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New designed nerve conduits with a porous ionic cross-linked alginate/chitisan structure for nerve regeneration

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Abstract. A new fabrication process for designing nerve conduits with a porous ionic cross-linked alginate/chitosan composite for nervous regeneration could be prepared. New designed nerve conduits with a porous ionic cross-linked alginate/chitosan composite were developed for nervous regeneration. Nerve conduits (NCs) represent a promising alternative to conventional treatments for peripheral nerve repair. NCs composed of various polysaccharides such as sodium alginate were designed and prepared by lyophilization as potential matrices for tissue engineering. The use of a porous ionic cross-linked alginate/chitosan composite could provide penetration channels that would lead to the products' increasing penetration rate properties. Furthermore, the use of a porous ionic cross-linked alginate/chitosan composite also has a highly cross-linked structure, which would give the products relatively good mechanical properties. Furthermore, the drug could be incorporated into nerve conduits as a new drug-carrying system for nerve regeneration because of its porous and cross-linked structures.

Keywords: Cross-linking reaction, drug-release systems, composite

1. Introduction

Repairing damaged nerves is a common clinical problem [1]. Artificial nerve conduits (NCs) that bridge the gap between severed peripheral nerve stumps are widely accepted as a useful alternative that creates a favorable micro-environment for nerve regeneration [2]. In general, natural-derived materials are useful in biomedical and clinical applications because the natural-derived materials provide

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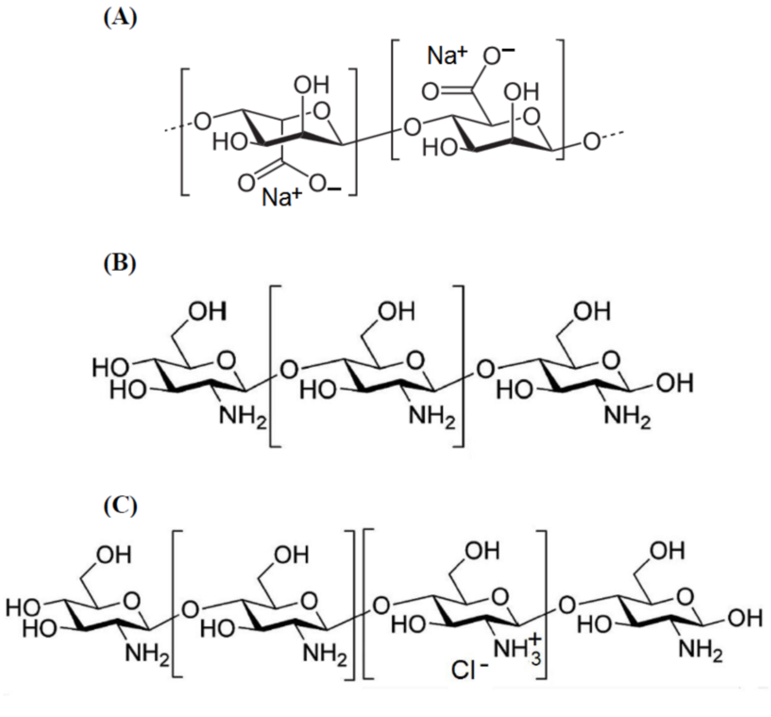
good cell compatibility and suitable interaction. Good cell compatibility and interaction resulted from the common hydrophilic property. However, natural-derived materials always suffer from batch-to-batch variability. Extensive purification and characterization before use is necessary and important. Natural-derived materials with good mechanical strength are difficult to find. Therefore, additional modifications, such as physical and chemical cross-linked reactions, are necessary to meet well mechanical requirements for biomedical and clinic application such as artificial nerve conduits (NCs).

Polysaccharides are widely employed in biomedical and clinic applications because of their excellent cell compatibility. Alginate is a kind of polysaccharide material that might be good for the clinic application of artificial nerve conduits (NCs). The good cell compatibility of alginate is due to the free carboxylic groups of alginate. For the application of artificial nerve conduits (NCs), it is necessary to enhance the mechanical properties of alginate. Calcium salt could be used to adjust mechanical strength through physically cross-linked reactions. Also, alginate could be used as a hydrogel inside the nerve conduit for clinic application of nerve regeneration [3]. On the other hand, a natural-derived material such as chitosan is another polysaccharide that has been considered for fabrication of artificial nerve conduits (NCs). To adjust their mechanical properties for clinical use, the natural-derived material of chitosan could be reinforced with additional cross-linked agents such as chitin [4] or formaldehyde [5] to prevent the nerve conduit from collapsing. The design, synthesis and development of new functional materials were studied to provide suitable materials for biomedical applications such as polyacrylate, polyester, polyurethane, polyamide, polyimide, polyester, polynorborene, polytetrafluoroethylene, polydiphenylacetylenes and polymeric resins [1-21]. Also, surface modification technology was considered to change the surface microenvironment of the materials [22-26]. Therefore, a suitable material and fabrication process can be selected, designed and established.



Scheme. 1. The chemical structures of (A) sodium alginate (NaAL), (B) chitosan (Ch) and (C) partially HCl quaternary ammonium chitosan (HClQCh).

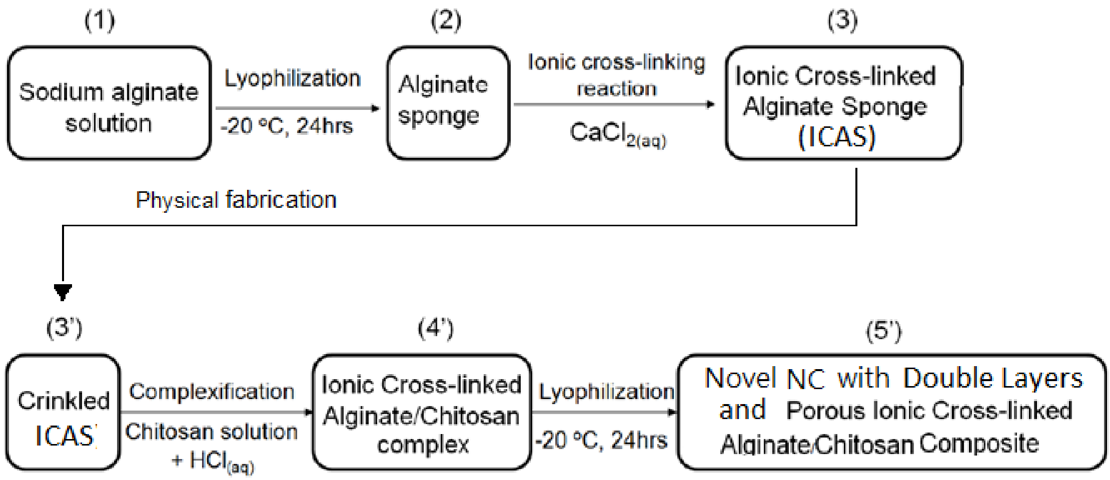
2. Experimental

2.1. Materials

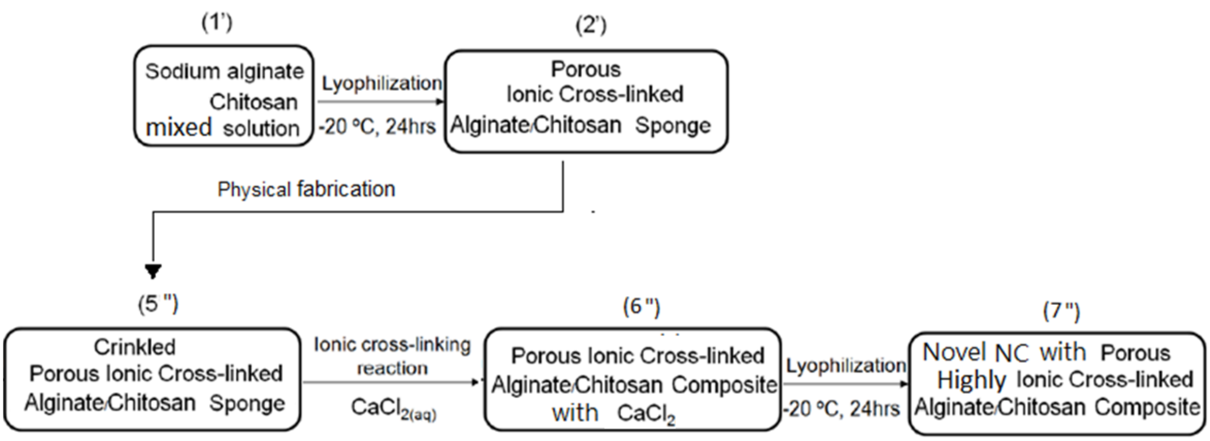
The materials of sodium alginate (NaAL), chitosan (Ch) and partially HCl quaternary ammonium chitosan (HClQCh) were employed in this study. The chemical structures of sodium alginate (NaAL), chitosan (Ch) and partially HCl quaternary ammonium chitosan (HClQCh) are shown in Scheme 1.

2.2. Preparation of porous ionic cross-linked alginate/chitisan composite

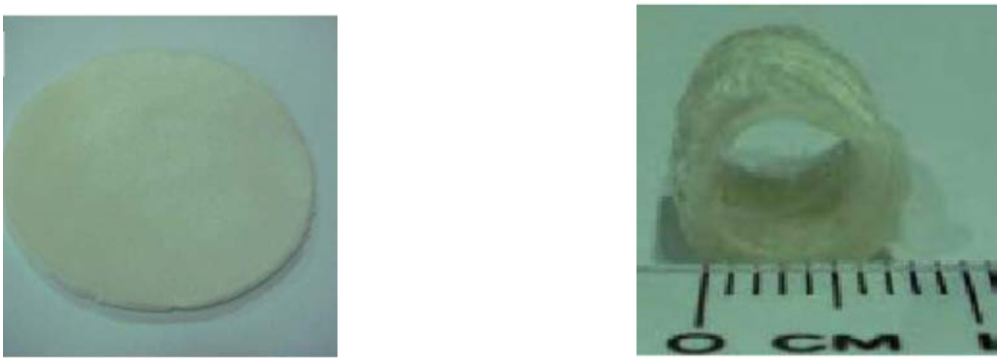
The preparation of the porous ionic cross-linked alginate composite and the porous ionic cross-linked alginate/chitosan composite was achieved as shown in Scheme 2.



Scheme. 2. Schematic diagram for new fabrication process of designed nerve conduits (NC) (5') with a porous ionic cross-linked alginate/chitosan composite.



Scheme. 3. Schematic diagram for new fabrication process of designed nerve conduits (NC) (7") with a highly ionic cross-linked alginate/chitosan composite.



(A) (B)

Fig. 1. Morphology of (A) an ionic cross-linked alginate sponge (ICAS) and (B) a new designed nerve conduit (5') with a porous ionic cross-linked alginate/chitosan composite, derived from a crinkled ionic cross-linked alginate sponge (crinkled ICAS) and combined with chitosan (Scheme 2).

2.3. Preparation of highly ionic cross-linked alginate/chitosan composite

The preparation of a highly ionic cross-linked alginate/chitosan composite and a porous ionic cross-linked alginate/chitosan composite was achieved and shown in Scheme 3.

3. Results and discussion

3.1. New designed nerve conduits with porous ionic cross-linked alginate/chitisan composite

We describe the preparation and characterization of nerve conduits made of alginate and a partially HCl quaternary ammonium chitosan complex. These nerve conduits fulfilled mechanical demands without further additives or chemical crosslinking reactions. The highly crosslinked alginate/ chitosan NC were expected to be suitable for cell in-growth and supplying nutrients through the wall of the NC. Polyelectrolyte complexes could be used for the delivery of proteins. Because of the agglutination characteristics of oppositely charged polymeric materials, the complex was firstly treated with the special method; that is, the sodium solution was lyophilized and the alginate sponge was obtained (Figure 1). After being treated with calcium ions, the crinkled ICAS hollow product (Scheme 2) was treated with chitosan HCl solution for 10 min. The ionic cross-linked alginate/chitosan complex was further lyophilized. The porous structure was obtained and is shown in Figure 2. The chitosan was partially quaternized with an HCl solution and the partially HCl quaternary ammonium chitosan (HClQCh) was obtained (Scheme 1). The partially HCl quaternary ammonium chitosan (HClQCh) provides multiple ammonium groups and ionic association between alginate and partially HCl quaternary ammonium chitosan. Nerve conduits with an outer diameter/inner diameter of 8 mm/5 mm were obtained (Figure 1).

3.2. New designed nerve conduits with a highly ionic cross-linked alginate/chitosan composite

In this study, the fabrication process for designing nerve conduits with a porous highly ionic cross-linked alginate/chitosan composite was designed to obtain various NC porosity and density. The mixed solution of sodium alginate and chitosan was prepared as shown in Scheme 3. After lyophilization, a porous ionic cross-linked alginate/chitosan sponge was obtained. Physical fabrication of the porous ionic cross-linked alginate/chitosan sponge was carried out, and a crinkled porous ionic cross-linked alginate/chitosan sponge (5”) was developed. To enhance strength and mechanical properties, an additional ionic cross-linking reaction was employed using an aqueous solution of $CaCl_2$, because these calcium ions can form a compacted highly ionic cross-linked structure that is a kind of porous ionic cross-linked alginate/chitosan composite with $CaCl_2$ (5”). Finally, novel nerve conduits (NC) (7”) with highly ionic cross-linked alginate/chitosan composite could be obtained (Figure 3). When the concentration of $CaCl_2$ was adjusted, a series of nerve conduits (NC) (7”) with different degrees of ionic cross-linked structures was designed. The different microstructures could be observed by treatment with low and high $CaCl_2$ (aq) concentration. The micrograph of the new nerve conduits with the porous crinkled ionic cross-linked alginate/chitosan composite and low and high $CaCl_2$ (aq) concentration treatment are shown in Figure 4(A). By treating with a low $CaCl_2$ (aq) concentration, relative low cross-linked density and loose structures could be obtained and found in Figure 4(A). By treating with high $CaCl_2$ (aq) concentration, relative high cross-linked density and compacted structures could be found in Figure 4(B). These results would be due to the high ionic cross-linking reaction of the designed nerve conduits with the porous ionic cross-linked alginate/chitosan composite in the $CaCl_2$ (aq) solution with high concentration and ionic strength. When the designed nerve conduits with the porous ionic cross-linked alginate/chitosan composites were treated with $CaCl_2$ (aq) solutions, calcium ions could interpenetrate the porous ionic cross-linked alginate/chitosan structures. Furthermore, additional ionic cross-linking reactions among calcium ions with the anionic groups of alginate would be carried out. Varying $CaCl_2$ (aq) concentrations provided different amounts of free cationic calcium ions and ionic strength. The strong additional ionic cross-linking reaction lashed the original porous structure of the designed nerve conduits, and the compacted structures were constructed from ionic interactions. Comparing the difference of two kinds of scaffolds (NC (5') and NC (7")), the nerve conduit (5’) might be more suitable for nervous regeneration because of its smooth porous structure. In this study, the method for preparing a nerve conduit (7”) could provide a solution to increase nerve conduit structure strength through additional ionic cross-linking reaction.

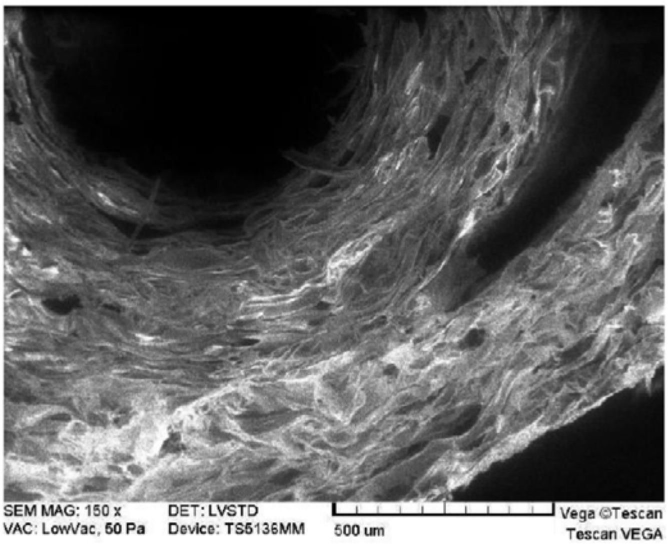


Fig. 2. Micrograph of new designed nerve conduit (5’) with double layers and porous microstructure.

Fig. 3. The morphology of a new designed nerve conduit (7”) with a porous ionic cross-linked alginate/chitosan composite derived from a crinkled ionic cross-linked alginate/chitosan sponge (crinkled ICAS) and additional cross-linking reaction with $CaCl_2$ (Scheme 2).

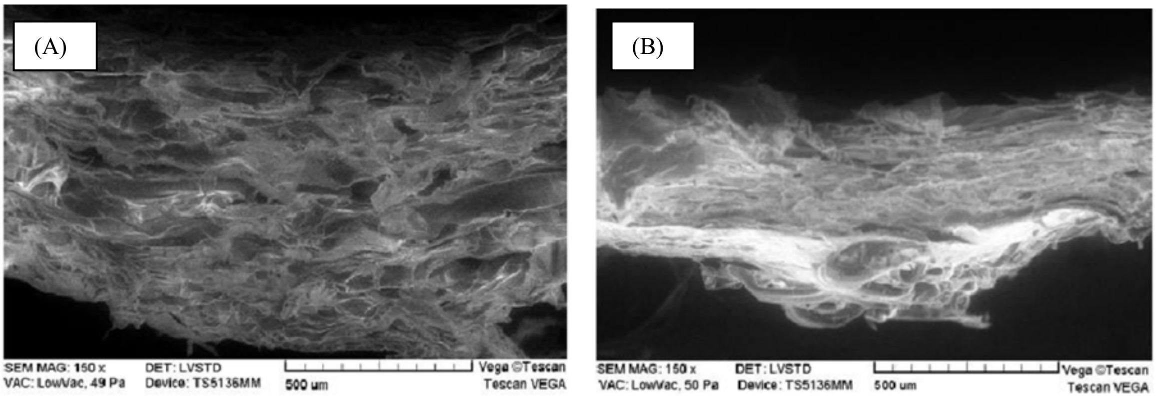


Fig. 4. (A) Micrograph of new designed nerve conduit (7”) with a porous highly ionic cross-linked alginate/chitosan composite treated with low $CaCl_2$ (aq) concentration. (B) Micrograph of new designed nerve conduit (7”) with a porous highly ionic cross-linked alginate/chitosan composite treated with high $CaCl_2$ (aq) concentration.

4. Conclusion

In this study, new designed nerve conduits (NCs) with a porous ionic cross-linked alginate/chitisan composite were successfully prepared for nerve regeneration. We developed a biodegradable nerve conduit made of a hydrophilic complex sponge, which consisted of oppositely charged chitosan and polysaccharides such as alginate. Furthermore, a new fabrication process for the designed nerve conduits was successfully established and developed. NCs composed of various polysaccharides, such as sodium alginate and chitisan, were designed and prepared as potential matrices for tissue engineering via lyophilization. Swelling ability and biocompatibility were served to characterize the NCs. The use of a porous ionic cross-linked alginate/chitisan composite, therefore, appears to allow the formula to manipulate both the mechanical properties and penetration rate properties of the products. Furthermore, a kind of new drug-carrying system with porous ionic cross-linked structure for nerve regeneration was successfully designed and established by incorporating drugs.

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