Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 8 (3) 217-231: Fall 2011
(J. Phys. Theor. Chem. IAU Iran)
ISSN 1735-2126

Physicochemical Characteristics and Biomedical Applications of Hydrogels: A Review

R. Sariri*

Department of Chemistry, Lahijan Branch, Islamic Azad University, Lahijan, Iran

Received October 2011; Accepted October 2011

ABSTRACT

Hydrogels are introduced to modern medicine as novel materials suitable for a variety of biomedical applications. Studying hydrogels as novel biomaterials has become a fast-developing and exciting research field during the last two decades. These interesting biomaterials have found a wide range of application including contact lenses, vehicles for drug delivery and scaffold in tissue engineering and protein delivery systems. Traditionally hydrogels are formed by chemical cross-linking of water-soluble polymers or by polymerization of water-soluble monomers. However, these cross-linking methods lack biocompatibility with fragile molecules like pharmaceutical proteins and living cells. In all types of applications, the biocompatibility of hydrogels is the most important factor to be considered. Many newly developed hydrogels are designed to gel spontaneously under physiological conditions. In these systems, hydrogel formation occurs *in situ*, at the site of injection, without the aid of potentially toxic or denaturizing cross-linking agents. This review paper presents the chemical nature and biomedical applications of hydrogels.

Keywords: Hydrogels; Tissue engineering; Protein delivery; Drug delivery; *In situ* gelation

INTRODUCTION

Hydrogels are hydrophilic polymer molecules which are cross-linked by water. They do not dissolve, but swell in water [1]. The capacity of hydrogels to absorb water is enormous and can be as much as 1000 times the weight of the polymer. The amount of water adsorbed by a hydrogel is expressed as the equilibrium water content (EWC) and is defined as:

$$\text{EWC} = \frac{\text{Weight of water in the gel}}{\text{Weight of the hydrated gel}} \times 100\%$$

The water in a hydrogel network exists in a state between two extremes. The "bound" or non-freezing water which is strongly associated with the hydrogel network through hydrogen bonds, whereas the "free" or freezing water has a much greater mobility and is unaffected by the polymeric environment. Hydrogels containing more than 95% water are termed super-absorbent and posses high biocompatibility due to their large degree of water retention and their physiochemical similarity with the native extra-cellular matrix both compositionally and mechanically [2].

Hydrogels were first introduced in 1960s and suggested for contact lens applications [3]. Since then research and development has rapidly increased on design, synthesis and application of hydrogels. In recent years these new materials are used in a broad range of pharmaceutical and biomedical applications [4-6]. Both natural and

* Corresponding author: sariri@guilan.ac.ir

synthetic polymers are used for the production of hydrogels. Cross-linking of the polymer chains can be achieved by various chemical or physical cross-linking methods [7]. Their wide range of application is mainly due to ease of preparation, high capacity of absorbing and releasing water, good biocompatibility and the excellent oxygen permeability.

Synthesis of Hydrogels

Hydrogels are formed by physical or chemical cross-linking of homo- or co-polymers, being appropriately used to give the necessary three-dimensional structures with specific mechanical and chemical characteristics. The cross-link can be formed by covalent, chemical [8] or non-covalent, physical interactions [9]. The cross-link can take place after or at the same time as the copolymerization [10]. The cross-link density is proportional to the equilibrium water content of hydrogels. Various cross-linking agents are used depending on the nature of polymer backbone.

Physical cross-linking

Physical gels are three-dimensional networks where the polymer chains bond through non-covalent interactions. One of the methods to form physical cross-linking is hydrophobic interaction in which hydrophobic blocks are coupled to hydrophilic blocks creating an amphiphile polymer. By increasing temperature, the hydrophobic blocks aggregate. It has been reported that the polymer concentration, the hydrophobic block length and the chemical structure of the polymer all affect the temperature in which the phase change takes place [2].

Chemical cross-linking

In this type of cross-linking, the bonds between polymers network are of covalent I nature. As covalent interactions are much stronger than non-covalent, excellent mechanical stability is obtained by this type of cross-linking. The practical methods include radical polymerization, chemical reaction of complementary groups, high energy irradiation and the use of enzymes [7].

Industrial applications of hydrogels

Hyrogels are used in pharmaceutical preparations to enhance the solubility or as biodegradable polymeric systems for control release of drugs. Biodegradable hydrogels have increasingly found wide applications in the improvement of existing dosage forms and development of new and more effective drug delivery systems. Some other industrial and medical applications of hydrogels are summarized as:

- ✓ Hydrogels are used in plant science and agriculture in order to provide a continuous release of moisture to plants.
- ✓ Hydrogels are used as thickening agents (e.g., starch and gelatin in foods), because they absorb a large volume of water from the food products.
- ✓ Technical and electronic instruments can be protected from moisture by enclosure in highly absorbent hydrogel-forming agents.
- ✓ Hydrogels can be used in electrophoresis and chromatography techniques, bearing in mind that they should possess a limited range of swelling for these purposes [11].
- ✓ Hydrogels are used in photographic technology because they are light-permeable and can also store light-sensitive substances.
- ✓ One of the most widely used applications of hydrogels is in the manufacture of soft contact lenses due to their desirable water content and biocompatibility.
- ✓ The use of hydrogels as synthetic articular cartilage has met with little clinical or commercial success, because of their relative poor mechanical properties [12].

- ✓ The new family of hydrogels based on interpenetrating polymer network (IPN) technology has been synthesized using composite structure of natural cartilage as a model.

Hydrogels in contact lens industry

One of the most widely area of hydrogel application is in the manufacture of soft contact lenses. Many commercial soft contact lenses are based on poly 2-hydroxyethyl methacrylate (PHEMA) more commonly referred to as HEMA, with EWC of 40%. Figures 1 and 2 show a range of monomers used in hydrogel synthesis. Hydrogel lenses are generally composed of four basic hydrophilic monomers: HEMA, glycerol methacrylate, vinyl pyrrolidone and methacrylic acid. In addition, cross-linking agents are typically added to produce mechanical strength and thermal stability. All of these hydrophilic monomers, with the exception of methacrylic acid, yield non-ionic polymers that interact with the polar molecules of water without generating a formal electrostatic change on the molecule. Low water content lenses of this type usually have water contents of 38-45%. High water content lenses, that are mostly vinyl pyrrolidone-based polymers, have water contents of 70-80%. These lens materials constitute the low and high water content non-ionic lens groups. On the other hand, ionic lens materials with low or high water contents are made using methacrylic acid, a charged, ionic monomer. Table 1 shows some of commercially available soft hydrogel lens materials.

The ionic lens materials have been shown to be more reactive with tear components and lens care products than non-ionic materials. Therefore, one of the major problems with hydrophilic contact lenses is their spoilage from tear film. The spoilage of contact lenses is due to different factors such as calcium films, organic plaques and protein films. The most important of these surface coatings appear to consist of proteins.

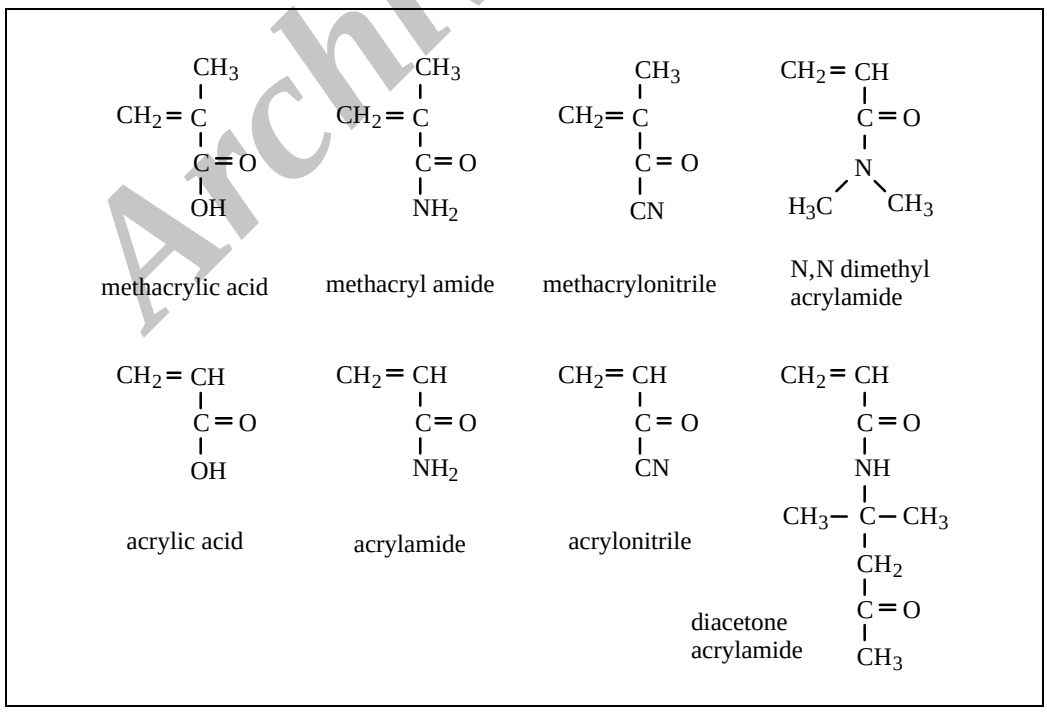

Fig. 1. Acrylic monomers used in hydrogel synthesis [13].

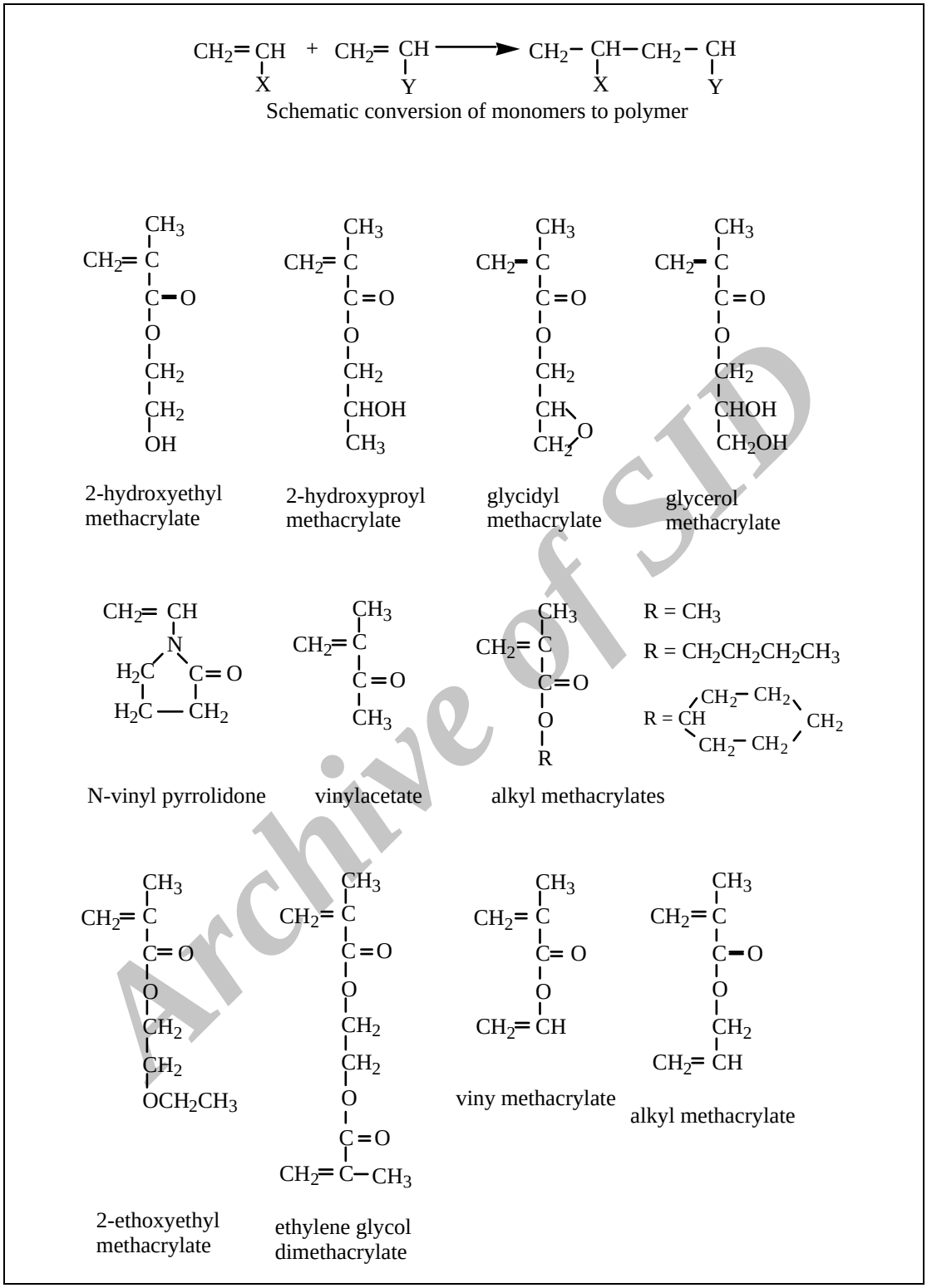

Fig. 2. Structures of some monomers used in hydrogel synthesis [13].

Table 1. Examples of soft contact lenses and lens materials

Name	Principle component	EWC [%]	Manufacturer	Nomenclature
Acuvue	HEMA, MAA	58	Vistakon	Etafilcon-A
A O	HEMA	38	American Optical	Telfilcon
Multivue	HEMA, PVP,	47	Strieter gel	Droxifilcon-A
Accugel	MAA	42.5	Pilkington Barens-	Tetraficon-A
Classic	HEMA, PVP,	41	Hind	Crofilcon-A
CSI	MMA	64	Pilkington Barens-	Atlafilcon
Excelens	MMA, GM	38	Hind	Polymacon
Frequency	PVA, MMA	38	Ciba	Polymacon
38	HEMA	38	Aspect	Polymacon
Hydron	HEMA	71	Hydron Europe	Perfilcon-A
Medalist	HEMA	38	Bausch & Lomb	Polymacon
Permalens	HEMA, PVP, MA	58	Pilkington Barens-	Etafilcon-A
Softlens	HEMA	46	Hind	Ocufilcon-A
Surevue	HEMA, MAA		Bausch & Lomb	
Tresoft	HEMA, MAA		Vistakon	
			Alcon Optics	

Smart hydrogels

This group of hydrogels is polymeric networks that could be degraded or swelled in response to various environmental factors. The most known factors that could act as stimuli are pH and temperature. More recently some synthetic hydrogels have been introduced with the ability to response to both stimuli.

pH-responsive hydrogel

These are hydrogels that sense the changes in pH of the environment. They have been studied and synthesized starting about 10-12 years ago [14-26]. It has been demonstrated that pH-sensitive hydrogel polymers can be produced by adding acidic or basic functional groups to the polymer backbone. They can response to any change in environmental pH by accepting or releasing protons. Variations in ionic strength of aqueous media would also trigger these hydrogels to change their structure. It is worth indicating that hydrogels with acidic groups become more ionized at higher pH values and basic hydrogels show more ionization at low pH. Therefore, acidic hydrogels tend to swell more as the pH of the surrounding solution increases, in contrast to basic hydrogels which shows more swelling behavior at lower pH values.

Temperature-responsive hydrogels

This group of polymeric hydrogels could show various degree of sensitivity to temperature alternations of the surrounding environment [14, 25, 26]. Once again, they may degrade gradually or swell in response to increasing or decreasing temperature. It has been demonstrated that thermo-responsive gels go through a phase transition in response to variation of temperature in their environment. Response of hydrogel to temperature may be positive i.e. the swelling increases with a rise in temperature [29] or negative which shrinks as temperature increases [27, 28]. The presence of hydrophilic groups induces water-solubility, while hydrophobic groups make the resulting hydrogel to be water-insoluble. For negative temperature sensitivity, the polymers are soluble in water at low temperatures due to their hydrophilic content, but they collapse above a certain temperature as hydrophobic interactions increase and they separate from the solution.

This temperature is termed the lower critical solution temperature (LCST).

N-alkyl acrylamides are among common monomers, used in the synthesis of temperature-sensitive hydrogels. For example, N-isopropylacrylamide (NIPAAm) with side chains which have favorable interactions with water in the form of hydrogen bonds. The efficiency of the hydrogen bonding process has negative temperature dependence. At lower temperatures, hydrogen bonding between hydrophilic segments of the polymer chain and the water molecules dominates, leading to enhanced dissolution in water. As the temperature increases, hydrophobic interactions among hydrophobic segments become strengthened, while hydrogen bonding becomes weaker. At higher temperatures the hydrogen bond based cross-links will break up causing the hydrogel to shrink [29].

Lastly, a group of hydrogels with multiple sensitivities have been synthesized and reported [14, 23, 25, 26]. The mechanism of sensitivity observed for various hydrogels may differ widely. For example, temperature- and pH-sensitive hydrogels are very different in their physical behavior and swelling mechanism. In order to design a hydrogel with multiple sensitivities, a combination of various factors must be taken into consideration.

The most requirement of a hydrogels for its application in medicine and surgery is its biocompatibility behavior. Besides, most of biocomplatible polymeric hydrogels can be made to be biodegradable. The biodegradable hydrogels are widely used for drug delivery purposes. They have an extremely low risk of rejection by the immune system and, when entering the human body, they slowly degrade to release the drug. However, poorly soluble drugs are difficult to be entrapped into the hydrogel matrix.

Hydrogels have also been employed for immobilization of therapeutically active cells [30]. It has been shown that immobilization of cells within a polymer matrix could protect them from possible rejection by immune system [31]. The design of cell-hydrogel capsules has provided a new type of biomimetic capsules suitable for controlled drug delivery [32]. It has been reported that these hydrogel capsules provide a very long-term functionality of the enclosed cells with improved mechanical stability of the capsules *in vivo*.

The use of natural polymers as biodegradable hydrogel has become a new area of interest for polymer scientists. For example, pachyman is a fungus polysaccharide used as a natural polymer for controlled release of protein drugs [33]. It has been reported that natural hydrogels could provide full preservation of protein's stability and activity. They have proposed that the technique is suitable for site-specific protein–drugs delivery [33].

Injectable hydrogels

During the last few years, many research interests have shifted from hydrogel implants to injectable formulations that form a macroscopic gel at the site of injection [34-36]. These novel hydrogels exhibit a number of advantages including patient comfort and very lower costs. *In situ* gelation can be obtained after UV-polymerization, introducing non-reversible covalent bonds, or via self-assembly by either reversible interactions or non-reversible chemical reactions.

The most interesting *in situ* gelling systems are self-assembling hydrogels which can be formed in time or in response to a known variable such as temperature and those hydrogels that release their content in response to a biochemical signal such as glucose concentration [37, 38]. An interesting strategy to create *in situ* gelling

systems is applied in pharmaceutical and biomedical fields. *In situ* gelling hydrogels are divided into two main categories: systems that are created upon irradiation with visible or UV-light and systems that self-assemble. Photopolymerizable hydrogels are formed *in situ* but are not self-gelling. Self-assembling hydrogels are formed spontaneously or after a known variable such as temperature.

Photopolymerizable hydrogels

As stated before, *in situ* photopolymerization has been used in biomedical applications for over more than a decade. Poly ethylene glycol (PEG) has been used as central block, flanked with oligo (α-hydroxy acids) and acrylate groups [39]. These have then been coupled to the terminal hydroxyl groups. The research team has stated that the acrylate end-groups could undergo rapid polymerization upon irradiation with visible light in the presence of a suitable photoinitiator. Incorporation of the oligo (α-hydroxy acids) oligomers guaranteed the degradability of the matrices under physiological conditions. It has been stated that the degradation time of the hydrogels could be varied from 1 day to 4 months by selecting appropriate α-hydroxy acids such as glycolic or lactic acid. They proved that their designed system was suitable for controlled delivery of bovine serum albumin (BSA) or any similar protein structure up to 2 months. One of the interesting results was that when the hydrogel mixture was polymerized prior to injection, the gel was not adhesive enough. On the other hand, *in situ* polymerization had created an adherent hydrogel film. It was assumed that formation of an interpenetrating network with extracellular proteins, present in tissues caused the adhesive properties observed. It has been shown that a group of PEG-based hydrogels are also suitable vehicles for delivery and release of proteins and oligonucleotides [40]. It was found that the release was depended on the molecular weight of the PEG and the type of α-hydroxy acid. They explained that combination of tailorable drug release together with *in situ* formation and adherence of the hydrogel to the tissues may allow localized drug release, precisely where needed.

Anseth and co-workers studied PEG and poly vinyl alcohol (PVA) based polymers, containing acrylate or methacrylate functionalities for the *in situ* generation of photopolymerized networks [41]. Their potential in cartilage tissue engineering was illustrated by using a cell-hydrogel construct formed rapidly after photopolymerization and temporarily served as a replacement for the damaged cartilage while new cartilage was formed. In a review paper, Nguyen and West indicated the advantages of photopolymerization together with polymerizable materials and photoinitiators [42].

Self-assembling hydrogels

Self-assembling hydrogels are formed spontaneously or in response to a biological variable such as temperature. Two types of strategies could be used to introduce cross linking and gel formation: enzyme-mediated gelation and chemical cross-linking of complementary groups.

Chemical cross-linking of complementary groups

Approved by Food and Drug Association (FDA) because of its biocompatibility and safety, PEG has been especially used in devices designed for *in vivo* applications [43]. However, PEG could be variously modified by functionalizing with different chemical groups for specific biomedical applications. PEG has been functionalized with thiol groups in order to design a group of self-assembling, chemically cross-linked

hydrogels [44]. In this research, diamino-PEG was copolymerized with 2-mercaptosuccinic acid and the resulting amide-linked copolymer with pendant thiol groups, reacted with a PEG-divinylsulfone (Fig. 3). The vinylsulfone (VS) groups react quite rapidly under mild conditions (pH 7.0) with thiol groups resulting in a hydrogels (EWC above 90%).

A continuous *in vitro* release of fluorescein-labeled BSA was observed during 25 days with no significant burst release. Quantitative release of the entrapped protein indicated that cross-linking conditions were mild. Subcutaneous injection of the gels in rats and rabbits caused minimal inflammatory cell response.

In a more recent research, heparin was functionalized with thiol groups (Fig. 4) and subsequently reacted with PEG-diacrylate to form a hydrogel [5]. The subsequent reaction of thiol groups with acrylates formed the network. It was shown that with increasing functionalization of heparin its affinity to antithrombin III decreased.

It was found that the mechanical properties such as storage modulus and gelation kinetics could be tailored by varying the degree of thiolation and the concentration of both heparin-SH and PEG-diacrylate. They found that while encapsulation was almost successful, cell proliferation was increased by addition of fibrinogen during gelation.

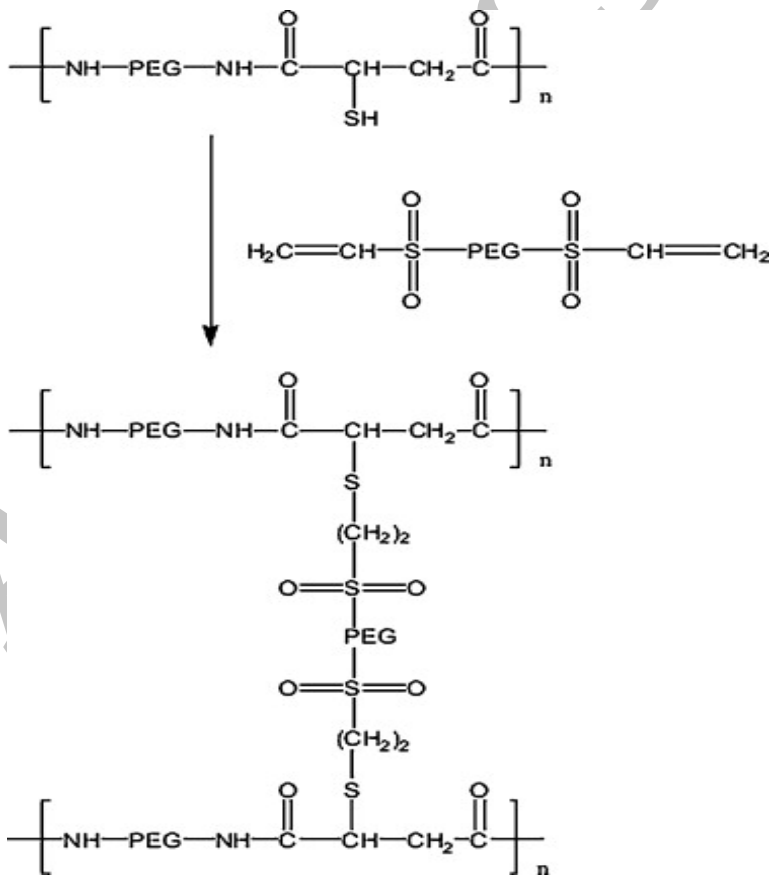

Fig. 3. Spontaneous chemical cross-linking of PEG copolymers [44].

A novel class of dextran-based *in situ* gelling system was introduced in 2007 [46]. In this research, dextran was functionalized with vinyl sulfone (dex-VS) followed by addition of 4-arm mercapto-PEG (PEG-4-SH) with equal molar ratio. In these

conditions, the hydrogel was formed at physiological conditions through a Michael-type addition. The gelation time decreased from 7 to 0.5 min when the degree of VS increased from 4 to 13. The strength of highly elastic hydrogels could be varied from 3 to 46 kPa, by changing the DS (number of VS groups per 100 glucopyranose units), concentration and molecular weight of dextran used.

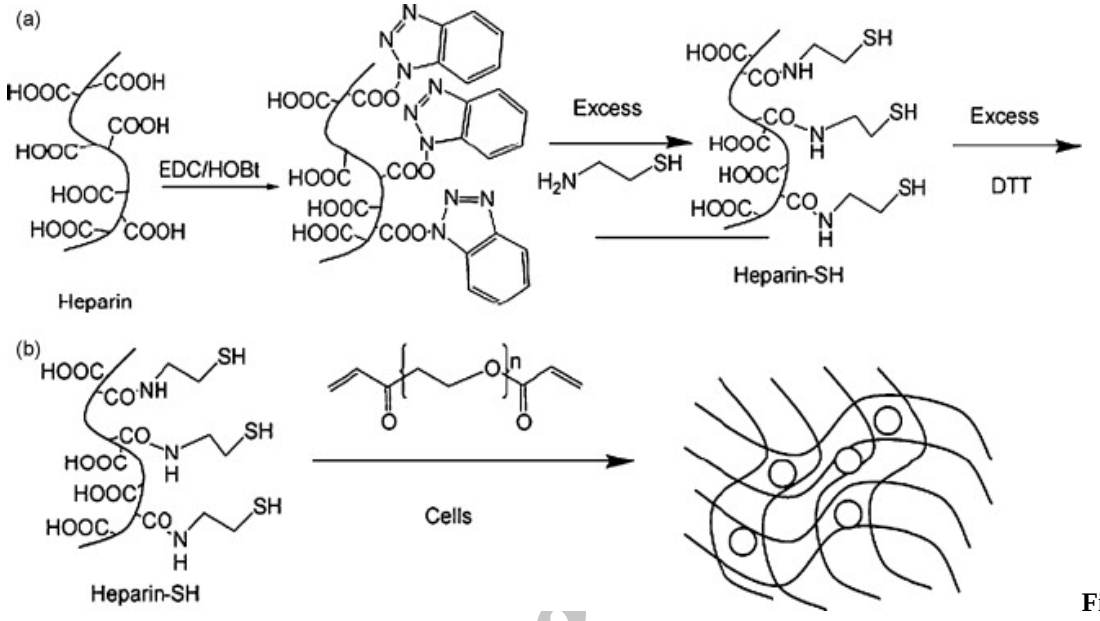

Fig. 4. Reaction of thiol-functionalized heparin with PEG-diacrylate in situ [45].

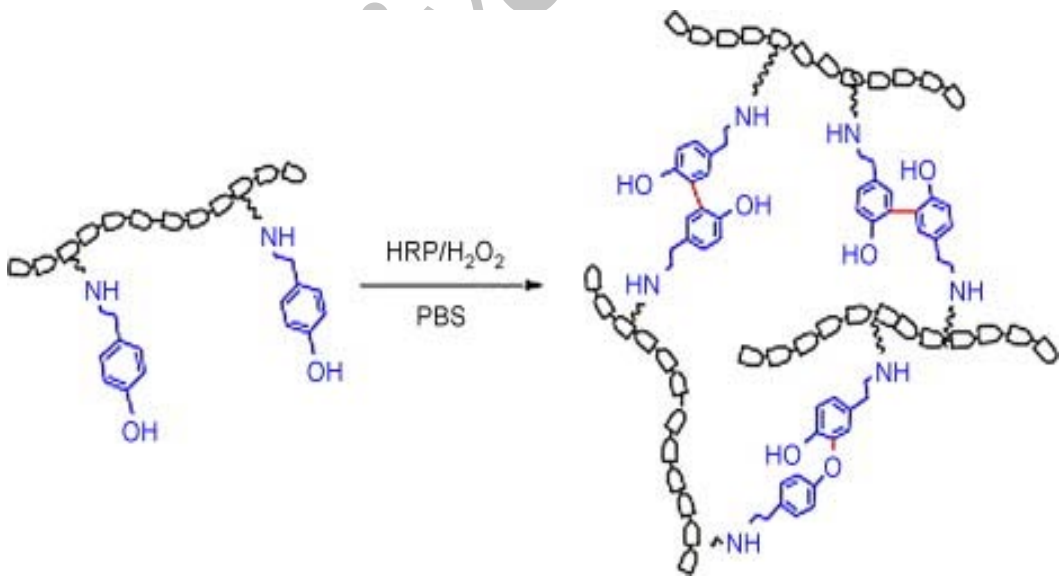

Fig. 5. Enzymatic cross-linking of dex-TA conjugates [48].

Hiemstra et al have reported that they had achieved an increase in the degradation time through *in situ* hydrogel formation. They have used thiol functionalized dextran (dex-SH) with either dex-VS or PEG-4-SH to obtain hydrogel *in situ* [47]. Using this strategy, the degradation times of the gels was prolonged up to 21 weeks.

Enzyme-mediated gelation

Gelation is achieved using certain enzymes that are able to introduce chemical cross-links. A research conducted in 2007, have used horseradish peroxidase (HRP) as the enzyme of choice for this purpose [48]. In this project, dextran-tyramine (dex-TA) was cross-linked in the presence of H_2O_2 and HRP. As a result, a chemically cross-linked, highly elastic and degradable hydrogel was formed *in situ*. It was stated that gelation time could be varied from 5 s to 9 min, depending on the polymer and enzyme or H_2O_2/tyramine ratios.

Hydrogels in tissue engineering

Due to their high water content and elasticity, hydrogels are able to mimic human tissue. However, for soft tissue applications, the *in vivo* response of hydrogel must be used as a model for designing the *in vitro* systems. This approach will ensure that the hydrogel retains its initial size and shape while remaining *in vivo*. On the other hand, the swelling behavior is essential in preserving the interface between matrix and needs to be controlled. It has been reported that PVA hydrogels are suitable biomaterials for tissue engineering applications. The viscoelastic behavior and non-degradable physically cross-linking of PVA hydrogels have provided their high comparablity with that of articular and meniscal cartilage [49].

Hydrogels have also found applications in joint replacement surgeries due to their high biocompatibility and desirable mechanical properties. One of the most common orthopedic injuries is tear and wear that occur to the meniscus. It should be recalled that meniscus is a semi-lunar fibrocartilage disc in human knee important for shock absorption and weight bearing [50]. The biomechanical functions of the meniscus stem from its physical characteristics and chemical composition as well as their three dimentional structure. PVA hydrogels have been used as biomaterials of choice for meniscal replacements applications. It has been demonstrated that reinforcement of PVA with ultrahigh molecular weight polyethylene (UHMWPE) and polypropylene (PP) fibers could produce a mechanically suitable material for meniscal replacements [51]. The meniscus was used as a model fibrocartilage tissue for mechanical property comparison. Evaluating the mechanical properties of PVA hydrogels have indicated that fiber-reinforced PVA hydrogels could be able to replicate the anisotropic modulus distribution present in the native meniscus. This was achieved through controlled fiber placement and material processing [52].

High water content hydrogels are also used successfully as scaffolds for regeneration of some soft tissues. Traumatic injury that occurs naturally with aging causes degeneration of the intervertebral disc (IVD). A disorder that is commonly associated with severe back pain. The IVD is composed of the collagenous, lamellar annulus fibrosus and the gelatinous nucleus pulposus (NP). The NP is a hydrated tissue, characterized by high proteoglycan and type II collagen content [53]. This region functions to resist compressive loads through the generation of a hydrostatic swelling pressure. It has been shown that high water content hydrogel networks could resemble the highly hydrated nature of the NP. This is the main reason for hydrogels to serve as scaffolds for NP regeneration. In practice, NP cells could be cultured by encapsulation in hydrogels. The type of hydrogel normally used for this purpose is alginate, a naturally derived polysaccharide originating from brown algae [5]. Alginate gelation could occur through ionic cross-linking and finally diffusion of divalent cations to carboxylic acid moieties on the polymer, would result in a cross-linked network.

Nature derived hydrogels in biomedical applications

Hydrogels derived from natural sources have been studied and utilized in the pharmaceutical and biotechnological industries for many years. In recent years, the high production of plastics and the problem of their waste disposal have attracted many scientists to the advantages of natural biopolymers. In the previous section the use of alginate as scaffolds for hydrated tissue regeneration was discussed. Alginate is an easily available anionic polysaccharide obtained from brown marine algae. It is a linear copolymer, composed of 1, 4-linked-β-d-mannuronic acid and α-l-guluronic acid residues (Fig. 6), that forms hydrogel in the presence of divalent cations such as calcium. The mechanism of gelation is based on the stacking of guluronic acid (G) blocks and the formation of calcium linked junctions are hemocompatible, they do not accumulate in any major organs, and undergo *in vivo* degradation [56]. In addition, alginate is used as the matrix for the microencapsulation of various drugs due to its biocompatibility and mild gelation mechanism [57, 58]. Lin et al have reported incorporation of polysaccharide nanocrystals, such as rod-like cellulose nanocrystals and chitin whiskers and platelet-like starch nanocrystals, into alginate-based nanocomposite microspheres [59]. It has been shown that mechanical strength has increased together with regulation of drug release. It has been concluded that the presence of polysaccharide nanocrystals increased the stability of the crosslinked network structure, and the nanocomposite microspheres consequently exhibited prominent sustained release profiles.

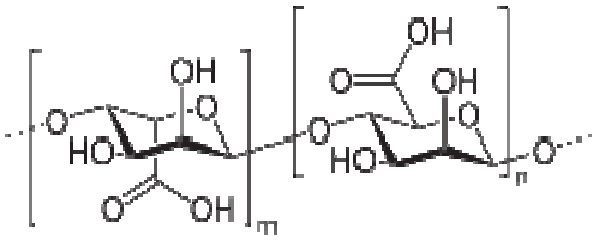

Fig. 6. The chemical structure of alginate backbone.

Nanoreinforced hydrogels could be prepared from wood cellulose whiskers coated with chemically modified wood hemicelluloses. Biocompatible polysaccharides have been used in food, packaging, agricultural chemicals, and biomedical applications due to their non-toxic, and biofunctional character. Cellulose whiskers, obtained by acid hydrolysis of cellulose, are a recent area of application for nanocomposites [60]. A group of novel hydrogels with improved mechanical properties have been recently designed using hemicellulose and cellulose whiskers derived from wood [61]. HEMA was used to chemically modify hemicellulose, isolated from aspen, followed by adsorption onto cellulose whiskers. The surface modified cellulose whiskers were used to prepare nanocomposite hydrogels using free radical polymerization of HEMA. It was shown that physical and mechanical properties of these PHEMA hydrogels, such as water holding capacity, mechanical properties and viscoelasticity, were similar to load-bearing natural tissue having hydrogel-like characteristics. It was suggested that the prepared nanoreinforced PHEMA hydrogels have potential for use in load-bearing biomedical applications such as cartilage replacement.

Physical hydrogels are a group of natural polymers that form a three-dimensional network of polymer chains, without any use of external chemical. In the last few years, chitosan based physical hydrogels have been obtained having intermolecular physical cross-links of low energy [62]. Boucard et al

have recently prepared a bi-layer physical hydrogel constituting only chitosan and water [63]. It was *in vivo* tested for the skin reconstruction after third-degree burns on pig back skins. Kinetics study of the healing proved that the material was able to promote full skin reconstruction. Physical hydrogels have also been reported to induce the production of a cartilaginous matrix for chondrocyte cultures *in vitro* [64]. It is, therefore, concluded that chitosan physical hydrogels are able to allow the dermis and dermal-epidermal junction reconstruction and the re-epithelialization on the full thickness skin defect.

Interpenetrating polymeric network (IPN) hydrogels

To engineer complex tissues, it is necessary to create hybrid scaffolds with micropatterned structural and biomechanical properties, in order to mimic the intricate body tissues. IPNs are polymer hybrids of two or more polymers, each present in physically or chemically cross-linked network forms and the two networks are entangled with each other [65]. They, therefore, can exhibit the properties of both individual component polymeric gels. Presence of two or more polymers also results in the strengthening and reinforcement of the overall scaffold [66]. The degradation rate of the hydrogel can be tailored depending on the properties of each polymer network. A semi-IPN (SIPN) consists of one cross-linked polymer with a second polymer entangled in the first, without being cross-linked. It should be emphasized that mechanical properties of SIPNs are poorer compared to IPNs as only one polymer network is crosslinked [67].

A novel interpenetrating polymeric network (IPN) hydrogel has recently been synthesized to be used for tissue engineering applications [65]. Collagen and hyaluronic acid (HA) were used to create the hydrogel and it presented unique advantages for tissue engineering applications because of the ubiquitous presence of both collagen and HA in the extracellular matrix (ECM) and its native biodegradability, participation in cell signaling events and biocompatibility. Further, the ease of fine-tuning the hydrogel properties, by simply changing the individual collagen and HA networks, renders these hydrogels useful for cartilage, bone, vascular grafts and soft tissue applications.

Hyaluronic acid (HA), a glycosaminoglycan and an important chemical building block of ECM, is composed of repeating disaccharide units of D-glucuronic acid and *N*-acetylglucosamine [68]. It is found in the highest concentrations in cartilage tissue [69], vitreous humor [70], synovial fluid of joints [71] and umbilical cord and [72], and is responsible for maintaining tissue homeostasis [73]. Among its important functions, HA plays a significant role in scar-free wound healing [74], tissue remodeling and morphogenesis [75]. On the other hand, collagen is the most abundant protein of the ECM and favors cell adhesion by interacting with cell surface integrins. Collagen has low antigenicity and excellent biocompatibility, and is biodegradable. Tissue engineering scaffolds composed of proteins and polysaccharides are being developed for various applications, such as cartilage [76-78], skin [79] and vocal folds [80]. However, only limited success has been achieved in regenerating the tissue completely. Most of research involving protein-polysaccharide combination hydrogels employ chemical cross-linking, which may produce potential toxic by-products and extensive washing steps [81] limiting fabrication of scaffolds in the presence of cells.

CONCLUSIONS

The growing application of hydrogels in tissue engineering, control release of drugs and protein delivery as led to the

development of a wide number of promising preparation strategies. Many of the strategies and systems innovated in laboratories, may eventually find clinical applications depending on their *in vivo* performance in specific applications. Clearly, the most important property expected from a novel hydrogel is good biocompatibility as well as reasonable biodegradability with the integrity to be tailored. Additionally, when in the case of drug delivery, controllable release rate and non-toxic products of biodegradation are important. However, in tissue engineering applications, such as scaffolds made from hydrogel materials for tissue repair, much lower degradation rates are required. As outlined in this review, those hydrogels that are formed *in situ*, at the site of injection, preferably by self-assembly of the building blocks, show most potential for further development. The use of hydrogel in contact lens industry still covers a wide range of application of these biomaterials. Therefore, their biocompatibility, stability towards spoilage in biological environment and strength are the most important features in this area of application.

REFERENCES

[1] N. A. Peppas, P. Bures, W. Leobandung and H. Ichikawa H, Eur. J. Pharm Biopharm. 50 (2000) 27.
[2] T. R. Hoare and D. S. Kohane, Polymer. 49 (2008) 1993.
[3] O. Wichterle and D. Lim, Nature. 185 (1960) 117.
[4] A. S. Hoffman, Adv. Drug Deliv. Rev. 43 (2002) 3.
[5] N. E. Fedorovich, J. Alblas, J. R. De Wijn, W. E. Hennink, A. J. Verbout and W. J. A. Dhert, Tissue Eng. 13 (2007) 1905.
[6] S. R. Van Tomme and W. E. Hennink, Expert Rev. Med. Devices. 4 (2007) 147.
[7] W. E. Hennink and C. F. van Nostrum, Adv. Drug Deliv. Rev. 54 (2002) 13.
[8] P. Edman, B. Ekman and I. Sjoholm I, J. Pharm. Sci. 69 (1980) 838.
[9] C. Xiao and M. Yang, Carbohydr. Polym. 64 (2006) 37.
[10] N. A. Peppas, Hydrogels in Medicine and Pharmacy, CRC Press: London, 1986.
[11] T. R. C. Boyde, J. Chromatogr. 124 (1978) 219.
[12] P. C. Corkhill, J. H. Fitton and B. J. Tighe, J. Biomater. Sci. Poly. Edit. 28 (1993) 33.
[13] P. H. Corkhill, C. J. Hamilton and B. J. Tighe, Crit. Rev. Biocompat. 5 (1990) 363.
[14] S. K. Sahoo, T. K. De, P. K. Ghosh and A Maitra, J. Colloid Interface Sci. 206 (1998) 361.
[15] L. H. Gan, Y. Y. Gan and J. R. Deen, Macromolecules 33 (2000) 7893.
[16] J. Varshosaz and M. Falamarzian Eur. J. Pharm. Biopharm. 51 (2001) 235.
[17] C. Ruan, K. Zeng and C. A. Grimes, Anal. Chim. Acta 497 (2003) 123.
[18] B. D. Johnson, D. J. Beebe and W. C. Crone, Mater. Sci. Eng. C. 24 (2004) 575.
[19] G. Gerlach, M. Guenther, J. Sorber, G. Suchaneck G, K. F. Arndt KF and A. Richter Sens. Actuators B. 111 (2005) 555.
[20] S. Herber, J. Bomer, W. Olthuis, P. Bergveld and A. van den Berg, Biomed. Microdev. 7 (2005) 197.
[21] A. C. Mack, J. Mao and M. J. McShane, Sensors IEEE 65 (2005) 912.
[22] Y. Y. Liu, X. D. Fan, B. R. Wei, G. F. Si, W. X. Chen and L. Sun, Int. J. Pharm. 308 (2006) 205.
[23] S. Bhattacharya, F. Eckert, V. Boyko and A. Pich, Small 3 (2007) 650.
[24] J. X. Sun, Y. F. Luo, H. Peng H and Z. W. Han, Chin. Chem. Lett. 19 (2008) 1475.
[25] C. Zhao, X. Zhuang, P. He, C. Xiao, C. He, J. Sun, X. Chen and X. Jing, Polymer 50 (2009) 4308.
[26] J. Zhang, R. Xie, S. B. Zhang, C. J. Cheng, X. J. Ju and L. Y. Chu, Polymer 50 (2009) 2516.
[27] H. Katono, A. Maruyama, K. Sanui, N. Ogata, T. Okano and Y. Sakurai, J. Control Rel. 16 (1991) 215.
[28] Y. H. Bae, T. Okano and S. W. Kim, J. Polym. Sci. Part B: Polym. Phys. 28 (1990) 923.
[29] L. D. Taylor and L. D. Cerankowski, J. Polym. Sci. Polym. Chem. 13 (1975) 2551.

[30] G. Orive, A. R. Gascon, R. M. Hernandez, M. Igartua and J. Pedraz, Trends Pharmacol. Sci. 24 (2003) 207.
[31] G. Orive, R. M. Hernandez, A. R. Gascon, R. Calafiore, T. M. S. Chang, P. D. Vos, G. Hortelano, D. Hunkeler, I. Lacik, A. M. J. Shapiro and J. L. Pedraz, Nat. Med. 9 (2003) 104.
[32] G. Orive, M. De Castro, H. J. Kong, R. M. Hernandez, S. Ponce, D. J. Mooney and J. L. Pedraz, J. Control Rel. 135 (2009) 203.
[33] Y. Hu, X. Zhou, Y. Lu Y, C. Hu and X. Hu, Int. J. Pharm. 371 (2009) 89.
[34] A. Hatefi and B. Amsden, J. Control Release. 80 (2002) 9.
[35] C. B. Packhaeuser, J. Schnieders, C. G. Oster and T. Kissel, Eur. J. Pharm. Biopharm. 58 (2004) 445.
[36] J. D. Kretlow, K. Klouda and A. G. Mikos, Adv. Drug Deliv. Rev. 59 (2007) 263.
[37] S. Tanna, M. Joan Taylor, T. S. Sahota and K. Sawicka, Biomaterials 27 (2006) 186.
[38] N. Kashyap, B. Viswanad, G. Sharma, V. Bhardwaj, P. Ramarao, M. N. V. Ravi Kumar, Biomaterials 28 (2007) 2051.
[39] A. S. Sawhney, C. P. Pathak and J. A. Hubbell, Macromolecules 26 (1993) 581.
[40] J. L. West and J. A. Hubbell, React. Polym. 25 (1995) 139.
[41] K. S. Anseth and J. A. Burdick,. MRS Bulletin 16 (2002) 1.
[42] K. T. Nguyen and J. L. West, Biomaterials 23 (2002) 4307.
[43] F. E. Bailey and J. V. Koleske. Poly (ethylene oxide); Academic Press: New York, 1976.
[44] B. A. Qui, Biomaterials 24 (2003) 11.
[45] G. Tae, J. L. Kim, W. I. Choi, M. Kim, P. S. Stayton and A. S. Hoffman, Biomacromolecules. 8 (2007) 1979.
[46] C. Hiemstra, L. J. vanderAa, Z. Zhong, P. J. Dijkstra and J. Feijen, Macromolecules. 40 (2007) 1165.
[47] C. Hiemstra, L. J. vanderAa, Z. Zhong, P. J. Dijkstra and J. Feijen, Biomacromolecules. 8 (2007) 1548.
[48] R. Jin, C. Hiemstra, Z. Zhong and J. Feijen, Biomaterials. 2007; 28: 2791-2800.
[49] M. Kobayashi, Y. Chang and M. Oka, Biomaterials. 26 (2005) 3243.
[50] P. Buma, N. N. Ramrattan, T. G. van Tienen and R. P. H. Veth, Biomaterials. 25 (2004) 1523.
[51] J. C. Bray and E. W. Merrill, J. Appl. Polym. Sci. 17 (1973) 3779.
[52] A. J. Almarza and K. A. Athanasiou, Ann. Biomed. Eng. 32 (2004) 2.
[53] A. E. Baer, J. Y. Wang, V. B. Kraus and L. A. Setton, J. Orthop. Res. 19 (2001) 2.
[54] A. Martlinson, G. Skjak Braek and O. Smidsred, Biotechnol. Bioeng. 33 (1989) 79. Martinsen A, Skjak-Braek G, Smidsrod O.
[55] A. Shilpa, S. S. Agrawal and A. R. Ray, J. Macromol. Sci. Poly. Revs. 43 (2003) 187.
[56] H. Zheng, Z. Zhou, Y. Chen, J. Huang anf F. Xiong, J. Appl. Polym. Sci. 106 (2007) 1034.
[57] X. Zhang, Z. Hui, D. Wan, H. Huang, J. Huang, H. Yuan and J. Yu, Int. J. Biol. Macromol. 47 (2010) 389.
[58] N. Lin, J. Huang, P. R. Chang, L. Feng and J. Yu, Collod. Surfs. B: Biointerfaces. 85 (2011) 270.
[59] Y. Habibi, L. A. Lucia, O. J. Rojas, Chem. Rev. 110 (2010) 3479.
[60] M. A. Karaaslan, M. A. Tshabalala, D. J. Yelle and G. Buschle-Diller, Carbohy. Poly. 86 (2011) 192.
[61] N. Boucard, C. Viton and A. Domard, Biomacromolecules. 6 (2005) 3227.
[62] N. Boucard, C. Viton, D. Agea, E. Mari, T. Roger, Y. Chancerelle and A. Domard, Biomaterials. 28 (2007) 3478.
[63] A. Montembault, K. Tahiri, C. Korwin-Zmijowska, M. T. Corvol, A. Domard A, Biochimie. 88 (2006) 551.
[64] S. Suri S and C. E. Schmidt, Acta Biomaterialia. 5 (2009) 2385.
[65] J. M. Phillips, W. J. Kao, Tissue Eng. 11 (2005) 964.
[66] X. Hou and K. S. Siow, Polymer. 42 (2001) 4181.
[67] B. P. Toole BP, Nat Rev Cancer. 4 (2004) 528.
[68] C. B. Knudson and W. Knudson, Semin. Cell Dev. Biol. 12 (2001) 69.
[69] R. G. Brewton and R. Mayne, Exp. Cell Res. 198 (1992) 237.

[70] A. Engstrom-Laurent A, J. Intern. Med. 242 (1997) 57.
[71] M. Yanagishita, EXS. 70 (1994) 179.
[72] J. R. Fraser, T. C. Laurent and U. B. Laurent, J. Intern. Med. 242 (1997) 27.
[73] E. A. Balazs, M. I. Freeman, R. Kloti, G. Meyer-Schwickerath, F. Regnault and D. B. Sweeney, Mod. Probl. Ophthalmol. 10 (1972) 3
[74] Toole BP. Hyaluronan in morphogenesis. Semin Cell Dev Biol 2001; 12[2]: 79-87.
[75] A. Tampieri, M. Sandri, E. Landi, D. Pressato, S. Francioli and R. Quarto. et al, Biomaterials. 29 (2008) 3539.
[76] R. M. Schulz, M. Zscharnack, I. Hanisch, M. Geiling, P. Hepp and A. Bader, Biomed. Mater. Eng. 18 (2008) S55.
[77] Y. Hong, Y. Gong, C. Gao and J. Shen, J. Biomed. Mater. Res. A85 (2008) 628.
[78] Z. Wang, H. Pan, Z. Yuan, J. Liu, W. Chen and Y. Pan Y, Tissue Eng. C. Methods. 14 (2008) 35.
[79] M. S. Hahn, B. A. Teply, M. M. Stevens, S. M. Zeitels and R. Langer, Biomaterials. 27 (2006) 1104.
[80] S. R. Jameela, A. Misra and A. Jayakrishnan, J. Biomater. Sci. Polym. Ed. 6 (1994) 621.
[81] T. Taguchi and J. Tanaka, J. Biomater. Sci. Polym. Ed. 13 (2002) 43