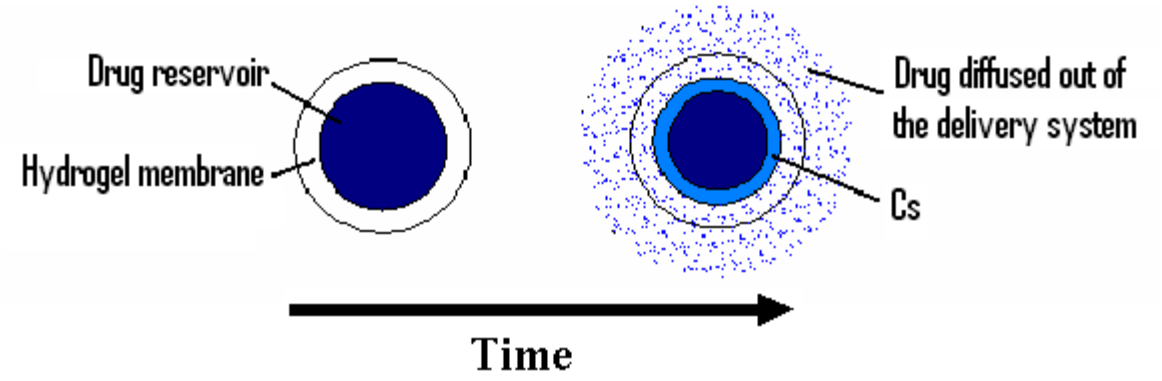


Figure 11. Drug delivery from typical reservoir device.

b. Matrix system:

In matrix type delivery system, the active agent is homogenously dispersed as a solid into a hydrogel matrix (Figure 12). The release of drug from the matrix depends on the properties of matrix. When the matrix is placed into the aqueous medium, water starts diffusing into the matrix thereby hydrating the same. The hydration of the matrix starts at the surface and continues towards the center of the core. The release of drug is dependent on the diffusion of water into the matrix followed by the dissolution of the drug and finally the diffusion of the dissolved drug from the matrix. Generally, inert polymer matrices are considered to prepare this kind of delivery systems. Of late bio-degradable polymers have also been used to design such delivery systems [45]. Polymer-drug interaction plays an important role in the release behavior of the drug. Hence, polymers interacting with drugs could be tried to modulate the release profile of the drug. The thickness of the hydrated matrix is considered as the diffusional path length of the drug. If we consider the polymer matrix to be inert and the drug release is diffusion-controlled, then the release rate of the drug could be described by the Higuchi equation [46], which relates drug release with the square root of time:

$$Q = \sqrt{2ADC_s t} \qquad (6)$$

where Q is the amount of drug released at time t, A is the total concentration of drug in the matrix, D is the diffusion coefficient and Cs is the saturation solubility of the drug in the mini-matrix.

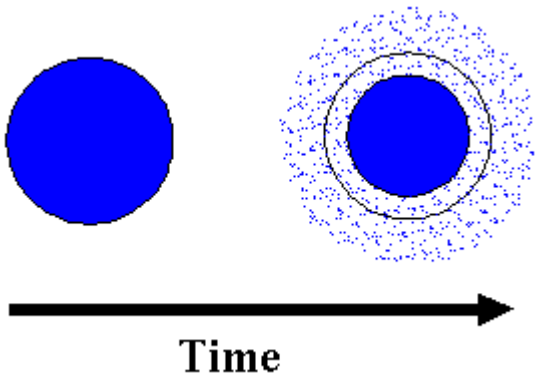


Figure 12. Drug delivery from a typical matrix drug delivery system.

If the drug delivery system is a true swelling-controlled system, then the diffusional exponent, n, assumes a value of 1 and results in zero-order release kinetics (Case II transport). However, if the drug release occurs due to a combination of macromolecular polymer chain relaxation of the matrix and Fickian diffusion, then the diffusional exponent has a value between 0.5 and 1 and results in anomalous or non-Fickian transport [47].

The chemically controlled release systems can be classified into (a) erodible systems and (b) pendant chain systems. In the erodible systems drug release mainly occur due to the degradation and/or dissolution of the matrix, which exposes the drug to the release media. While in the pendant chain systems, the drug is covalently bonded to the polymer chains of the matrix. The drug is released due to the degradation of these linkages in the physiological environment.

Hydrogels may change their equilibrium water uptake due to the change in the environment, viz. temperature, pH, ionic strength and temperature, of the release media [42, 48]. These kinds of hydrogels can be used for the development of controlled delivery systems. Depending upon the design of the delivery system, the drug may be released either by diffusion while the matrix is in the swollen state or by squeezing during the syneresis process.

Researchers are working on new strategies to develop delivery systems which can deliver the drug in a controlled fashion. For the purpose, hydrogels offer them a wide variety of properties, viz. bio-adhesive and environment sensitive nature, to achieve the goal. Hydrogels have already been successfully used to develop oral, rectal, ocular, transdermal and implantable drug delivery

systems. Figure 13 illustrates various sites that are available for the application of hydrogels for drug delivery.

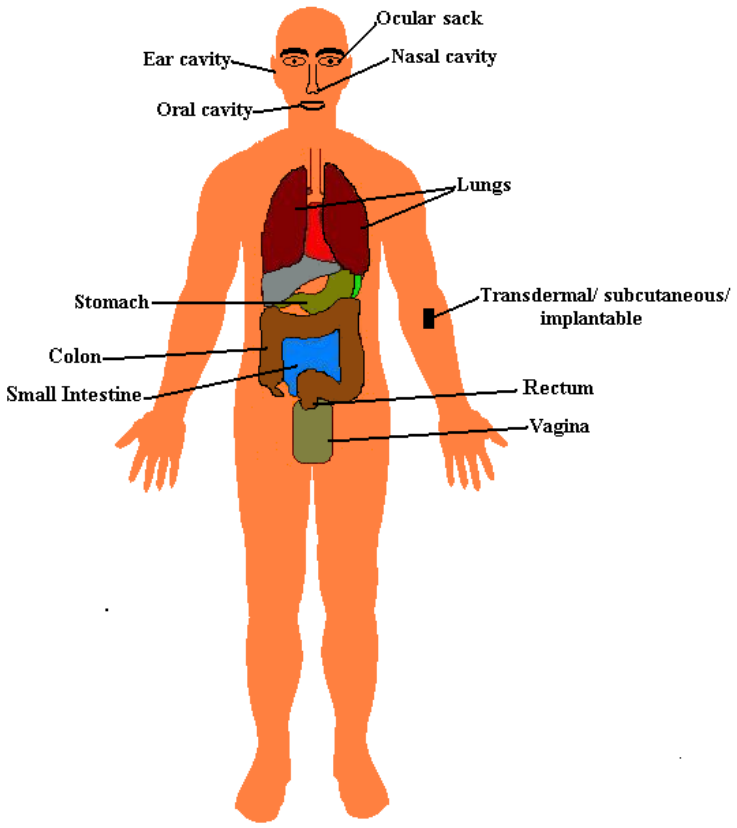


Figure 13. Different sites in body where Hydrogels can deliver drug [modified from 49]

Oral delivery of drug is cheap and allows maximum patient compliance. Through oral delivery system one can target mouth, stomach, small intestine and colon [49]. The bioadhesive property of the hydrogels could help to deliver drugs to the oral cavity or at the specific sites of gastro-intestinal tract (GIT). These hydrogels have been used to locally treat periodontal diseases, fungal and viral infections and oral cavity cancers. The main challenge in the local treatment of diseases of the oral cavity is to keep the delivery system at the site of infection for a long period of time. These delivery systems can also be used for the delivery of liposomes in addition to the topical treatment into the oral cavity. Orabase® (a sodium carboxymethyl cellulose, pectin and gelatin combination in a polyethylene-paraffin base), Carbopol 934P and neutralized poly

(MAA-co-methyl methacrylate (MMA)) have been successfully employed for the delivery of liposome into the oral cavity by Petelin and co-workers [49-50]. The drugs susceptible to high first pass metabolism can also be delivered systemically by sub-lingual/buccal route using this type of delivery system. Pitaressi and co-workers investigated the release behavior of amoxicillin in simulated buccal and gastric conditions [51]. The bioadhesive hydrogels increase the gastric residence time of the delivery system thereby ensuring the release of most of the drug at the delivery site and increase in the bioequivalence. Lectin has been used for long in such delivery systems.

The environment sensitive hydrogels have been effectively used to deliver drug at specific sites of the GIT. Enteric polymers like Eudragits have been used for long to deliver drugs at various sites of intestine. It has been stated earlier that the enteric polymers are generally used either to protect the acid-labile drugs (e.g. peptides and penicillin-G) from the harsh environment of the stomach or to avoid the contact of the gastric mucosa with the gastric-irritant drugs (e.g. ibuprofen, indomethacin), which might lead to gastric mucosa perforations. Akiyama and co-workers developed such an enteric system using poly (acrylic acid) product, which inhibited the hydrolytic activity of trypsin [52]. The oral delivery of insulin employing the use of enteric polymers is gaining importance [53]. A complex system of alginate-chitosan microparticles was developed by Hari et al. for the controlled release of bioactive peptides including insulin. In the study, the bioactive peptide dispersed in the crosslinked alginate formed the core with a subsequent layer of chitosan over the core [54]. Thermal crosslinking of the anionic hydrogel comprising of poly (vinyl alcohol) and poly (γ-glutamic acid) was found to be pH-sensitive in nature and was found to be compatible with the 3T3 fibroblast cell line. The drug diffusion in the hydrogel indicated its probable use for the oral delivery of the bioactive agent [55]. A controlled release system consisting of N-succinyl chitosan/alginate prepared by ionic gelation indicated a pH-dependent release profile of nifedipine [56]. Microspheres of interpenetrating networks of poly(methacrylic acid) and poly(vinyl alcohol), which were crosslinked with glutaraldehyde were able to deliver ibuprofen into the intestine [57]. Cationic hydrogels has the potential to deliver the drug in the stomach and not releasing the drug in the colon and intestinal environment. Such kind of delivery system was developed by Patel and co-workers for the

treatment of *H. Pylori* infection with an antibiotic [58]. Due to the presence of lower proteolytic activity in colon, it is becoming a hot spot to deliver peptide drugs.

Of late, the rectal route of administration of drug is also gaining attention even though the patient acceptability is low. The main reason for this can be attributed to the rich blood supply to the region, which helps in increased bio-availability of the drug as the first-pass metabolism is partially bypassed. Rectal route of administration has been used for a long time for the local treatment of hemorrhoids but the main limitation of the delivery route was the migration of delivery device either towards the colon or out of the body. With the discovery of bioadhesive hydrogels and subsequent use of the same in the rectal administration have reduced the chances of delivery system migration and thereby increased the bioavailability of the administered drug [59].

Administration of aqueous drops in ocular cavity is the preferred way to administer drug in the ocular cavity. But most of the drug is removed from the ocular cavity due to tear drainage and blinking. In addition to this, the low permeability of the cornea worsens the situation. Though the use of suspension and ointments increase the ocular retention time, they produce a gritty feeling thereby reducing the patient compliance. The use of in-situ-gelling systems can increase the ocular retention time and ocular availability of the drug to a greater extent. The advantage of this kind of delivery system lies in the fact that it is liquid while dispensing and administering, but forms a drug depot after it is administered in the ocular cavity [49, 60].

Human skin can be easily accessed by a person and has got a large surface area which makes it a potential site for administering drugs, both locally and systemically. Systemic delivery of drug by this route of administration helps in bypassing the first–pass metabolism and delivery of the drug for prolonged period of time at a constant rate [49, 61]. In addition to the above advantages, the hydrogels provide a soothing effect on the skin as compared to occlusive/oily feeling caused by the application of ointments. The various drugs used in this type of delivery system include nitroglycerin and hydrocortisone [62]. Hydrogels have also been proposed as a delivery system to wound surface and in-situ gel forming hydrogels are preferred due to the relative ease of application and increased contact between the hydrogel and wound surface [63]. In spite of the above advantages, the main concern is the permeation of the drug through the keratinized

epidermis. Currently research is being carried out to increase the drug permeation through the keratinized layer using either electrical force (iontophoresis) or physical force of ultra-sound (sonophoresis). The drugs whose permeability can be increased by iontophoresis include luteinizing hormone, sodium nonivamide acetate, nicotine and enoxacin while the permeability of insulin and vasopressin can be increased using sonophoresis.

The delivery of drug to the ear cavity is mainly carried out by the use of aqueous or oil drops. The main limitation in the use ear drops is the retention time of the drops in the cavity while the person is standing. The use of hydrogel for the delivery of drugs to the ear cavity can be done easily. Lee and co-workers were successful in delivering recombinant human insulin-like growth factor I (rhiGF-1) locally using gelatin hydrogel. The group found that by delivering the rhiGF-1 by this method can be useful in the treatment of noise-induced hearing loss [64]. Of late scientists are working on the local delivery of the drugs in the ear cavity using hydrogels.

The local delivery of drugs to the lungs is generally achieved either by powder insufflators or by inhalational aerosols. The limitation of these types of delivery systems includes the immediate absorption of the drug from the site of application. The use of biodegradable hydrogels for the delivery of active agents may help in this regard. Tomoda and Makino studied the effect of lung surfactants on the release properties of rifampicin loaded in inhalable PLGA microspheres on the tubercle bacilli. They found that with the change in the surface properties of the PLGA microspheres there is a change in the uptake efficiency of the drug by the alveolar macrophages, the site where the tubercle bacilli resides in lungs [65].

Due to the presence of wide variation in properties, the hydrogels have been used in a wide variety of pharmaceutical applications. In addition to this, the hydrogels are generally biocompatible and can be tried as an implantable delivery system. Of late the implantable delivery system is being directed to biodegradable matrices, which will be eliminated from the physiological system after the drug supply is depleted. A semi-interpenetrating structure developed by Cho and co-workers comprising poly (ε-caprolactone) and PEG macromer terminated with acrylate groups is one of the examples of the degradable matrices, which released clonazepam in a controlled manner for a period of 45 days [66].

B. Applications of hydrogels in wound healing

The use of hydrogels in the healing of wounds dates back to late seventies or early eighties. As mentioned earlier, hydrogel is a crosslinked polymer matrix which has the ability to absorb and hold water in its network structure. Hydrogels act as a moist wound dressing material and have the ability to absorb and retain the wound exudates along with the foreign bodies, such as bacteria, within its network structure. In addition to this, hydrogels have been found to promote fibroblast proliferation by reducing the fluid loss from the wound surface and protect the wound from external noxae necessary for rapid wound healing. Hydrogels help in maintaining a micro-climate for biosynthetic reactions on the wound surface necessary for cellular activities [67]. Fibroblast proliferation is necessary for complete epithelialisation of the wound, which starts from the edge of the wound. Since hydrogels help to keep the wound moist, keratinocytes can migrate on the surface. Hydrogels may be transparent, depending on the nature of the polymers, and provide cushioning and cooling/ soothing effects to the wound surface. The main advantage of the transparent hydrogels includes monitoring of the wound healing without removing the wound dressing. The process of angiogenesis can be initiated by using semi-occlusive hydrogel dressings, which is initiated due to temporary hypoxia. Angiogenesis of the wound ensures the growth of granulation tissue by maintaining adequate supply of oxygen and nutrients to the wound surface. Hydrogel sheets are generally applied over the wound surface with backing of fabric or polymer film and are secured at the wound surface with adhesives or with bandages [68].

C. Applications of hydrogels in tissue engineering

Tissue engineering (TE) is a multidisciplinary approach and involves the expertise of materials science, medical science and biological science for the development of biological substitutes (tissue/ organ). It is emerging as an important field in regenerative medicine. It has got three basic components namely, cells/tissues, scaffolds and implantation and/or grafting. The principles of TE have been used extensively to restore the function of a traumatized/malfunctioning tissues or organs [10, 69]. In practice, the patient's cells are generally combined with a scaffold for generating new tissue. A scaffold can be made up of either ceramic or polymer, which can be either permanent or resorbable. The pore size of the scaffolds should be >80 μm [70]. This is necessary for the cell migration into the core of the

scaffolds, angiogenesis, and supply of nutrients to the cells and to take away the metabolic products away from the cells. The scaffolds made up of polymers are generally hydrogels. Every year thousands of people are victims of tissue loss and organ failure caused either due to disease or trauma. Also, there is a shortage of organ donors because of the religious beliefs and/or medical complications. Keeping the above facts in mind, TE can be a useful tool to replace the damaged/malfunctioning organs or tissues. Recently the use of resorbable hydrogels in TE has gained much importance because (a) it is easy to process the polymers; (b) the properties of the hydrogels can be tailored very easily; and (c) resorbable polymers like polylactic acid (PLA), polyglycolic acid (PGA), and their co-polymers (PLA-co-PGA; PLGA) are being used for biomedical application since long time. Sterilization of the hydrogels is very tricky, which may alter the characteristics of the scaffold. Hence, due consideration on the sterilization method should be given before selecting a particular sterilization method [10].

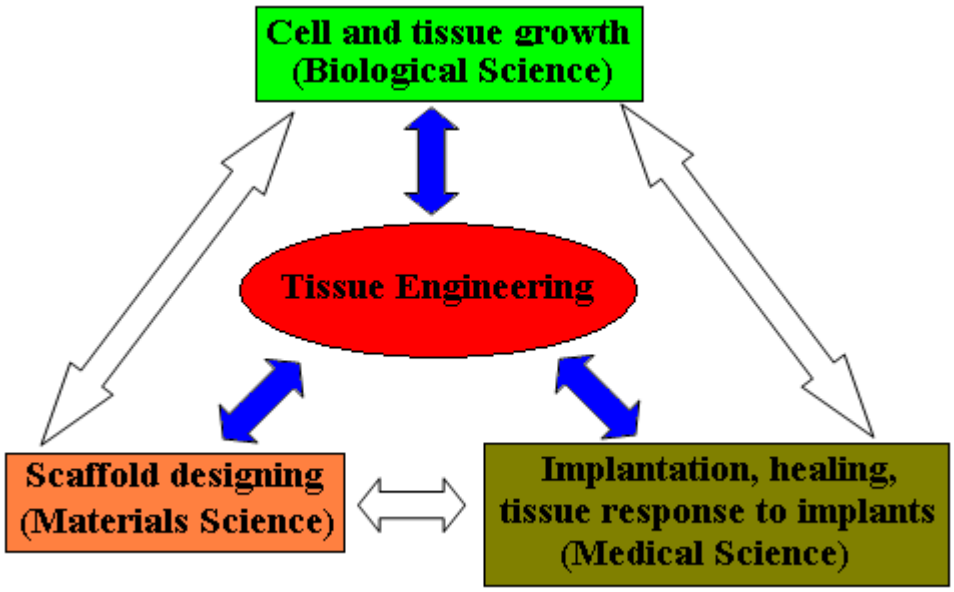


Figure 14. Schematic diagram showing multidisciplinary approach of tissue engineering

Examples of various tissue engineering employing various hydrogels have been provided below:

- Collagen-coated tissue culture inserts are used for growing three- dimensional corneal implant, tracheal gland cells etc [71].
- Poly (lactic-co-glycolic acid) (PLGA) polymer foams are seeded with preadipocytes for the epithelial cell culture of the breast [71].

- Porous scaffolding (e.g. filter, swatch of nylon, transwell, biodegradable microcarrier) coated with fibrillar collagen,ideally type III collagen mixed with fibronectin or with Matrigel are used for the culture of the normal mature liver cells (polyploidy liver cells) [71].

D. Application of hydrogels for gene delivery

Gene delivery is defined as the incorporation of foreign DNA particles into the host cells and can be mediated by viral and non-viral methods. The delivery of gene into the host cells by utilizing a virus uses the capability of a virus to incorporate its DNA into the host cells. For the purpose retroviruses and adenoviruses have been used. These viral vectors are used as they can provide efficient transduction and high gene expression. At the same time, the use of viral vectors is quite limited as they can produce immunogenic reactions or mutagenesis of transfected cells. Hence, scientists are tuning their interest towards the available non-viral techniques, which produces less complexity. The non-viral techniques include the use of a gene gun, electroporation and sonication. Of late researchers have started the use of polymers, viz. poly-L-lysine (PLL), polyamidoamine dendrimer (PAMAM), polyethylenimmine (PEI), PGA, PLA and PLGA, for gene delivery [72]. Though PAMAM and PEI can provide high transfection efficiency, their use is limited due to their poor degradability. This is why the use of biodegradable polymers, viz. PLA, PLGA and PGA, has gained importance. The use of PEG-PLGA-PEG hydrogel for the delivery of plasmid-beta 1 gene increased the wound healing process in diabetic mouse model [73]. Meilander- Lin and co-workers reported similar results with agarose hydrogels. They concluded that agarose gels can be useful in the wound-healing and TE applications [74]. Mageed and co-workers reported the use of recombinant silk-elastin like polymer hydrogels (SELP) for the delivery of pRL-CMV for the treatment of human breast cancers. Their results suggested an increase in the transfection efficiency when SELP hydrogels were used [75]. A recent study describes encapsulation of C2C12 myoblasts in a biocompatible permselective hydrogel such as alginate –poly-L–lysine- alginate (APA) to protect the cell from host immune response; while allowing diffusion of gene products. Inclusion of basic fibroblasts growth factor (BFGF), insulin growth factor II (IGF- II) and collagen within the microcapsules showed proliferation and differentiation of encapsulated C2C12 myoblasts. When tested against tumor

induced by B16-Fo/neu tumor cells in mice, the APA microcapsules had an 80% reduction in tumor volume at day 21 [76].

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