Transactions of the Materials Research Society of Japan 33[2] 417-423 (2008)

Preparation, Characteristics and Applications of Chitinous Hydrogels

Rong Huei Chen

Department of Food Science, National Taiwan Ocean University
#2, Pei-Ning Road, Keelung, Taiwan.
Fax: +886-2-24631977; rhchen@mail.ntou.edu.tw

The review introduce hydrogel, responsive hydrogel briefly, then describe the characteristics of chitin, chitosan, and chitinous materials. Different chitinous physical hydrogels, chemical hydrogels, polyelectrolytes complex, semi-interpenetrating polymer networks, blends and complex are introduced. Why prepared different shapes of hydrogel are also introduced. The applications of chitinous hydrogels in areas of biosensor, biocarrier, controlled release systems, absorption of heavy metal/ harmful materials, immobilized enzyme, medical device, tissue engineering, wound dressing and the others are listed. Recently developments in tissue engineering, microfabrication, hydrogel nanoparticles are stressed.

Keywords: chitinous hydrogels, microfabrication, nanoparticle, tissue engineering,

1. HYDROGEL, RESPONSIVE HYDROGEL

Hydrogels are usually formed by cross-linking of linear hydrophilic polymers to form a 3D network of material capable of absorbing a very large amount of water or biological fluids, yet still remaining insoluble [1]. Their hydrophilic character is due to the presence of functional groups such as: alcohols, carboxylic acids, or amines [2].

Hydrogels are able to provide ideal aqueous conditions for biocompatible applications [3], particularly attractive as a wound dressing [4,5] matrix for enzyme immobilization. Because, the hydrophilic microenvironment favourable for the inclusion and the stability of the enzymes. Dumitriu et al. [6] demonstrated that the co-immobilization of protease and xylanase led to a synergistic effect. Lipase immobilization into this matrix doubles its enzymatic activity in aqueous media [7]. Moreover, the immobilization of lipase protects the enzyme against thermal degradation and excellent activity is obtained at 55 °C [7] and for environmentally sensitive bioactive materials such as proteins [8]. Crosslinks within polymeric hydrogels can be created either chemically or physically. The use of crosslinking agents to chemically form polymeric hydrogels may lead to toxic side effects (owing to residual crosslinking agents) or to unwanted reactions with drugs. Heterogeneous polymer mixtures may also be used to form hydrogels without the need for covalent cross-linking [9].

The ability of polymer hydrogels to undergo a volume transition between swollen and collapsed phases as a function of their environment is one of the most remarkable and universal properties of these materials. Besides volume transition, permeability, or mechanical strength of some polymer hydrogel can be induced by temperature, pH, solvent composition, ionic strength, electric field, light, stress, and the presence of specific chemical stimuli. Polymer hydrogel possess those capacity are categoried as smart, responsive, stimuli-sensitive, or intelligent hydrogel can be tuned to change their above-mentioned physical properties in predictable and pronounced ways. Smart gels as potential actuators, sensors, controllable membranes for separations, and modulators for the delivery of drugs. Smart gels are also great potential for biomedical and bioengineering applications: pulsatile drug release, molecular separation processes, diagnostics, cell culture, and bioreactions. [10]

In the design of oral delivery of acid vulnerable drugs, pH-sensitive hydrogels have attracted increasing attention. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. The extent of swelling increases as hydrogels passes down the intestinal tract due to increase in pH. Alginate is widely used in biomedical applications, non-toxic when given orally [11]. Nevertheless, the swelling of the calcium-crosslinked alginate beads at pH 7.4 was minimal due to the relatively strong ionic interaction between the carboxylic groups on alginate and Ca^{2+}. This may limit the drug release at the intestinal tract. To overcome this problem, a complex composed of alginate blended with a water-soluble chitosan (N,O-carboxymethyl chitosan, NOCC) was prepared to form microencapsulated beads. These microencapsulated beads were used as a pH-sensitive-based controlled release system for protein drug delivery [11].

2. CHITIN, CHITOSAN, CHITINOUS HYDROGELS

2. 1. Chitin

It is the most abundant naturally polysaccharide after cellulose. Chitin has low toxicity and is inert in the gastrointestinal tract of mammals, it is biodegradable, owing to widely distributed of chitinases in nature. Chitin has been used to prepare affinity chromatography column to isolate lectins. Chitin and 6-O-carboxymethylchitin activate peritoneal macrophages in vivo, chitin suppress the growth of tumor cells in mice, stimulate nonspecific host resistance against *Escherichia coli* infection. Chitin was used to immobilize enzymes such as α- and β -amylases, invertase or whole cells, chitin film and fiber as dressing material for accelerates wound healing, controlled drug release; an excipient and drug carrier in film, gel or powder form [12-15].

2. 2. Chitosan

Chitosan is the N-deacetylated derivative of chitin. Chitosan is bioabsorbable, haemostatic and bacteriostatic. Moreover, it plays an important role in the cell regulation and tissue regeneration. Chitosan is a nontoxic polymer biodegradability environment friendly, biocompatible material structural similarity to natural GAGs degradation by chitosanase and lysozyme slowly to harmless products (amino sugars) that are absorbed completely in body. Chitosan can induce desquamation of pig urothelium to remove all diffusion barriers; Glycosaminoglycans, membrane plaques, and tight junctions of umbrella cells. This ability has been proven in vitro on nasal, buccal, vaginal, and urinary bladder mucosa of different animals, thus making this polymer a promising agent in the development of controlled drug delivery systems. Chitosan aqueous acid solution reacted with polyanion aqueous solutions (heparin, sodium alginate, carboxymethyl chitin, polyacrylic acid) to give polyelectrolyte complexes. Chitosan has a primary amino group at C2 and hydroxyl group at C6 positions, can undergo a host of chemical reactions under mild conditions to N-acyl, N-carboxyalkyl, N-carboxyacyl, and O-carboxylalky (epichlorohydrin cross-linked O-carboxymethyl) substituted chitosan with scores of potential uses. [2,12,13,15].

2. 3. Chitinous materials

Chitin and it's major derivatives, with different degrees of deacetylation and different molecular weights chitosans and their chemically modified derivatives are called chitinous materials [16]. As mentioned in the beginning, hydrogels are usually formed by cross-linking of linear hydrophilic polymers. Chitinous materials are linear polymers with many desired physical and biological properties. Chitinous hydrogels have been explored in many aspects. There are couple reviews of chitinous hydrogels in the literatures. Applications of chitinous hydrogel in biomedical areas are promise.

3. CHITINOUS HYDROGEL

3. 1. Chitinous physical hydrogel

Physical gels are formed by various reversible links. These can be ionic interactions as in ionically crosslinked hydrogels and polyelectrolyte complexes or secondary interactions as in chitosan/poly (vinylalcohol) complexed hydrogels, grafted chitosan hydrogels and entangle hydrogel [17, 18]. These physical gels exhibit two major advantages: 1. the possible reversibility in the solvents used, 2. the chemical structure of polymer chains is preserved. Consequently, the whole of the physicochemical and biological properties of chitin chains, especially the biocompatibility and the bioactivity, are maintained [19].

3. 1. 1. N-acyl-chitosan gels by Late Prof. Hirano

N-acylchitosan gels by acylation of chitosan with various anhydrides in aqueous alcohol acetic acid solution [20]. N-acylchitosan gels are of interest for various potential applications, particularly in the field of pharmacy or biomaterials, because they exhibit good biocompatibility and bioresorption properties. They can also be used in chromatography as a stationary phase [21]. The influence of different parameters on their formation, such as the nature of the acylating agent, the solvent, and cosolvent, the concentration of the acylating agent and chitosan content, and the temperature [22-24]

3. 1. 2. Chitosan hydrogel by Domard et al.,

The mechanism of gelation simply consisted in the modification of the hydrophilic/hydrophobic balance allowing the formation of both hydrophobic interactions and hydrogen bonding. Several parameters had an important role on this mechanism: 1. the apparent charge density of chitosan, modified by the degree of neutralisation, 2. the dielectric constant of the solvent, related to the composition of the medium, 3. the degree of acetylation, 4. temperature, playing a role on the interactions responsible for the physical cross-linking and the molecular mobility, and, 5. the molecular mobility depending on possible changes of conformation, steric hindrance and viscosity of the medium [25]. The influence of various parameters on gelation, such as the temperature, the molecular weight, the initial degree of acetylation of chitosan, the stoichiometry by means of the molar ratio R (anhydride over free amine of chitosan), and the nature of the solvent [21].

3. 2. Chitinous chemical hydrogels

Chemical hydrogels are formed by irreversible covalent links, as in covalently crosslinked chitosan hydrogel [17, 18]. The composition and the cross-linking density are controlled to induce a precise swelling and specific mechanical properties. The disadvantages of such systems include the difficulty of their processing, the presence of a significant solvent fraction, and their low biocompatibility [2]. The use of crosslinking agents to chemically form polymeric hydrogels may lead to toxic side effects (owing to residual crosslinking agents) or to unwanted reactions with drugs [11]. The most common crosslinkers used with chitosan are dialdehyde such as glyoxal and in particular glutaraldehyde by a Schiff base reaction. However, these preparatory conditions are not ideal because of the toxic nature of any remaining reagents used for cross-linking. Genepinis, a naturally occurring cross-linking agent which is significantly less cytotoxic than glytaraldehyde has been used as an alternates [26].

3. 3. Chitinous Polyelectrolyte complex (PEC), semi-interpenetrating polymer network (semi-IPN networks), blends and composites

PEC between chitosan and natural or synthetic polymers are cited in the literature: e.g. xanthan, carrageenan, alginate, pectin, heparin, hyaluronan (HA) , sulfated cellulose, dextran sulfate, N-acylated

chitosan/chondroitin sulfate, polyacrylic acid sodium salt (PAA), carboxymethylcellulose (CMC). The main applications of these electrostatic complexes are: controlled release systems, encapsulation of drugs, immobilization of enzymes, cells, and gene carriers, anti-thrombogenic materials [12].

Semi-interpenetrating polymer network (semi-IPN) hydrogels have been produced between chitosan: polyethylene oxide diacrylate-cross linked by U.V. irradiation [27], glyoxal cross-linked chitosan: polyethylene oxide [28] and glutaraldehyde cross-linked chitosan combined with silk fibroin [29] or polyacrylic

acid [30]. Heterogeneous interpolymer hydrogen bonds are the main source of interpolymer cohesion in these semi-IPN hydrogels [9].

Blends and composites systems are reported in the literature: chitosan/polyamide 6 [31], chitosan/cellulose fibers [32], chitosan/polyelthylene glycol [33], chitosan/polyvinylpyrrolidone and chitosan/polyvinyl alcohol [34].

4. SHAPES OR FORMS OF CHITINOUS HYDROGELS

Spheric or bead shape is the most common shapes or forms of the hydrogel because of its stability (smallest surface area over volume ratio) and easy to be prepared by spray, stirring, homogenation etc. hallow fiber or tube type hydrogel were prepared to suit the scaffolds in tissue culture especially for never cell culture [35]. Film or sheet type hydrogel were common used in wound dressing or artificial skin, artificial kidney membrane application [5, 36]. Injectable thermosensitive hydrogel were prepared for joint treatment [37]. Some special form for novel applications such as thermosensitive chitosan-based hydrogel containing liposomes for the delivery of hydrophilic molecules [38]. A chitosan-containing multiparticulate system for macromolecule delivery to the colon [39], increase of saturation solubility and dissolution velocity, improving the bioavailability of poorly soluble drug buparvaquone. Another feature of nanosuspensions is the adhesion properties to surfaces, e.g. mucosa [40].

5. APPLICATIONS OF CHITINOUS HYDROGELS

5. 1. General applications

Table 1 shows chitinous hydrogel can be applied in biosensor [41-44]; as a biocarrier [45-46]; controlled release of drugs, insulin, growth factors etc.[39, 47-57]; adsorption of heavy metal or harmful materials [58-60]; immobilized enzymes [61-63]; applied in medicial devices [64]; tissue engineering/ stem cell culture [25, 65-70]; wound dressing [5, 71-73]; and others [74-76]. Among these applications, recent development in tissue engineering, microfabrication, hydrogel nanoparticle will elaborate further more detail.

5. 2. Tissue Engineering Applications

Boucard, Viton, Agay, Mari et al. [5] reported bi-layered physical hydrogels only constituted of chitosan and water were processed and applied to the treatment of full-thickness burn injuries to assess whether this material was totally accepted by the host organism and allowed in vivo skin reconstruction of limited area third-degree burns. A first layer constituted of a rigid protective gel ensured good mechanical properties and gas exchanges. A second soft and flexible layer allowed the material to follow the geometry of the wound and ensured a good superficial contact. All the results showed that chitosan materials were well tolerated and promoted a good tissue regeneration. They induced inflammatory cells migration and angiogenetic activity favouring a high vascularisation of the neo-tissue. At day 22, type I and IV collagens were synthesised under the granulation tissue and the formation of the dermal–epidermal junction was observed. After 100 days, the new tissue was quite similar to a native skin, especially by its aesthetic aspect and its great flexibility.

Montembault, Tahiri, Korwin-Zmijowska, Chevalier,et al [25] reported to develop a biomaterial formulation by combining fragments of chitosan hydrogel with isolated rabbit or human chondrocytes. Morphological data showed that chondrocytes were not penetrating the hydrogels but tightly bound to the surface of the fragments and spontaneously formed aggregates of combined cell/chitosan. A significant amount of neoformed cartilage-like extracellular matrix (ECM) was first accumulated in-between cells and hydrogel fragments and furthermore was widely distributed within the neo-construct. Results showed that a chitosan hydrogel does not work as a scaffold, but could be considered as a decoy of cartilage ECM components, thus favoring the binding of chondrocytes to chitosan. Such a biological response could be described by the concept of reverse encapsulation.

Freier, Montenegro, Koh, Shoichet [35] reported chitin and chitosan supported adhesion and differentiation of primary chick dorsal root ganglion neurons in vitro. Chitosan films showed significantly enhanced neurite outgrowth relative to chitin films, reflecting the dependence of nerve cell affinity on the amine content in the polysaccharide: neurites extended 1794.7 ± 392.0 $\mu m/mm^2$ on chitosan films vs. 140.5 ± 41.6 $\mu m/mm^2$ on chitin films after 2 days of culture. This implies that cell adhesion and neurite extension can be adjusted by amine content, which is important for tissue engineering in the nervous system.

Sechriest, Miao, Niyibizi, Westerhausen–Larson [77] reported the quality of articular cartilage engineered using a cell–polymer construct depends, in part, on the chemical composition of the biomaterial and whether that biomaterial can support the chondrocytic phenotype. The combined chondroitin sulfate-A (CSA) and chitosan to develop a novel biomaterial to support chondrogenesis and form discrete, focal adhesions to the material and maintain many characteristics of the differentiated chondrocytic phenotype, including round morphology, limited mitosis, collagen type II, and proteoglycan production. The results suggest CSA–chitosan may be well suited as a carrier material for the transplant of autologous chondrocytes or as a scaffold for the tissue engineering of cartilage-like tissue

Dang, Sun, Shin-Ya, Sieber et al. [78] reported temperature-responsive polymers was evaluated the potential of hydroxybutyl chitosan (HBC) as a biomaterial for the culture of human mesenchymal stem cells (hMSC) and cells derived from the intervertebral disk, with the eventual goal of using the HBC polymer as an injectable matrix/cell therapeutic. Formulations of HBC gels presented in this study have gelation temperatures ranging from 13.0 to 34.6 °C and water contents of 67 - 95%. Minimal cytotoxicity in MSC and disk cell cultures was observed with these polymers up to a concentration of 5 wt%. Detection of metabolic activity, genetic analysis of synthesized mRNA, and histological staining of MSC and disk cell cultures in these gels collectively indicate cell proliferation without a loss in metabolic activity and extracellular matrix production. This study suggests the potential of HBC gel as an injectable carrier for future applications of delivering therapeutics to encourage a biologically relevant

reconstruction of the degenerated disk.

5. 3. Microfabrication

Fukuda, Khademhosseini, Yeo, Yang, et al. [74] reported a novel method for preparing a spheroid microarray on microfabricated hydrogels for alone or in co-cultures. Cells were initially microarrayed within low shear stress regions of microwells. Human hepatoblastoma cells, Hep G2, seeded in these wells formed spheroids with controlled sizes and shapes and stably secreted albumin during the culture period. The change of cell adhesive properties in the chitosan surface facilitated the adhesion and growth of a second cell type, NIH-3T3 fibroblast, and therefore enabled co-cultures of hepatocyte spheroids and fibroblast monolayers. This co-culture system could be a useful platform for studying heterotypic cell–cell interactions, for drug screening, and for developing implantable bioartificial organs.

Karp, Yeo, Geng, Cannizarro et al. [79] reported a simple and rapid system for creation of patterned cell culture substrates via (1) printing a mask on a standard overhead transparency, (2) coating a thin layer of a photocrosslinkable chitosan on a slide, (3) exposing the slide and mask to ultraviolet (UV) light, and (4) rinsing the uncrosslinked polymer to expose the underlying cell-repellent patterns. Patterns of various shapes (lanes, squares, triangles, circles) were created on glass and tissue culture polystyrene. The pattern size could be varied with a mm resolution using a single mask and varying UV exposure time. Cardiac fibroblasts formed stable patterns for up to 18 days in culture. Cardiomyocytes, patterned in lanes 68–99 mm wide, exhibited expression of cardiac Troponin I, well developed contractile apparatus and they contracted synchronously in response to electrical field stimulation. Osteoblasts (SAOS-2) localized in the exposed glass regions (squares, triangles, or circles; 0.063–0.5mm2). They proliferated to confluence in 5 days, expressed alkaline phosphatase and produced a mineralized matrix.

Khalil and Sun [80] report the recent research on development of a novel multi-nozzle biopolymer deposition system for freeform fabrication of biopolymer-based tissue scaffolds and cell-embedded tissue constructs. The process of the biopolymer deposition is conducted in a biocompatible environment which allows the construction of scaffolds with bioactive compounds and living cells. The system configuration and the process for fabrication of bioactive scaffolds through the biopolymer depositions systems under different nozzle systems were described. The deposition feasibility and 3D structural formability of alginate-based tissue scaffolds was reported. A semi-empirical model, developed based on the Poiseulle's equation for non-Newtonian fluids to predict the deposition flow rate and the deposition geometry, along with comparison of experimental data also presented. Deposition of cell embedded tissue scaffold as well as the cell viability was introduced. The effect of the process parameters on the structural, mechanical and cellular tissue engineering properties for freeform fabricated 3D alginate tissue scaffolds was presented.

5. 4. HYDROGEL NANOPARTICLES

5.4. 1. Pros and Cons on the Nanocarriers [81]

Nanocarriers overcome the resistance offered by the physiological barriers in the body because efficient delivery of drugs to various parts of the body is directly affected by particle size. Nanocarriers aid in efficient drug delivery to improve aqueous solubility of poorly soluble drugs [82, 83] that enhance bioavailability [84] for timed release of drug molecules, and precise drug targeting [85]. The surface properties of nanocarriers can be modified for targeted drug delivery [85-87] for e.g. small molecules, proteins, peptides, and nucleic acids loaded nanoparticles are not recognized by immune system and efficiently targeted to particular tissue types [88].

Targeted nano drug carriers reduce drug toxicity and provide more efficient drug distribution [89]. Nanocarriers holds promise to deliver biotech drugs over various anatomic extremities of body such as blood brain barrier, branching pathways of the pulmonary system, and the tight epithelial junctions of the skin etc. Nanocarriers better penetrate tumors due to their leaky constitution, containing pores ranging from 100-1000 nm in diameter.

However, nanocarriers exhibit difficulty in handling, storage, and administration because of susceptibility to aggregation. It has unsuitability for less potent drugs. Its small size can gain access to unintended environments with harmful consequences, e.g. it can cross the nuclear envelope of a cell and cause unintended genetic damage and mutations [90].

Nanoparticles have a special role in targeted drug delivery in the sense that they have all the advantages of liposomes including the particle size, but unlike liposomes, nanoparticles have a long shelf life and can usually entrap more drugs than liposomes [91]. Nanoparticles made of hydrophobic polymers are usually taken up by RES and have short residence time in blood [92]. The hydrophilic nanoparticles can evade RES and remain in circulation for a couple of hours without PEG conjugation on the particle surface. Such systems should allow the control of the rate of drug administration that prolongs the duration of the therapeutic effect, as well as the targeting of the drug to specific sites. These hydrogel polymers can have reactive groups on the surface which enable the nanoparticles to be converted to stimuli responsive particles and they can also be made targetable by attaching receptor specific ligands or utilizing its mucoadhesive properties to transport drugs and DNA across mucosal surfaces [93, 94].

Tsai et al. [95] reported the cavitation effects versus stretch effects resulted in different size and polydispersity of ionotropic gelation chitosan–sodium tripolyphosphate nanoparticle. The particle size and reduction rate of ionotropic gelation chitosan– TPP can be manipulated by varying different mechanical energy of ultrasonic radiation or mechanical shearing. Effects of solution temperature on resulted nanoparticle size by ultrasonic treatment or by mechanical shearing were different. The higher the solution temperature, the lower the solution viscosity that facilitates the sporadic cavitation effect exerted on the chitosan molecules producing smaller molecular weight fragments than that produced by stretching effects. Mean diameter of nanoparticles decreased with increasing solution temperature in ultrasonic radiation samples.

SUMMARY

Hydrogel is a dynanic, multidiscipline science, chitinous materials can be an important material to apply in various fields such as tissue engineering, nanoparticles, Chitinous materials are the 2nd most abundant biodegradable, renewable, naturally polysaccharide. They are biodegradable, bioabsorbable, biocompatibility, furthermore, having a primary amino group at C2 and hydroxyl group at C6 positions, chitosan undergoes a host of chemical reactions under mild conditions. Many methods to prepare hydrogel chemical, physical, PEC complex, semi-IPN, photo, thermal, Can be prepared in different shapes such as bead, sheet, film, tube, nanoparticle, etc. to suit the applications. Numberous biomedical application such as DDS, wound healing, New front fields methods and application such as biosensor, microfabrication are developing.

References

[1] N. B. Graham and M. E. McNeil, *Biomaterials,* 5, 27–36 (1984)

[2] N. Boucard C. Viton and A. Domard, *Biomacromolecules,* 6, 3227-3237 (2005)

[3] S. Dumitriu and E. Chornet, *Advanced Drug Delivery Reviews,* 31,223–246 (1998)

[4] T. Kiyozumi Y. Kanatani M. Ishihara D. Saitoh J. Shimizu H. Yura S. Suzuki Y. Okada and M. Kikuchi, *Burns,* 3, 3642–3648 (2007)

[5] N. Boucard, C. Viton, D. Agay, E. Mari, T. Roger, Y. Chancerelle and A. Domard, *Biomaterials*, 28(24), 3478-3488 (2007)

[6] S. Dumitriu P. Magny D. Montane P. F. Vidal and E. Chornet, *J Bioactive Compatible Polym,*. 9, 184–209 (1994)

[7] D. Magnin S. Dumitriu P. Magny and E. Chornet, *Biotechnology Progress,* 17, 734–738. (2001)

[8] T Park. G. Lu W. and G. Crotts, *J. Control Release*, 33, 211–222 (1995)

[9] L. A. Noble I. Gray L. Sadiq, I. F. Uchegbu, Intn. J. Pharmaceu. 192, 173–182 (1999)

[10] F. M. Goycoolea W. M. Argüelles-Monal J. Lizardia, C. Peniche, A. Heras, G. Galed and E. I. Díaz *Polym Bull* 58, 225–234 (2007)

[11] Y-H. Lin H-F. Liang, C-K. Chung, M-C. Chen and H-W. Sung, *Biomaterials,* 26, 2105-2113 (2005)

[12] M. Rinaudo, Prog. Polym. Sci., 31, 603–632 (2006)

[13] M. N.V. Ravi Kumar *React. Funct Polym.*, 46, 1–27 (2000)

[14] D. Magnina J. Lefebvreb E. Chorneta, S. Dumitriua, *Carbohydr. Polym.*, 55, 437–453 (2004)

[15] D. K. Singh and A. R. Ray, *J. M. S.- Rev. Macromol. Chem. Phys.*, C40(1), 69–83 (2000)

[16] M. L. Tsaih L. Z. Tseng and R. H. Chen, *Polym. Degrad. Stab.* 86, 25-32 (2004)

[17] J. Berger M. Reist J.M. Mayer O. Felt N.A. Peppas and R. Gurny, *Euro. J. Pharma. Biopharma.*, 57(1), 19-34. (2004),

[18] J. Berger M. Reist J.M. Mayer O. Felt and R. Gurny, *Euro. J. Pharma. Biopharma.*, 57(1), 35-52. (2004)

[19] L. Vachoud, N. Zydowicz, and A. Domard, *Intn. J. Biolog. Macromol.*, 28, 93–101 (2001)

[20] S. Hirano, S. Kondo, and Y. Ohe, *Polymer,* 16, 622 (1975)

[21] L. Vachoud, N. Zydowicz, and A. Domard, *Carbohydr Res.*, 302 (1997) 169 - 177

[22] S. Hirano and Y. Ohe, *Agr. Biol. Chem.,* 39, 1337-1338 (1975)

[23] S. Hirano, Y. Ohe, and H. Ono, *Carbohydr. Res.,* 47, 315-320 (1976)

[24] S. Hirano, O. Miura, and R. Yamaguchi, *Agric. Biol. Chem.,* 41(9), 1755-1759 (1977)

[25] A. Montembault K. Tahiri C. Korwin-Zmijowska X. Chevalier M-T. Corvol and A. Domard, *Biochimie* 88(5), 551-564 (2006)

[26] F. L. Mi H. W. Sung and S. S. Shyu, *J. Polym. Sci., 38*, 2804-xxxx 2000,

[27] Y. M. Lee S. S. Kim and S. H. Kim, *J. Mater. Sci.-Mater. Med.*, 8, 537–541 (1997)

[28] V. R. Patel and M. M. Amiji, *Pharm. Res.*, 13, 588–593 (1996)

[29] X. Chen W. J. Li W. Zhong Y. H. Lu, and T. Y. Yu, *J. Appl. Polym. Sci.,* 65, 2257–2262. (1997)

[30] H. F. Wang W. J. Li Y. H. Lu and Z. L. Wang, *J. Appl. Polym. Sci.*, 65, 1445–1450 (1997)

[31] M. J. Ko W. H. Jo H. C. Kim and S. C. Lee, *Polym J.,* 29, 997–1001 (1997)

[32] J. Hosokawa M. Nishiyama K. Yoshihara and T. Kubo, *Ind. Eng. Chem. Res.,* 29, 800–805 (1990)

[33] M. Mucha J. Piekielna and A. Wieczorek, *Macromol. Symp.,* 144, 391–412 (1999)

[34] T. H. M, Abou-Aiad K. N. Abd-El-Nour I. K. Hakim and M. Z. Elsabee, *Polymer* 47, 379–89 (2005)

[35] T. Freier R. Montenegro, H. S. Koh, M. S. Shoichet, *Biomaterials* 26, 4624–4632 (2005)

[36] C. Chatelet O. Damour and A. domard, *Biomaterials* 22, 261-268 (2001)

[37] N. Bhattarai H. R. Ramay J. Gunn F. A. Matsen and M. Q. Zhang, *J. Control. Rel.* 103, 609-624 (2005)

[38] E. Ruel-Garie´py, G. Leclairb, P. Hildgen, A. Guptac and J.-C. Lerouxa, *J. Control. Rel.* 82, 373–383 (2002)

[39] H. Zhang, I. A. Alsarra and S. H.Neau, *Intn. J. Pharma.* 239(1-2), 197-205 (2002)

[40] R. H. Mu¨ller and C. Jacobs, Intn. J. Pharma. 237, 151–161 (2002)

[41] Y-H. Bai, Y. Du, J-J. Xu H-Y. Chen, *Electrochem. Commun.,* 9, 2611-2616 (2007)

[42] D. Du J-W. Ding J. Cai and A-D. Zhang, *J. Electroanaly. Chem.,* 605(1), 53-60 (2007)

[43] X-L. Luo, J-J. Xu Y. Du, H-Y. Chen, *Analy. Biochem.,* 334(2), 284-289 (2004)

[44] J. Roche, K. Curry, PCT Int. Appl. (2007), WO 2007100588 A1 20070907

[45] K. L. Douglas and M. Tabrizian, *J. Biomaterials Sci. Polym. Ed.* 16(1), 43-56 (2005)

[46] Q-L. Li, Z. Chen, G. Ou L-k Liu, H-B. Jiang Q. Zeng G, Li G. He A-C. Mo and B. W. Darvell, *Key Engineering Materials (Advanced Biomaterials VI)*, 288-289, 75-78. (2005)

[47] A. Alsarra, S. H. Neau and M. A. Howard. *Biomaterials* 252, 645–2655 (2004)

[48] J. W. Bae D. H. Go K. D. Park, and S. J. Lee, *Macromol Res.*, 14(4), 461-465 (2006)

[49] E. Eljarrat-Binstock, F. Raiskup, J. Frucht-Pery, and A. J. Domb, *J. Biomat Sci., Polym. Ed.* 15(4), 397-413 (2004)

[50] M. Ishihara M. Fujita K. Obara H. Hattori S.

Nakamura M. Nambu T. Kiyosawa Y. Kanatani B. Takase M. Kikuchi T. Maehara, *Current Drug Del.*, 3(4), 351-358 (2006)
[51] M. Ishihara K.Obara, S. Nakamura M. Fujita K. Masuoka Y. Kanatani B. Takase H. Hattori Y. Morimoto M. Ishihara T. Maehara and M. Kikuchi, *J. Artificial Organs*, 9(1), 8-16 (2006)
[52] K. Kofuji H. Akamine H. Oshirabe Y. Maeda Y. Murata and S. Kawashima, *J. Biomater Sci., Polym. Ed.*, 14(11), 1243-1253 (2003)
[53] T-Y. Liu T-Y. Liu S-Y. Chen S-C. Chen and D-M. Liu, *J Nanosci. Nanotech.*, 6(9/10), 2929-2935. (2006)
[54] Z. Liu Y. Jiao and Z. Zhang, *J. Applied Polym. Sci.*, 103(5), 3164-3168 (2007)
[55] A. P. Rokhade N. B. Shelke S. A. Patil and T. M. Aminabhavi, *J. Microencapsul.*, 24(3), 274-288 (2007)
[56] C-J. Tsai L-R. Hsu J-Y. Fang and H-H. Lin, *Biolog & Pharma. Bull.*, 22(4), 397-401 (1999)
[57] J. Viyoch T. Sudedmark W. Srema and W. Suwongkrua, *Intn. J. Cosmetic Sci.*, 27(2), 89-99 (2005)
[58] N. Li and R. Bai, *Indust. & Eng Chem. Res.*, 44(17), 6692-6700 (2005)
[59] Z. Modrzejewska W. Sujka M. Dorabialska and R. Zarzycki, *Sepa. Sci. Tech.*, 41(1), 111-122. (2006)
[60] W. L. Yan and R. Bai, *Water Res.*, 39(4), 688-698 (2005)
[61] J. Li Y. Du L. Sun H. Liang T. Feng Y. Wei and P. Yao, *J. Applied Polym Sci.*, 101(6), 3743-3750 (2006)
[62] X-L. Luo J-J. Xu Q. Zhang G-J. Yang and H.-Y.Chen, *Biosensors & Bioelectron.*, 21(1), 190-196 (2005)
[63] Y.-Q. Xia, T-Y. Guo M-D.Song B-H. Zhang and B-L.Zhang, Biomacromolecules, 6(5), 2601-2606 (2005)
[64] M. Fujita M. Kinoshita M. Ishihara Y. Kanatani Y. Morimoto M. Simizu T. Ishizuka Y. Saito H. Yura, T. Matsui B. Takase H. Hattori M. Kikuchi and T. Maehara, *J. Surg Res.*, 121(1), 135-140 (2004)
[65] K. E. Crompton J. D. Goud R. V.Bellamkonda T. R. Gengenbach D. I. Finkelstein M. K. Horne and J. S. Forsythe, *Biomaterials*, 28(3), 441-449 (2007)
[66] A. Domard M.-T. M. Corvol F. Guillot X. L. Chevalier I. Morfin and D. J.Vacher, WO 2002078761 A1 20021010
[67] L. A. Pfister M. Papaloizos H. P. Merkle and B.Gander, *J. Biomed. Materials Rese., Part A*, 80A(4), 932-937 (2007)
[68] I. I. Selezneva I. V. Savintseva E. F. Vikhlyantseva G. A. Davydova and B. K. Gavrilyuk, *Bull. Exp Biol. Med.*, 142(1), 119-122 (2006)
[69] W. Tachaboonyakiat D. Ogomi T. Serizawa and M. Akashi, *J. Bioact. Compat. Polym.*, 21(6), 579-589 (2006)
[70] X. Wu L. Black G. Santacana-Laffitte and C. W. Jr Patrick, J. Biomed. Materials Res., Part A, 81A(1), 59-65 (2007)
[71] M. Ishihara, Trends *Glycosci. Glycotech.*, 14(80), 331-341 (2002)
[72] T. Kiyozumi Y. Kanatani M. Ishihara D. Saitoh J. Shimizu H. Yura S. Suzuki Y. Okada and M. Kikuchi, *J. Biomed. Materials Res., Part B: Applied Biomateerials*, 79B(1), 129-136 (2006)
[73] S. M. Strukova T. N. Dugina I. V. Chistov M. Lange E. A. Markvicheva S. Kuptsova V. P. Zubov and E. Glusa, *Clinic. Applied Thrombo./Hemosta.*, 7(4), 325-329 (2001)
[74] J. Fukuda A. Khademhosseini Y. Yeo X. Yang J. Yeh G. Eng J. Blumling C-F. Wang D. S. Kohane and R. Langer, *Biomaterials*, 27(30), 5259-5267 (2006)
[75] T. Koyano N. Minoura M. Nagura and K-I. Kobayashi, *J. Biomed Materials Res.*, 39(3), 486-490 (1998)
[76] K. Masuoka M. Ishihara T. Asazuma H. Hattori T. Matsui B. Takase Y. Kanatani M. Fujita Y. Saito Y. H. Yura K. Fujikawa and K. Nemoto, *Biomaterials*, 26(16), 3277-3284 (2005)
[77] V. F. Sechriest Y. J. Miao C. Niyibizi A. Westerhausen–Larson H. W. Matthew C. H. Evans F. H. Fu and J-K Suh, *J Biomed Mater Res*, 49, 534–541, (2000)
[78] J. M. Dang D. D.N. Sun Y. Shin-Ya, A. N. Sieber, J. P. Kostuik, K. W. Leong, *Biomaterials*, 27, 406 - 418 (2006)
[79] J. M. Karp Y. Yeo W. Geng C. Cannizarro K. Yan D. S. Kohane G. Vunjak-Novakovic R. S. Langer M. Radis, *Biomaterials*, 27, 4755–4764 (2006)
[80] S. Khalil, W. Sun. *Materials Sci. Eng.*, C27, 469–478 (2007)
[81] M. Rawat D. Singh, S. Saraf and S. Saraf, *Biol. Pharm. Bull.*, 29(9), 1790-1798 (2006)
[82] J. E. Kipp, *Int. J. Pharm.*, 284, 109-122 (2004)
[83] L. Ould-ouali M. Noppe X. Langlois B. Willems P. T. Riele P. Timmerman M. E. Brewster A. Arien and V. Preat, *J. Control. Rel.*, 102, 657-668 (2005)
[84] P., Arbos O. Campaner A. Miguel M. A. Arangoa and J. M. Irache, *J. Contr. Rel.*, 96, 55- 65 (2004)
[85] H. Yamamoto Y. Kuno S. Sugimoto H. Takeuchi and Y. Kawashima, *J. Contr. Rel.*, 102, 373-381 (2005)
[86] L. B. Peppas and J. O. Blanchette, *Adv. Drug Del. Rev.*, 56, 1649-1659 (2004)
[87] J. Kreuter, *Adv. Drug Del. Rev.*, 47, 65-81 (2001)
[88] H. Cohen R. J. Levy J. Gao I.. V. Fishbein V. Kousaev S. Sosnowski S. Slomkowski and G. Golomb, *Gene Ther.*, 7, 1896-1905 (2000)
[89] R. Lobenberg and J. Kreuter, *AIDS Res. Hum. Retroviruses*, 12, 1709-1715 (1996)
[90] H. M. Hoet Peter I. B. Hohlfeld and O. V. Salata, *J. Nanobiotechnol.*, 2, 1-5 (2004)
[91] T. Banerjee, S. Mitra, A. K. Singh, R. K. Sharma and A. Maitra, *Intn. J. Pharma.*, 243, 93–105 (2002)
[92] S. J. Douglas S. S. Davis and L. Illum, *Int. J. Pharm.*, 34, 145–152 (1986)
[93] M. J. Alonso P. Salve C. Remunan-Lopez and J. L.Vilajato, European Patent 0860-166-A1 (1998)
[94] K. Roy, H. Q. Mao S. K. Huang and K.W. Leong, *Nat. Med.* 5, 387–391 (1999)
[95] M. L. Tsai S. W. Bai and R. H. Chen, *Carbohydr. Polym.*, 71, 448-457 (2008)

Table 1. Applications of chitinous hydrogels

Applied in biosensor MnO_2 nanoparticles modified electrodes to H_2O_2 [41] hydrogel for biosensor [42] oxidase-gold nanoparticles biocomposite [43] intravenous amperometric biosensor [44]	Immobilized enzymes immobilized neutral protease [61] horseradish peroxidase immobilization through gold nanoparticles [62] recognize Hb, by molecularly imprinted method [63]
As a carrier as DNA carrier [45] as an osteoblast carrier [46]	Applied in medical devices inhibition of vascular prosthetic graft infection [64] Tissue engineering /Stem cell culture application to cartilage tissue engineering [25] for neural tissue engineering [65] cartilaginous neo-tissue capable of being grafted/Method for preparing a cartilaginous neo-tissue [66] hydrogel nerve conduits [67] culturing of mouse embryonic stem cells [68] cell adhesion and proliferation [69] for adipose tissue engineering [70]
Controlled release macromolecule delivery to the colon [39] prolonged delivery of diclofenac sodium [47] injectable carrier for local drug delivery [48] iontophoresis drug delivery to the eye [49] releases of FGF-2 and paclitaxel for wound repair [50] drug delivery carrier to control angiogenesis [51] retention and release behavior of insulin [52] hydroxyapatite nanoparticles on ibuprofen release [53] protein drug delivery system [54] controlled release of 5-fluorouracil [55] transdermal delivery of berberine [56] controlled release of alpha-hydroxy acid [57]	Wound dressing as a wound dressing and a biological adhesive [71] adhesive and dressing in autologous skin grafts [72] Immobilized thrombin receptor accelerates wound healing [73] skin regeneration following third-degree burns [5]
adsorption of heavy metal or harmful materials enhanced Metal Adsorption [58] adsorption of Cr(VI) [59] adsorption of lead and humic acid [60]	Others for spheroid microarray and co-cultures. [74] attachment and growth of cultured fibroblast cells [75] fibroblast growth factor-2 protection [76]

(Recieved February 19, 2008 ; Accepted February 26, 2008)