Advance Journal of Food Science and Technology 5(10): 1318-1321, 2013
DOI:10.19026/ajfst.5.3103
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: May 16, 2013 Accepted: July 05, 2013 Published: October 05, 2013

Research Article

Research on Water Bath Dynamic Cross-linking Properties of Bone Scaffold Made of Gelatin and Sodium Alginate

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Abstract: This study presents a novel water bath dynamic cross-linking method which involves cross-linking process when the bone scaffold is being manufactured. Biomaterial possesses good biocompatibility, but possesses poor mechanical properties. Thus, the uncross-linked bone scaffold usually degrades rapidly, contracts easily and can't meet the requirements of scaffold for tissue engineering. After being cross-linked by Ca^{2+} ions, scaffold made of Gelatin and Sodium Alginate (SA), one kind of gel-type scaffolds, can present much better mechanical properties. However, Ca^{2+} ions can react with SA solution and the gel can limit the flowing of Ca^{2+} ions in the entire system, hence making cross-linking process uneven for the inner part. Applying the novel approach, the designed experiment verifies the scaffold can posses higher porosity, contain more water, has lower degradation rate and better mechanical properties.

Keywords: Cross-linking, Ca^{2+} ions, mechanical properties, water bath dynamic

INTRODUCTION

Collagen, one kind of protein, belongs to high polymer material and is one kind of natural polymers existing in human body. It involves about 25% of all protein in all human tissues. Among these, extracellular matrix possesses the largest portion of the protein. Collagen exists in the form of collagenous fiber and the basic composition unit is tropocollagen molecule. Collagenous fiber is formed by multi-stagely polymerizing tropocollagen molecules. The complexus of structure and function is formed by collagenous fiber and other elements in ECM. This plays an important role in the development, growth, cell differentiation and conglutination, chemotaxis and conjugation reaction process. Gelatin (Bangyao *et al*., 2007; Yutao and Fanglian, 2005; Claire *et al*., 2009) is degenerating product of collagen, due to lack of mechanical strength, It is hard to mould and quick to degrade. Its physical property is unstable. Because of these properties, the gelatin hard to used as host material alone for cultivating bone cell. Thus, during actual process, gelatin together with other materials is used as host material to promote the osteogenic cell as well as improve the mechanical property and deformability.

Sodium Alginate, one kind of natural polysaccharide, is extracted from brown seaweed. it is one kind of Random linear block copolymer and formed by a-L-guluronic acid glucosidic bonding with B-D-mannuronic acid. By chelation cross-linking method, Sodium Alginate together with divalent ion such as Ca^{2+} can form a gel structure which has good elastic property and can be widely used in tissue engineering and medical domain (Xiujuan *et al*., 2008).

Sodium Alginate is one kind of Random linear block copolymer and formed by a-L-guluronic acid glucosidic bonding with B-D-mannuronic acid and has three structural units: MM, MG and GG. Among these, GG structure can easily be boned with Ca^{2+} ions, forming "egg-box" structure. Meanwhile the liquid state is changed into gel state (Feng *et al*., 2006). Because Ca^{2+} ions is one of the required ions for sustaining cells' life, during cross-linking (Wei *et al*., 2011; Hang *et al*., 2008; Michael *et al*., 2012; Wei *et al*., 2012; Hong and Chen, 2007) and solidity process, addition of Ca^{2+} ions can't effect physiological function of cells. The "egg-box" structure provides the channel for trading energy and material with extracellular environment. The mesh structure of bone scaffold at gel molecule level can bear lots of cells.

The purpose of this study is mainly study the effect of water bath dynamic cross-linking method in degradation rate, porosity, water content of bone scaffold made of Gelatin and Sodium Alginate. What is more, as the novel method can effectively solve the problem that Ca^{2+} ions can't move freely within the scaffold because of speedy cross-linking reaction between gelatin and Alginate mixture, thus affect the whole mechanical properties of bone scaffold.

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MATERIALS AND METHODS

Dissolve 2 g gelatin granule into a certain amount of hot-water to form gelatin solution with the concentration of 20%. Dissolve 0.4 g Sodium Alginate into a certain amount cold water and mix uniformly to form Sodium Alginate solution with the concentration of 4%. Mix gelatin solution and Sodium Alginate solution with the ratio of 1:1 and then make the mixed solution even with the aid of magnetic stirrer to obtain gelatin and Sodium Alginate solution. Put solid calcium chloride into a certain amount of water until can't solve to form saturated calcium chloride solution.

Methods:

Making of gelatin and sodium alginate scaffold: After viscosity of the mixed solution reach the requirement; put the material into charging barrel of the experiment device. The gelatin and Sodium Alginate scaffold (Xiaoli *et al*., 2009; Yang, 2008; Yang *et al*., 2012) having three dimensional holes can be manufactured by Rapid Prototyping (Hua *et al*., 2008) experiment device. The device composes of moving modules of X, Y, Z axis, PACRX3i controlling module, pneumatic type of feeding module and Water Bath module. The movement in XY directions and the lifting movement can be realized by utilizing the RX3i controlling module. Pressure source is provided by air pump in the feed module and number of pressure can be regulated by reading the pressure value of pressure transducer to realize the stable and ordered feed velocity. Water bath module composes of a cistern and receiver plate L which can move in the Z direction. The system can effectively accomplish the manufacturing process of scaffold. The experiment device is shown in Fig. 1.

Fig. 1: Experiment device

Experiment process: Firstly, provide air by starting air feeder and making the mixed solution perform according to the predesigned routine, the scaffold can be formed in receiving plate. During the additive forming process, when certain lay number has reached, the receiving plate descends fixed height, making formed scaffold soak into the mixed solution containing Ca^{2+} ions and performed cross-linked reaction. Finally, the timely cross-linked scaffold can be obtained by additive forming process. Process flow diagram is shown in Fig. 2.

Grouping method: The experiment is classified into two groups: the water bath dynamic cross-link group and soaking cross-link group. The water bath dynamic cross-link is adding saturated calcium chloride solution to the Square water bath cistern. During the forming process, L receiving plate descends a fixed height to make the scaffold material cross-linked with calcium chloride. When the process is finished, scaffold can be taken out form L receiving plate. In the soaking cross-link group, the water bath is cleared out and scaffold is formed in L receiving plate directly then the formed scaffold is soaked in the saturated calcium chloride solution for the same time as the first group. After that, take out the scaffold.

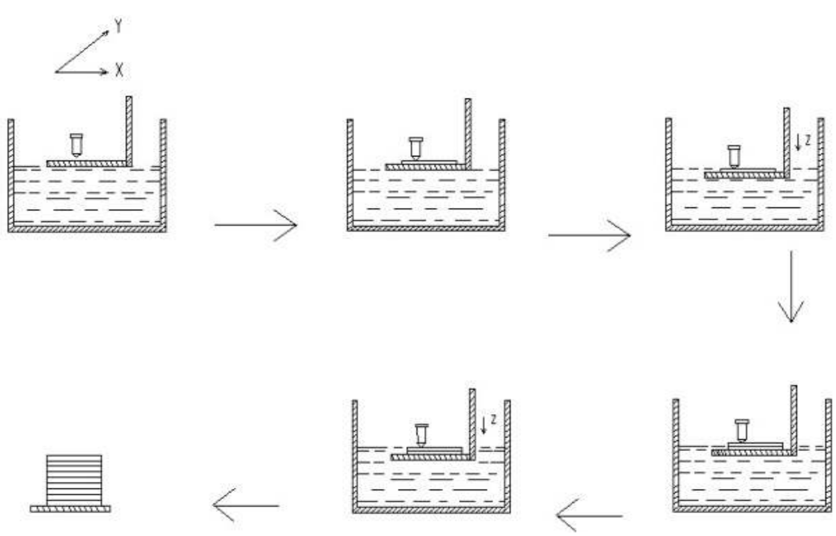

Fig. 2: Process flow diagram

ANALYSIS METHODS

Measure degradation rate Lin *et al*. (2012).record the weight (W0) of material, respectively and put them into PBS solution with 2 mg/mL lysozyme. pH value is set as 7.4 and temperature 35°C Take out the scaffold in the 1^{th}, 2^{th}, 3^{th}, 4^{th} week, respectively and repeatedly wash scaffolds with distilled water. Measure the weight after freeze drying. The degradation rate is calculated as (W0-W1)/W0×100%.

Measure porosity Xuming *et al*. (2007) put scaffold material with fixed volume (V0) and weight (W0) into absolute ethyl alcohol until it is saturated. Take out scaffold and wipe out solution and record the Weight (W1). The density of absolute ethyl alcohol is ρ. Porosity is calculating as (W1-W0) ρ/V0×100%.

Measure water content Mingbing *et al*. (2012) put scaffold material into PBS solution and soaked for 24 h and record the weight (W0). Record weight (W1) after the material be freeze dried. Water content is calculate as (W0-W1)/W0×100%.

RESULTS AND DISCUSSION

The Scaffold Made of Gelatin and Sodium Alginate is shown in Fig. 3, we can see that the scaffold present porous structure, pore size is around 200-300 um and the size is conducive to cell adhesion, gelatin and sodium alginate as gel material are well to form bone scaffold.

Degradation rate in vitro: Measure the degradation rate of the two-group scaffolds after 1, 2, 3 and 4 weeks, respectively. The result shows that data from soaked group is larger than the water bath dynamic group (Fig. 4). From the first week the degradation rate of soaking group is (21.8±1.6)% and the water bath dynamic is (12.3±1.8)%, after 4 weeks, degradation rate in water bath dynamic group is (38.3±1.54)% and the other is (57.7±1.2)%.

Table 1 show that porosity from the soaked group is 90.21±0.49 and the water bath dynamic group 91.23±0.28. Experiment shows that scaffold with large number of porosity are more in favor of the cells growth.

The water content in the water bath dynamic is 87±1.12 and soaked group 80±0.85. The result show that water bath dynamic group content more water than the soaked group.

Currently, materials meeting requirement of tissue engineering can be classified into two categories (Lin *et al*., 2012):

- Natural materials, such as collagen, gelatin, hyaluronic acid, chitosan and alginate.
- Artificial synthetic material, such as polylactic acid and hydroxyapatite. Because of good biocompatibility, degradability, convenience and wide use, most researcher pick up natural materials for tissue engineering scaffold. However, how to overcome the deficiency that natural material has poor mechanical properties has become the research focus on this field. Now, the most widely used method is utilizing cross-linking approach to acquire better mechanical properties. By traditional method, the scaffold is soaked in cross-linking solution after the scaffold is made. Because Ca^{2+} ions can easily perform cross-linking reaction and gelling with Sodium Alginate, but gelling process limit the flow of Ca^{2+} ions within the scaffold, these can make internal structure of scaffold uneven. Compared with traditional method, the presented method have better anti-degradation property and better mechanical properties, moreover, the internal structure of scaffold is more even.

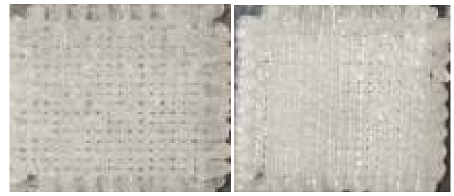

Fig. 3: Scaffold made of gelatin and sodium alginate

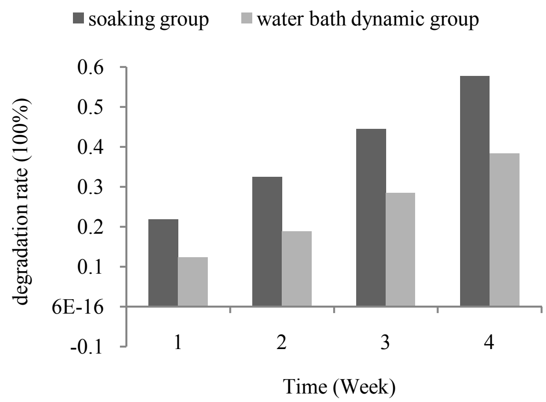

Fig. 4: The degradation rate comparison between water bath dynamic group and soaked group after 1 week, 2 weeks, 3 weeks and 4 weeks, respectively

Table 1: Porosity and water content for the 2 groups

	Porosity	Water content
Water bath dynamic	91.23±0.28	87±1.12
Soaking	90.21±0.49	80±0.85

Utilizing the presented method, the scaffold material can be quickly solidified during forming process and the three-dimensional pore structure can be preserved, thus improving the entire mechanical property of scaffold. Meanwhile, Ca^{2+} ions can flow more sufficiently because of L receiving plate descends in the water bath. Thus, break through the limit of Ca^{2+} ions flow because of the gelling of scaffold material. So, making the Ca^{2+} ions get into the internal structure of scaffold and cross-linking more even in the entire scaffold.

CONCLUSION

With the water bath dynamic cross-linking method, real-time cross-linking for gelatin and Sodium Alginate mixture can be realized. The scaffold made by this method possesses more advantages in degradation, porosity and moisture content. Moreover, this method can effectively solve the problem that Ca^{2+} ions can't move freely within the scaffold because of speedy cross-linking reaction between gelatin and Alginate mixture, thus making the cross-linking of scaffold more even. The entire mechanical properties can be improved, thus effectively solve the defect that natural biological material possesses insufficient mechanical properties.

ACKNOWLEDGMENT

The authors thank the National Natural Science Foundation of China (No.51075253, No.51105239), Rapid Manufacturing Engineering Center of Shanghai University for support.

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