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CHITOSAN IN APPLICATIONS OF BIOMEDICAL DEVICES

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ABSTRACT

Chitosan is a natural polysaccharide with great potential for biomedical applications due to its biocompatibility, biodegradable capability, and nontoxicity. Various techniques used for preparing chitosan microspheres/membranes and evaluations of these fabrications have also been reviewed. The hydrophilicity of chitosan provides unique characteristics of hydrogel formation with the acidic media and may entrap the drug content inside of the matrix for controlled release. In order to improve upon the scope of preparation of chitosan microspheres, we had successfully employed and incorporated a high-voltage system into the direct pumping injection process. The wide range of drug release profiles could be attributed to the surface characteristics, porosities, and various structures of chitosan microspheres upon treatment with $Na_5P_3O_{10}$/NaOH solutions of various volume ratios. We also demonstrated that with the addition of chitosan/β-TCP microspheres as a constituent into the PMMA cement significantly decreases the curing peak temperature and increases the setting time. The excellent gelforming property of chitosan offers another biomedical application in membrane separation fields. Chitosan membranes were prepared by a thermal induced phase separation method, following treatment with nontoxic NaOH gelating and $Na_5P_3O_{10}$, Na_2SO_3 crosslinking agents. In order to further improve the mechanical strength and biocompatibility and to expand the potential of chitosan GTR membranes in periodontal applications, various chitosan membranes incorporating with negatively charged alginate, bioactive tricalcium phosphate, and platelet rich plasma, respectively, were also prepared and characterized. Moreover, we had also utilized chitosan, which with good blood-clotting, cheap, and easy preparation characteristics, as the raw material to prepare rapid clotting wound dressing and tooth plug.

Keywords: Chitosan; Microspheres; Membrane; Guided tissue regeneration.

INTRODUCTION

Chitin is a naturally occurring biopolymer material that belongs to polysaccharide family with β-1,4-linkages throughout the linear chain. This renewable material in abandonment is second only to the cellulose material in the biosphere. The material is derived mainly from the shell of marine and terrestrial invertebrates such as shrimps, crabs, insect, and the fungal cell walls, and is considered to be inexpensive and very much underused raw material. With the utilization of green-conceptual materials on the rise in the global communities, the biomedical and renewable applications of

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chitin and its derivatives deserve close attention for their technological, food, and healthcare merits. Furthermore, chitin and its derivatives, being highly biocompatible, biodegradable and nontoxic, ought to find ways to enter the food, textile, biomedical, and environmental management industries more vigorously. Their unique physical-chemical characteristics and high value-added prospects could plant firm footprints on the path leading to the "green utilization" of global resources.

Chitin, poly-(beta-1-4-*N*-acetyl-D-glucosamine), upon full or partial deacetylation (DA) of the side groups by aqueous sodium hydroxide solution at high temperature, forms chitosan, poly-(beta-1-4-D-gucosamine), which carries free amino group that are present together with hydroxyl groups on the same monomer residual unit. The coexistence of these two types of functional groups provides vast alternatives of chemical modifications and/or crosslinking sites, such as esterification, amidation, and Schiff-base reaction of the polymer chains. In general, there is no clear line drawn in defining chitosan as far as the degree of deacetylation (DDA) of chitin is concerned. For practical purpose, chitin with DDA above 60%, the accepted chitosan material would stay soluble in weak alkaline medium The chitosan material exhibits wide range of solubility with solvents of different polarity and pH media, and appreciable mechanical strength depending on the overall molecular weight and DDA. Specificlly, we have found that, to take full advantage of its characteristics, chitosan materials with molecular weight in the range of 70 KD to 300 KD, and DDA in the range of 85–90% exhibit excellent physiological responses for biomedical applications.[1]

Because of the presence of mildly reactive groups, namely, hydroxyl:-OH^-, amino-NH_3^+, and amido-NH-$CO(CH_3)$, chitosan provides excellent sites of hydrogen bonding, and cationic charge binding for various phenomenological expressions of molecular interactions, such as uniform polypositive charge distribution, hydrophilic bulk and surface, charge-to-charge, charge-to-dipole interactions, etc. Chitosan material has shown to be readily reshaped and processed into designated forms for various applications with unique inter-facial characteristics and physiological responses.

As a result, when chitosan products encounter cells, tissues, body fluids, and other components, they have shown to be with low antigenic and aseptic responses, good compatibilities to wound site and its healing,[2] lowering systemic cholesterol re-adsorption, resistive to inflammation, and promoting pharmacological efficiency.[3] Impressively, the widely spread polypositive charges, NH_3^+s, on the surface promote extreme fast rate in blood clotting due to the strong interaction to the polynegative charges on the surfaces of platelet and red blood cell (RBC). It is postulated that the antibacterial aspects of chitosan material is probably due to its polycationic and the counter-ionic sites on the polymer chain, which act as adsorption or reception sites for the cell walls of invading parasites. In addition, this alters the properties of their cell membrane that causes suppression of growth and eventually destroys the cell altogether. In other words, the strong adsorption and attraction of cell walls to the chitosan molecular surface eventually cause the depletion of cellular fluid of bacteria. Moreover, the chitosan pH environment ought to be less than pKa 6.3 in order to afford the expression of $-NH_3^+$ that provide aseptic expression.

Retrospectively, there are similarities in characteristics between chitosan and hyauronic acid in terms of schemical structure, wound healing capability, and effective stimulation of osteogenesis. Chitosan has shown effectiveness in stimulating the adsorption of calcium and the precipitation of calcium phosphate for a composite material with hydroxyapatite in the application of bone-repairing procedures.[4]

In recent years, there have been extensive efforts devoted to the further propagation of science and technology of processing chitosan material for biomedical applications.[5,6] Taking advantage of chitosan material being able to be easily processed in wide range of conditions, and fabricated into various forms of membranes, filaments, or particles by casting, molding, injection grinding, spreading or pulverizing techniques. In terms of biomaterial applications in healthcare, the most appreciative results have been in categories of medical devices, such as wound dress, artificial skin, surgical space-filling, osteogenesis, control-release systems, etc.[7,8]

THE APPLICATIONS OF MICROSPHERES FROM CHITOSAN

For most orally taken medication according to a fixed schedule, to maintain average or "appropriate" concentration of the medicine in the blood serum is necessary in order to control and or cure a symptom in a given timeframe. However the drug metabolic profile varies, due to the scheduling, with alternation of bursting and decaying of concentrations of the drug in the blood serum. This so-called "over-dose" and "cold-turkey" phenomenon is inevitable unless the medication is delivered to the system steadily and continuously So, we have, undoubtedly, entered the era of concept and

practice of controlled release of drugs by encapsulation of polymer coating over drug.

With the controlled release of effective dosage to a systemic drug delivery techniques in practice, the next step will be to target a drug to be delivered to particular sites without going through a circularly systemic, i.e. blood serum, route. This route is a dream scheme of a pharmacologist-designer for prescribing a given medication. In fact, to avoid a circularly systemic application of a given medical treatment by employing controlled release with deliver-to-target technique is to save the patient from the unnecessary burden which would, otherwise, subject the "unwilling" parts of the body to a potentially harmful or even toxic environment including the vast volume of the blood serum of the individual involved. In conclusion, the key to overcome the side effects and reduce the risks for traditional systemic treatment is to control the toxic level to minimum and maintain the minimum effective concentration of the prescriptions on the localized target.

In general, most drugs taken by a patient are in the form of powder, tablets, capsules, or injections, and the effective components, either to act directly in the gastrological tracks or to be adsorbed into the blood serum first and to be transported forward to the target sites. Of course, in order to target the medication to the designated site and time, the coating of the polymer system needs to be engineered for degradation thus allowing the drug content to be released as planned. This is further verified by *in vitro* and *in vivo* tests. The most popular and consistent polymer encapsulated drug systems are in microsphere forms with diameter from 1 μm to several millimeters. This is because, for a given thickness of the polymer coatings, the smaller the diameter of a coated sphere, the greater the surface area of a given quantity of material to be coated. These are done for the sake of ease of applying diffusion aspects and mass transfer phenomenon to a given polymer coating formulation system. As for microsphere fabrication techniques, there are agitating and ultrasonic emulsifications, spreading, injection, etc. processes available There are advantages and disadvantages for each fabrication method. For instance, size distribution of microspheres in agitating emulsification is usually too broad for critical application; ultrasonic emulsification renders the microspheres out of shape; spreading when coupled with drying offers fast turn around but with much too wider size distribution; and direct and pump injection processes all lead to mostly large sized spheres, etc. Furthermore, there are double coating processes developed: for instance, with an acid-resistant polymer coated outerlayer providing protection of the drug content during passing through the medium in stomach and followed by another porous polymer layer for controlled release for intestine track adsorption of the drug content. Along the same vein, there are preliminary studies involving the microspheres with multiple layers or multiclusters of polymer coatings being carried out.

As indicated, chitosan is biodegradable and nontoxic with one amino and two hydroxyl groups on each residual unit readily for further chemical modifications for extended applications.[9–11] The hydrophilicity of chitosan provides unique characteristics of hydrogel formation with the acidic media including with the stomach. Further, the hydrogel may entrap the drug content inside of the matrix, which still allows for slow release of the content via diffusion process and eventual dissolution of polymer structures. In addition, these properties provided the pharmacologic bases for the slow control release microspheres, particles dosages, pills, and clusters. In addition, the polypositive ionic characteristics, and the ease of formations of gel and membrane provide the engineering potentials for the controlling of speeds, mass transfer, reaction medium, etc. of the drug contents. For microspheres larger than 100 μm in size, are used for encapsulating anti-inflammatory drugs such as didofenac sodium, and cancerous suppressant mitoxantrone, and other medications.[12–14] Whereas for chitosan microspheres of smaller than 10 μm in size are used for coating of anticancerous drug such as 5-fluorouracil.[15]

In order to improve upon the scope of preparation of chitosan microspheres by the direct pumping injection process, we have successfully employed and incorporated a high-voltage system into the process (Fig. 1 to be referred for schematic representation). This unique process provided us with extremely narrow range of size distribution of microspheres with nearly true spheroid. Incidentally, we deliberately avoid using glyceraldehyde as the crosslinking agent for fear of it potential toxicity; instead, we employ 1N NaOH and 5% $Na_5P_3O_{10}$ as a curing agent. The results indicate that the chitosan microspheres exhibit excellent spheroid, narrow particle-size distribution, and smaller in particle size limits. The entire process is straightforward with simple steps to operate, well-defined parameters, such as the applied voltage, pumping rate, electrode gap distance, etc. consistently provides reproducible results As for the characterizations of the chitosan microspheres, as expected that the ones from 1N NaOH solution curing can be dissolved into a weak acidic solution, whereas those from 5% $Na_5P_3O_{10}$, being properly crosslinked by phosphate ions, would stay in perfect spherical shape and insoluble. We found that each reagent alone will not

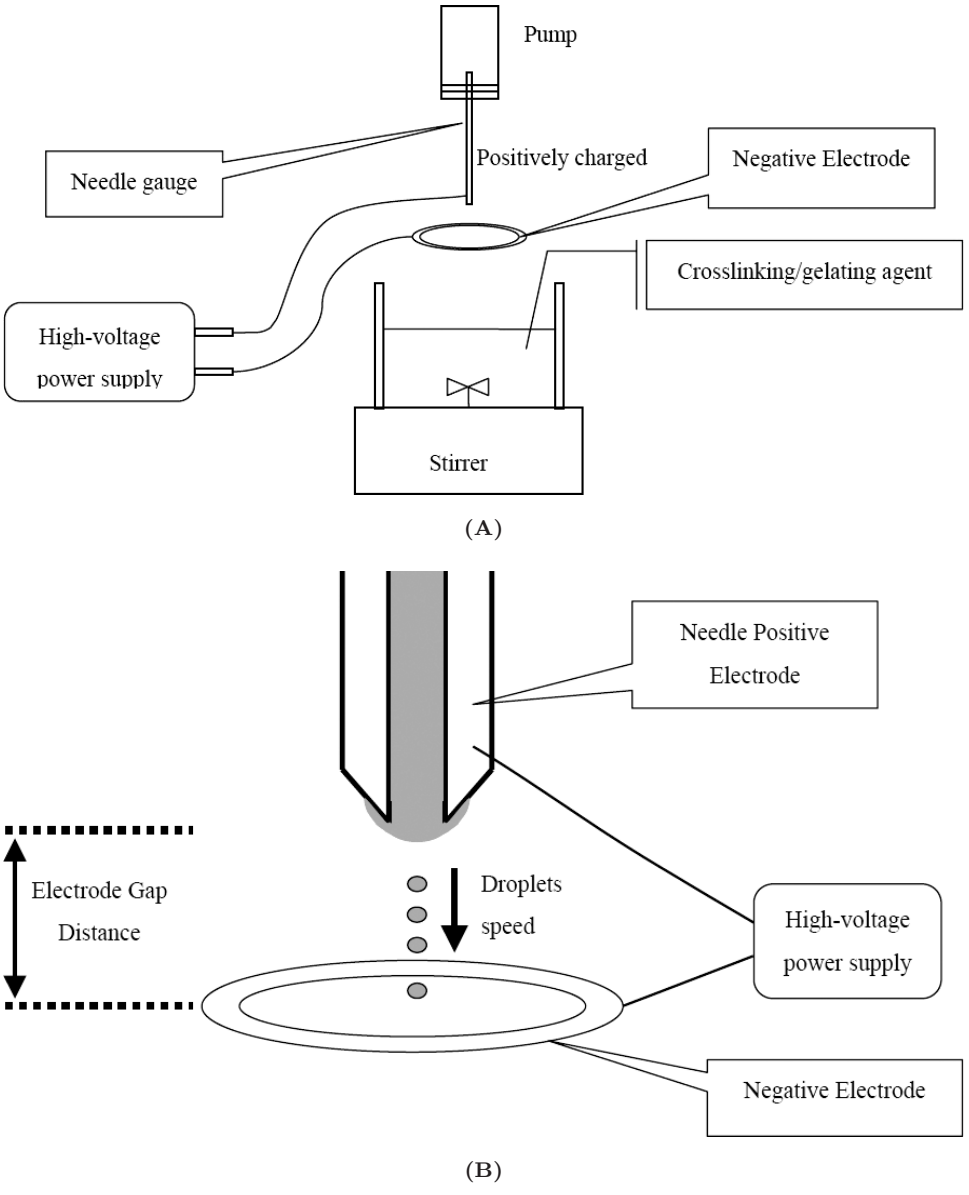


Fig. 1 **(A)** Schematic presentation of chitosan microsphere generator; and **(B)** Close-up view of electrodes. From Kuo SM, Niu GCC, Chang SJ, *et al.*, A one-step method for fabricating chitosan microspheres, *J Appl Polym Sci* **94**:2150–2157, 2004.

act properly in the crosslinking process. Microspheres treated with just an aqueous NaOH solution became flattened and lost the spherical shape over time. On the other hand, microspheres treated with only an aqueous $Na_5P_3O_{10}$ solution exhibited a thinner and more fragile membrane structure.[16] However, the chitosan microspheres treated with $Na_5P_3O_{10}$/NaOH solution at ratios of 17:3 and 19:1 exhibited layered morphological structure (Fig. 2(A)).[17]

It is believed that chitosan droplets could be first precipitated by gelation in NaOH to form a gelating microsphere. Subsequently, the ionic crosslinking reaction with $Na_5P_3O_{10}$ occurs on the outer part upon treatment with $Na_5P_3O_{10}$/NaOH mixture to form a skin structure. Afterward, the masses of chitosan gelating droplets were gradually exposed to further infiltration of $Na_5P_3O_{10}$/NaOH solution. The two anionic species: $P_3O_{10}{}^{-5}$ (a larger ion) and OH (a smaller ion) that would compete to each other in a diffusion-controlled process. Consequently, the outer layer of microsphere was largely crosslinked with $P_3O_{10}{}^{-5}$ being the dominant species in the mixed reaction solution and, in the internal portion, a weakly gelled chitosan might be subjected to a dynamically controlled ionotropic crosslinking reaction on the polymer chains. This most distinctive display of the two major concurrent reactions is illustrated on Fig. 3. Furthermore, the mechanical strength of the microspheres was also improved

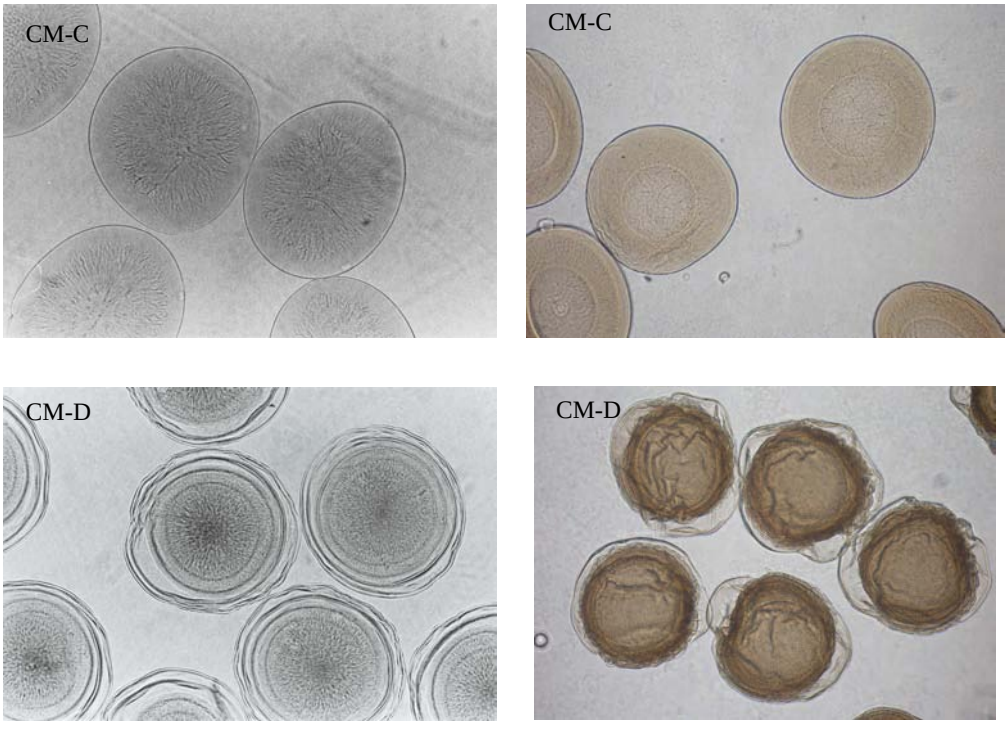


Fig. 2 Micrographs of **(A)** chitosan microspheres treated with various ratios of $Na_5P_3O_{10}$/NaOH (v/v) solutions; and **(B)** chitosan microspheres after immersion in acetic acid (0.1N) for 8 h with CM-C as $Na_5P_3O_{10}$/ NaOH = 17/3 and CM-D as $Na_5P_3O_{10}$/ NaOH = 19/1, respectively. From Chang SJ, Niu GCC, Kuo SM, *et al.*, Preparation and preliminary characterization of concentric multi-walled chitosan microspheres, *J Biomed Mater Res Part A* **81**:554–566, 2007.

with the increase of $Na_5P_3O_{10}$/NaOH ratios This result could be due to a more efficient crosslinking reaction with higher concentration of $Na_5P_3O_{10}$ to chitosan, which would strengthen the polymer network of the outer part of microsphere to yield greater mechanical strength. In general, these ionotropic crosslinked microspheres could be shrunk in size after immersion in acetic acid (0.1N) solution for 8 h. Only the ones treated with 17:3 ratio of $Na_5P_3O_{10}$/NaOH solution still exhibited an intact membrane structure and with an obviously two-layered different textures. As for the microspheres treated with 19:1 ratio of $Na_5P_3O_{10}$/NaOH solution, the surface became irregular with floppy and loosening membrane structure (Fig. 2(B)).

Release studies were carried out to evaluate the kinetic profiles of two model drugs (5-fluorouracil and cytochrome C) from these prepared chitosan microspheres. When chitosan microspheres treated with $Na_5P_3O_{10}$/NaOH ratio at 17:3, the release of cytochrome C was found to be the slowest as compared to those treated by the same $Na_5P_3O_{10}$/NaOH solution of other mixing ratios, after a period of 35-day "endurance" test. However, in one case, 5-fluorouracil released quite quickly in a period of 30 min (about 80% completion). The wide range of drug release results might be attributed to the unique and wide range of surface characteristics, porosities, and various structures of chitosan microspheres upon treatment with $Na_5P_3O_{10}$/NaOH solutions.

Due to its biodegradable and osteoinduction characteristics, chitosan shows high potential as an encapsulation material incorporating with bone matrix components or growth factors used in orthopedic applications.[18,19] Polymethylmethacrylate (PMMA) cement has widely been used as a fixation filler that is injected into the gaps between the prosthesis and the surrounding bone.[20] Despite its wide acceptance as a grouting material in dentistry and orthopedic applications, PMMA cement has some inherent problems. First, the high heat generated during its hardening polymerization process, which has been shown to cause damage to the surrounding bone tissue. Second, poor compatibility with bone: PMMA does neither adhere to the bone nor induce bone formation and may contribute to mobility of the implant and, later on, aseptic loosening may occur after long-term implantation.[21] Several attempts to improve the situation were made by the addition of small quantities of ingredients such

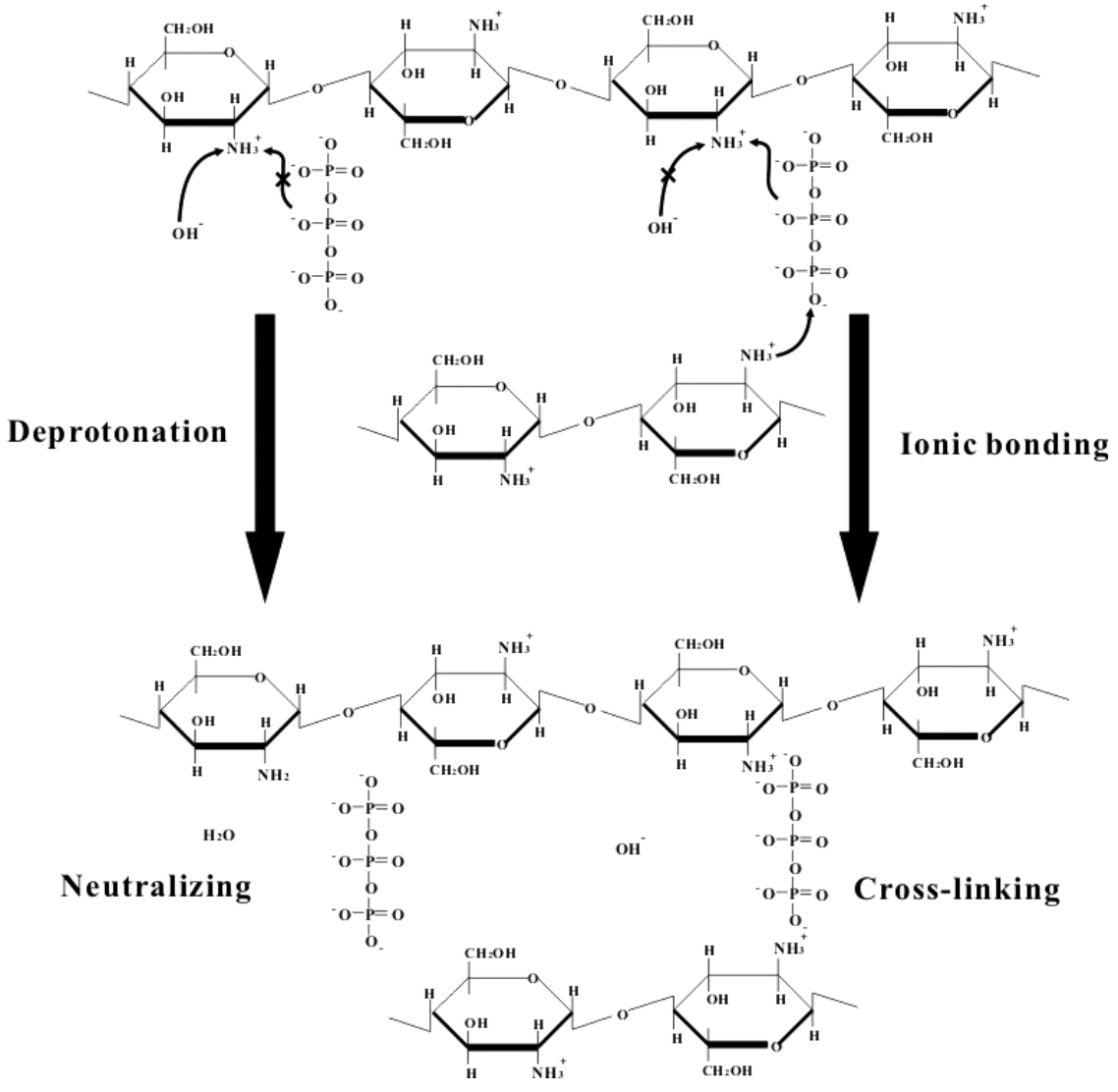


Fig. 3 Mechanism of the formation of chitosan chains network structure by ionic bonding and deprotonation. From Chang SJ, Niu GCC, Kuo SM, *et al.*, Preparation and preliminary characterization of concentric multi-walled chitosan microspheres, *J Biomed Mater Res Part A* **81**:554–566, 2007.

as bioactive tricalcium phosphate (TCP) or hydroxyapatite (HA) into the matrix of PMMA bone cement.[22,23] From clinical practice and experience, β-TCP has a more favorable resorption pattern and osteotransduction property than HA. Besides, β-TCP can also be gradually adsorbed following new bone formation and is often incorporated as an additive to bone grafting and dental materials. However, the β-TCP ceramic in particulate form, being incoherent to each other, may be easily washed off from the wound area. It seems that to group the particulate ceramic into manageable packages by a process of capping or encapsulating would be highly desirable.

The chitosan/β-TCP microspheres prepared by high voltage electrostatic system exhibited a narrow size distribution with good sphericity and the β-TCP was trapped well inside the chitosan gel. These microspheres were then used as an added constituent to commercially available PMMA bone cement. The characteristics of these materials indicate that with the addition of chitosan/β-TCP microspheres as a constituent into the PMMA cement significantly decreases the curing peak temperature. Furthermore, the setting time increases from 3.5 to 9 min, as compared to the pure PMMA cement.

These changes could be beneficial for the handling of the bone cement paste and causing less damage to the surrounding tissues. However, the diminished ultimate compressive strength and bending strength due to the presence of chitosan/β-TCP microspheres in the prepared composites is a major limitation of these composites when considering as a load-bearing material.

From the degradation test and SEM observations, the modified chitosan/β-TCP/PMMA composites could be degraded gradually and provided rough and porous spaces that would be beneficial to cell adherence and growth. Rough and porous chitosan/β-TCP/PMMA cement enabled bone ingrowth, which made these composites promising for and consistent to clinical considerations and applications.[24]

GUIDED TISSUE REGENERATION BY CHITOSAN MEMBRANE

Periodontal lesions are one of the most widely spread oral disorders. They are caused by the uncontrolled growth of dental plaques in the oral cavity of the adult population. If the parasitic dental plaque is not properly cleaned, the minerals in saliva will slowly be deposited onto the plaque site to form the dental debris, which invades and cause inflammation or bleeding of the dentine tissue. The clinical treatment in the early stage involves debridement of the teeth for removal of dental plaques and debris, and the wound site may heal by itself. However, for more severe cases, i.e. with deeper down growth of debris to the softer tissue zone the wound healing may develop differently: the bony area growth cannot keep up with the faster paths of growth of epithelial cells and connective tissues, and thus may further leave the dentine exposed without the umbrella protection of epithelial cells and connective tissues.

Guided tissue regeneration (GTR), techniques involved procedures utilizing membranes to serve as mechanical barriers to create a space around the defects, have been successfully applied in the treatment of periodontal diseases and provided an opportunity for the formation of new bone.[25,26] In general, an effective barrier membrane should have appropriate mechanical strength to occlude the rapid growth of repairing tissues (epithelium and gingival connective tissue) away from the root surface and maintain a protective space over the defect that will allow migration of bony cells from the surrounding alveolar bone into the targeted area. Ideally, effective materials should be safe, nontoxic, and nonantigenic ones, which induce little or no inflammatory responses around the host tissue. Furthermore, the barrier function must be established and maintained for a period of time long enough for tissue guidance to take effect. Among the materials used in GTR applications, nonresorbable membranes, such as expanded polytetrafluoroethylene (e-PTFE) membrane, and resorbable membranes, such as collagen membranes, have been successfully applied and are commercially available. However, they might exhibit disadvantages (regarding the use of e-PTFE membrane), which would require a second surgical procedure to remove it, and of collagen membrane, which might cause localized chronic inflammatory response and fast degradation behavior.[27,28]

Highly deacetylated chitosan (e.g. >85%) exhibit low degradation rate and may last several months.[29] Another promising feature of chitosan in biomaterial application is its excellent gelforming properties and can be processed into different shapes simply by thermal induced phase separation method.[30] This extra property offers another application in membrane separation fields and thus increases its potential in biomedical applications. Chitosan membranes were prepared by a thermal induced phase separation method, following treatment with nontoxic NaOH gelating and $Na_5P_3O_{10}$, Na_2SO_3 crosslinking agents. The results showed that the gel content of chitosan membranes crosslinked with $Na_5P_3O_{10}$ and Na_2SO_3 was 53.5% and 52.2%, respectively. The high gel content of chitosan membrane indicated that chitosan molecules could be crosslinked with each other to form an insoluble network. Owing to the dense characteristics of this chitosan membrane prepared by ovendried method, the reaction agent could not diffuse into the subinner part of the matrix readily, thus yielded only about 5% of gel content on the chitosan membrane. Contrarily, chitosan molecules simply gelated under NaOH base solution and thus the chitosan matrix gelated with NaOH dissolved completely during gel content measurement.[31]

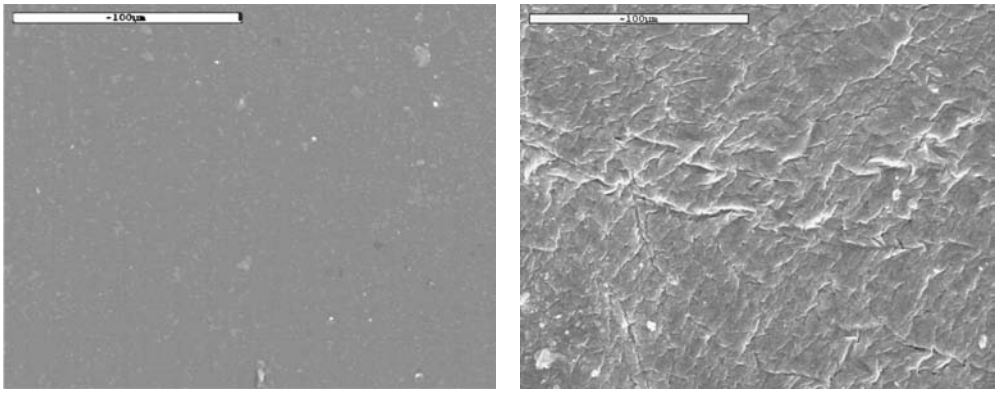
When considering the development of guided tissue regeneration barrier materials, the basic bulk and mechanical properties must be noted. Besides, the appropriate degradation rate of material also has to fit the requirement of tissue regeneration. The chitosan membrane gelated with NaOH agent was stiffer than the crosslinked chitosan membranes. All the three chitosan membranes degraded by about 23–28% of the initial weight after a 90-day *in vitro* shaking test. In general, chitosan membranes crosslinked by $Na_5P_3O_{10}$ and Na_2SO_3 agents had similar basic properties. In addition, $Na_5P_3O_{10}$ crosslinked chitosan membrane showed better cell adhesion and proliferation results for osteoblastic cells *in vitro* cultures. Subsequently, we applied these chitosan membranes into animal GTR study models. Critical sized skull defects were made in adult rats and the defective regions were covered with the specifically prepared chitosan membranes. After four weeks of recovering, varying degrees of bone healing were observed beneath the chitosan membranes in comparison to the control group. The chitosan-covered regions

showed a clear boundary space between connective tissues and bony tissues. Among the chitosan membranes tested in this study, chitosan membrane gelated with NaOH agent provided a higher percentage of new bone formation. It could be concluded that the chitosan membranes prepared in this study appeared to be of great promise for application in GTR.

Alginate is a naturally derived polysaccharides composed of (1–4)-linked β-D-mannuronic acid and α-L-guluronic acid monomers. Alginates have been extensively used as an ideal matrix material to prepare synthetic extracellular matrices or for immobilization of enzymes and microencapsulation of tissues and cells.[32−34] Because of the highly hydrated anionic surface characteristics, alginate matrix could resist cell adhesion and spread.[35] Thus, we take advantage of the specific properties of chitosan (with positively charged and osteoinduction potential) and alginate (with negatively charged and resisting cell adhesion potential) to prepare alginate/chitosan (A/C) barrier membrane (Fig. 4). The results revealed that the bulk properties of the membrane were not extensively altered after coating one side with solution of alginate content from 0.05 to 0.1 wt%. The A/C membrane had a higher water content of 71.8% in comparison to the neat chitosan membrane of 61.8%. In addition, A/C membrane became stiffer and expressed higher Young's modulus and mechanical strength. However, A/C membrane exhibited different hydrophilicity and surface morphology. The contact angle on the alginate-modified side dropped substantially to 34.28 as compared to the untreated side of 88.48. Furthermore, this alginate-coated surface exhibited a 50% improvement in the resistance to 3T3 cells adhering under low shear rate. Our studies implied that the alginatemodified chitosan membrane could be an ideal GTR material that presumably provides a means to regulate the growth of soft tissue, such as gingival tissue, over a competing hard tissue, such as alveolar bone.[36]

Among many commercially available ceramics, beta-tricalcium phosphate (β-TCP) is frequently used as bone repairing and replacing materials because of their biocompatibility, bioactivity, mechanical strength, and nontoxicity. Thus, we take advantage of the unique properties of chitosan and β-TCP to prepare a composite membranes system (Fig. 5). Briefly, β-TCP was annexed to chitosan matrix to prepare the composite membranes with high mechanical strength. Following the process, β-TCP also altered morphological structure and degradation behavior of the chitosan membranes. These changes probably are attributed to that β-TCP could bind with chitosan through ionic bonding, and subsequently crosslinked to the different parts of chitosan polymer chains. Subsequently, we applied these β-TCP/chitosan composite membranes to the animal GTR study models. The β-TCP/chitosan membranes covered regions showed a clear boundary space between connective tissues and bony tissues.[37] Over all, good cell occlusion and beneficial osteogensis effects by these bioabsorbable materials toward the wound recovery were indicated.

There has been a great deal of interest in oral bone grafting procedures by the use of platelet-rich plasma (PRP) to enhance bone regeneration and graft healing.[38] The basis of using PRP is that, upon engaging with thrombin, the platelets in PRP would release various growth factors, including platelet-derived growth factors (PDGFs), transforming growth factors (TGFs-β), epidermal growth factors (EGFs), and vascular endothelial growth factors (VEGFs).[39,40] Chitosan itself alone is not sufficient enough to induce rapid bone regeneration at least at the initial bone



(A) **(B)**

Fig. 4 SEM micrographs of A/C membrane: **(A)** chitosan side; and **(B)** alginate side. Magnification: ×300. From Chen TW, Chang SJ, Niu GCC, *et al.*, Alginate-coated chitosan membrane for guided tissue regeneration, *J Appl Polym Sci* **102**:4528–4534, 2006.

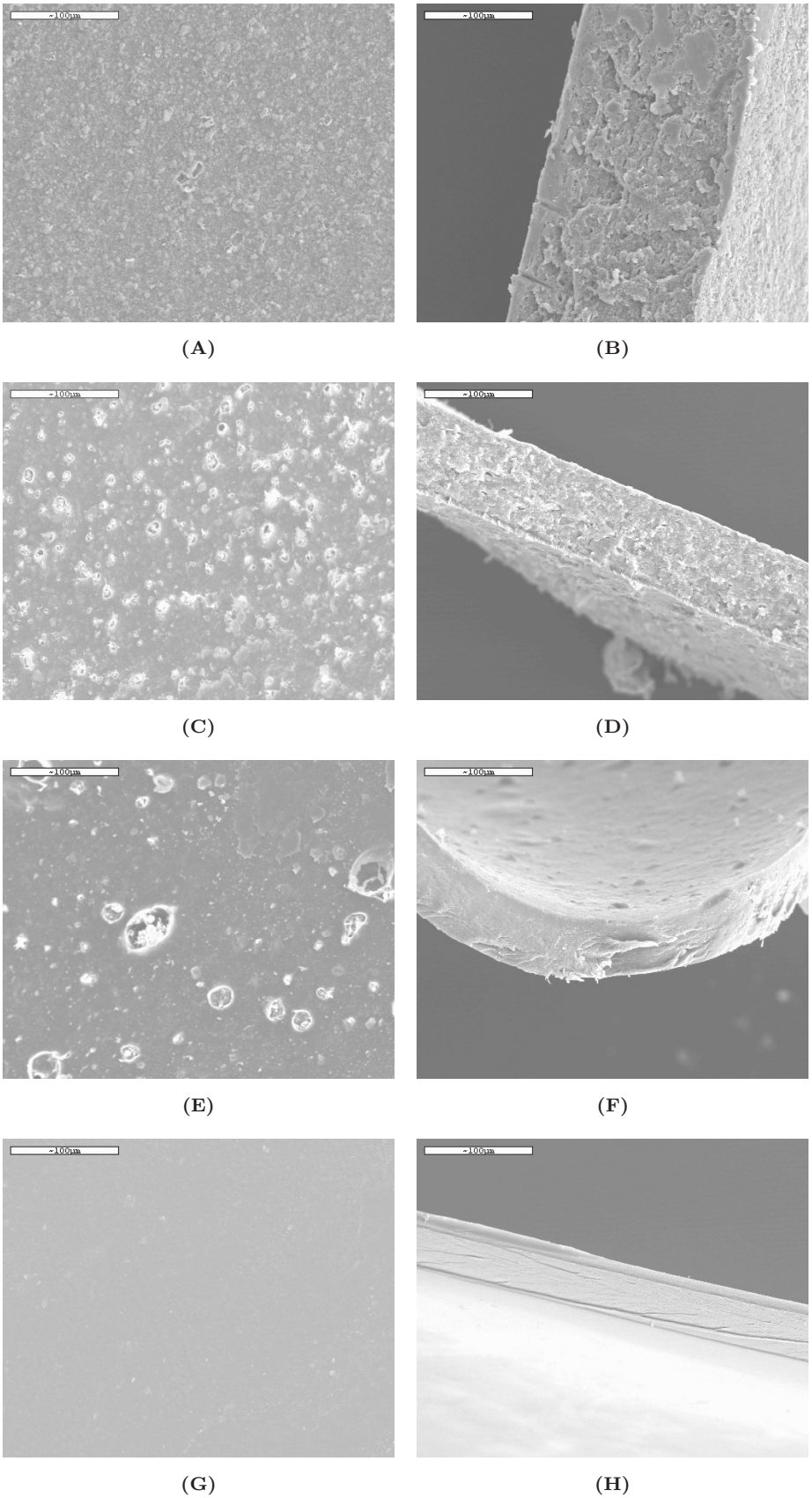


Fig. 5 SEM micrographs of the membrane: **(A, B)** a β-TCP/chitosan (65:35) membrane; **(C, D)** a β-TCP/chitosan (33:67) membrane; **(E, F)** a β-TCP/chitosan (10:90) membrane; and **(G, H)** a plain chitosan membrane. From Kuo SM, Chang S J, Niu GCC, *et al.*, Guided tissue regeneration with use of β-TCP/chitosan composite membrane, *J Appl Polym Sci* **112**:3127–3134, 2009.

healing phase. In order to conserve the activity of growth factors and enhance the bone regeneration in application, we have utilized PRP to coat onto the chitosan and thus prepared a novel barrier membrane that is used in GTR applications.[41]

In summary, various chitosan GTR membranes were prepared incorporating with negatively charged alginate, bioactive tricalcium phosphate, and platelet rich plasma to further improve the mechanical strength and biocompatibility and to expand the potential of chitosan GTR membranes in periodontal applications.

CHITOSAN AS RAPID CLOTTING WOUND DRESS

One of the major challenges in a surgical operation that involving massive bleeding symptom is to control the bleeding during operation and to promote appropriate clotting procedure postoperationally. With the advancement in expectation and creative execution of surgical procedures, blood coagulation by traditional application of clippers, compression of plain dressing pad, and suturing of blood vessel have been deemed inadequate. Consequently, laser coagulation procedures have been introduced but with limited success because of massive damage to the surrounding tissues. Typically, in an endoscopic operation or areas of massive concentration of blood vessels such as biopsy or surgical procedures of breast, the anticoagulant and coagulant situations have to be addressed concurrently to avoid post-operational complications. A need of composing a proper coagulation system ought to be developed such that chitosan polymer material in combination with other biomaterials may provide a treatment regiment of nearly instant vessel coagulation procedure. There are several commercial wound dress pad products available for usage of compression to the wound site with very rapid clotting effectiveness. Chitosan material is one of the key elements that promote the rapid blood clotting on wound sites.[42]

Breast is composed of tissues much rich in blood vessels with high turn-over volume rate. The bleeding controls for routing and extended biopsy and surgical procedures have presented some levels of risk.[43] We have attempted to compare a novel process with the use of a commercial product "Instant Clot Pad" (Bio-Sponge Technology Co., Ltd., Taiwan), a chitosam polymer material preparation, to the standard process with ordinary cotton dressing. The "Instant Clot Pad" is a spongy-like porous material with average pore size about 200–250 μm. The results showed that the material can accumulate a vast number of RBCs, which further promote the adhesion of platelets. Moreover, there is fibrin formation all over the surface of chitosan pad. Statistically, from 1 to 10-min period, with the use of chitosan pad, the average clotting time (ACT) is 3.75 min., whereas, with use of ordinary cotton pad only, the ACT is 6.40 min. Apparently, with the use of chitosan, the pressed-on ACT has been greatly reduced.

The main purpose of tooth plug is to maintain or protect the original vacancy after tooth extraction to prevent the excessive growth of gingival tissue to occupy the space of alveolar bone. Also, the tooth plug is expected to be able to stop bleeding after the tooth extraction, and promote the dental healing. We utilized chitosan, which with good blood clotting, cheap and easy preparation characteristics, as the raw material to prepare tooth plug. The chitosan tooth plugs were treated by 1 N NaOH gelating agent and/or 5% $Na_5P_3O_{10}$ crosslinking agents The preliminarily results revealed that the prepared chitosan tooth plug all with porous structures and the opening was about 200$\sim$250 μm in diameter. The NaOH-treated chitosan tooth plug had better water content and quicker swelling behavior.

CONCLUSIONS

The applications of chitosan either in particulate or membrane forms in biomedical engineering are increasing in number every day, where the range of possibilities is also extending. In the past five years, we have built various techniques used for preparing chitosan microspheres/membranes and evaluations of these chitosan devices as an ingredient of PMMA cement or in GTR applications or as a rapid blood clotting dressings. The biocompatibility of chitosan devices produced in our laboratory, when compared with those produced by emulsion methods, is inherently higher due to the few chemicals, nontoxic reagents, and the aqueous fabrication environment used. Furthermore, we had built a high-voltage system and incorporated it into the direct pumping injection process, and hence we were able to produce chitosan microspheres with extremely narrow size distribution in the size from 200 μm to 800 μm. The wide range of drug release profiles could be attributed to the various surface characteristics, porosities, and structures of chitosan microspheres upon treatment with $Na_5P_3O_{10}$/NaOH solutions of various volume ratios. Recently, we also prepared a chitosan sponge, which with good blood clotting, cheap and easy preparation characteristics, and the evaluations of these sponges in the application as clotting wound dressing and tooth plug are still ongoing in our laboratory.

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