Fabrication of Scaffold based on Chitosan – Poly-2-Acrylamido-2-Methylpropane Sulfonic Acid (PAMPS) Polyelectrolyte Complexes

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Abstract. Chitosan is considered as one of the best biopolymers for bone tissue engineering application due to its appealing properties such as biocompatible, non-toxic, non-immunogenic, able to accelerate growth rate of bone tissue, possess antibacterial activity and able to form porous structure. Herein, polyelectrolyte complexes (PECs) prepared by simple mixing of chitosan solution and poly-2-acrylamido-2-methylpropane sulfonic acid (PAMPS) solution were investigated. In this study, chitosan concentration were varied from 1.0 to 2.0% (w/v), while concentration of AMPS was fixed at 0.1% (w/v) to know the influence of chitosan concentration on swelling capacity and mechanical properties of obtained PECs. Swelling test and compressive test results showed that the most stable PECs in aqueous environment with highest ultimate compressive strength was PECs obtained from chitosan 2.0% (w/v) – PAMPS 0.1% (w/v) with swelling capacity 3,326% for 2 hours and ultimate compressive strength 1.22 kPa.

Keywords: cancellous bone implant, chitosan, poly-2-acrylamido-2-methylpropane sulfonic acid, polyelectrolytes complexes, tissue engineering.

1. Introduction
Recently, bone fractures cases raise significantly due to higher number of road traffic accidents and bone weakening disease such as osteoporosis. Consequently, the demand of human bone implant will also increase in the following trend as the organ donor shortage.¹ However, the recent bone implant mainly focus on replacing hard and dense tissue of bone, namely cortical bone. While bone implant to replace sponge and soft tissue, namely cancellous bone, is still limited. To fulfil this demand, new materials with high porosity, similar mechanical strength (σu compressive = 1 - 12 MPa),² induce cell-attachment and immune-acceptance is needed.
One of attractive materials to overcome this challenge is chitosan because of its appealing properties, such as biocompatible, non-toxic, non-immunogenic and has good cell attachment. However, chitosan has some disadvantages when being used for physical support because it is very stiff, brittle and low mechanical resistance ($\sigma_{\text{compressive}}$ (2% w/v, dry) = 0.967 MPa).\textsuperscript{2,5} Besides, chitosan is also unstable in the porous form that it cannot maintain its shape that will disturb the tissue regeneration and make the scaffold difficult to function as the support.

In order to optimize the mechanical properties and stability of chitosan scaffold, crosslinking agents are used to bond the polymeric chains to create a 3D network in order to enhance the mechanical properties. Even so, most of crosslinking agents are toxic to human body thus it is not safe.\textsuperscript{6} The other way to stabilize the chitosan scaffold is pairing the cationic chitosan with negatively charge biopolymer hence they are only bonded by electrostatic interaction to form polyelectrolyte complexes (PECs) which results a non-toxic bond. The electrostatic interaction in polyelectrolyte complexes also might enhance the mechanical properties and possess better stability of chitosan. One of known negatively charged polymers is poly-(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS), which is a synthetic biocompatible polymer that has distinctive behaviour in complement system in order to delay the hyperacute rejection.\textsuperscript{7} Previously, Zhang et al has been prepared chitosan-PAMPS based PECs using a polymer-monomer pair reaction system.\textsuperscript{8} In this research, chitosan-PAMPS based PECs was prepared by simple mixing of PAMPS solution with chitosan solution. This method might offer easier and faster strategy to obtain chitosan-PAMPS based PECs, because it can be prepared directly from their polymers solution. It is expected that mechanical strength of chitosan based scaffold could be elevated by formation of PECs that is useful for bone implant application.

2. Experimental Section

2.1 Materials and Raw Materials Characterizations

Chitosan was purchased from CV. Biochitosan Indonesia. Before used, chitosan was characterized by FTIR in order to determine the degree of deacetylation (DD) using baseline method.\textsuperscript{9,10} The determination of DD is important to ensure whether purchased chitosan can be classified as chitosan with DD above 70%.\textsuperscript{11} FTIR measurements were recorded with KBr pellets on Prestige 21 Shimadzu. The spectra were measured at wavelength 4500 to 400 cm\textsuperscript{-1}. After the FTIR spectra were collected, DD of sample was calculated using Eq. 2.1:

$$DD[\%] = 100 - \frac{A_{1655}}{A_{3450}} \times 115$$

(2.1)

2.2 Preparation and Characterization of CS/PAMPS PECs

CS/PAMPS PECs were prepared by simple mixing of 0.1% (w/v) PAMPS in 150 mM sodium chloride solution and CS in 2% acetic acid solution with various concentration (1.0%, 1.5%, 2.0% (w/v)). Briefly, 50 mL CS solution was added to 50 mL PAMPS solution using dropper pipette while the solution was stirred at 300 rpm for two hours. The obtained samples were freeze dried at -50 °C overnight to obtain dried samples without destroying their structures.

Morphologies of obtained samples were characterized using SEM Hitachi SU3500. Samples were gold coated and measured at acceleration voltage between 3 – 10 kV. Mechanical properties of obtained samples were determined by performing compression test using Tensilon®. Dimension of freeze dried samples with cylindrical shape were measured by caliper. The load cell was 10 kN with test speed 1 mm/min.

Water uptake properties of obtained samples were determined by performing swelling test on four specimens of each freeze dried samples. After measuring the dry weight of test specimens ($W_0$), they were immersed in SBF (pH 7.1 at 37 °C) at room temperature for predetermined time intervals, and wet weights ($W_w$) were determined at these time intervals after removing excess surface water by tissue paper. Degree of swelling of specimens were calculated using Eq. 2.2:
3. Results and Discussion

3.1 FTIR result
To ensure that purchased chitosan can be classified as chitosan that was defined with their DD should be above 70%, purchased chitosan was characterized using FTIR method and FTIR spectra of sample can be seen in Fig. 1. As can be seen in Fig. 1, FTIR spectra of sample of purchased chitosan is associated to the chitosan with the characteristic intense absorption bands at ~3400 cm\(^{-1}\) that indicates presence of the vibrate stretching band of O-H functional groups and/or secondary band for N-H groups, as well as to intermolecular hydrogen bonding within the polyssacharide. The FTIR spectrum of chitosan itself showed some features of amide groups: amide I and amide II bands at 1656 and 1600 cm\(^{-1}\), respectively. Thus, these spectrum peaks showed the distinctive functional groups in chitosan, which is secondary amide.\(^9,10,12,13\)

Based on baseline method, DD of sample was 77% using Eq. 2.1. Considering that DD of sample is above 70%, it can be claimed that purchased chitosan is classified as chitosan and can be used for this experiment. DD is above 70% means the deacetylated D-glucosamine in the sample is more dominant than the acetylated glucosamine, which is important to obtain chitosan with positive charge (polycations).

3.2 Visual and SEM observation
Visual observation of obtained freeze dried sample (Fig. 2) showed that bulkier and denser sample were observed as chitosan concentration increased. By increasing chitosan concentration, solute content increase and the amount of solvent to be sublimed decrease, lead to formation of bulkier and denser sample after freeze drying process.
Further observation of sample morphologies were done by SEM observation (Fig. 3). As can be seen in Fig. 3, sample with chitosan concentration 1.0% (Fig. 3a) showed loose structure, while sample with chitosan concentration 1.5% and 2.0% (Fig. 3b and 3c respectively) showed more compact structure. This compact structure is caused by the presence of excessive chitosan, which possesses rigid structure, that lead to the bigger remaining solute in solution that was not carried away during freeze drying process.

3.3 Swelling capacity
The swelling test was performed to examine the stability of freeze dried PECs in aqueous environment. After soaked in SBF for such intervals, it was found that PECs sample with chitosan concentration 1.0% could not maintain its shape and already swollen in 1 hour. On the other hand, PECs samples with chitosan concentration 1.5% and 2.0% could maintain their shape while absorbing water. After 2 hours, no more weight gaining were observed at those two samples. Thus, it could be said that the swelling maximum capacity of those samples reached in 2 hours. Swelling capacity of PECs sample with chitosan concentration 2.0% (3,332%) is higher than PECs sample with chitosan concentration 1.5% (2,823%). Therefore, it can be said that the higher the chitosan concentration in the mixture, the bigger the swelling capacity of scaffold could get.
3.4 Compressive Test
Mechanical properties of obtained samples were examined by compressive test and the results were presented in Fig. 5. As can be seen in Fig. 5, the ultimate compressive strength of the PECs samples increase as chitosan concentration increase. This trend happens due to the denser structure as the chitosan concentration increase (that were revealed by SEM observation), leading to the more strength when being compressed. Even though ultimate compressive strength of PECs sample with chitosan concentration 2.0% (1.22 kPa) still far from ultimate compressive strength of cancellous bone (1 – 12 MPa), but PECs sample with chitosan concentration 2.0% showed higher ultimate compressive strength than sample of pure chitosan 2.0% (0.73 kPa), which means the PECs form is successfully enhance the mechanical strength of chitosan scaffold.

Figure 4. Swelling capacity of freeze drying sample with chitosan concentration 1.5% and 2.0% in aqueous environment

Figure 5. Ultimate compressive strength of pure chitosan 2.0% compared with PECs samples with chitosan concentration 1.0%, 1.5% and 2.0% respectively
4. Conclusion
Scaffold based on chitosan-PAMPS PECs has been successfully prepared using simple mixing method directly from their polymer solution. Based on swelling test and compressive test, PECs consist of chitosan 2.0% (w/v) and PAMPS 0.1% (w/v) shown the optimum stability in SBF medium amongst all PECs and highest ultimate compressive strength. Also, formation of PECs between chitosan and PAMPS is successfully enhance mechanical strength of chitosan scaffold, which is attractive initial information for future application of this material for bone implant scaffold.

5. References
[1] Amini, A. R., Laurencin, C. T., Nukavarapu, S. P. 2012 Critical Reviews™ in Biomedical Engineering, 40 363 – 408
[2] Levengood, S. L., Zhang, M., 2014 Journal of Materials Chemistry B, 21 3161–3184
[3] Li, Z., Ramay, H. R., Hauch, K. D., Xiao, D., Zhang, M., 2005 Biomaterials 26 3919-3928.
[4] Venkatesan, J., Kim, S.-K. 2010 Marine drugs 8 2252-2266
[5] Han, J., Zhou, Z., Yin, R., Yang, D., Nie, J., 2010 International Journal of Biological Macromolecules 46 199-205
[6] Niknejad, H., Mahmoudzadeh, R. 2015 Iranian Journal of Pharmaceuticals Research, 14 385 – 394
[7] Setoyama, H., Murakami, Y., Inoue, K., Iwata, H., Kitamura, H., Shimada, T., Kaji, H., Ikada, Y., Imamura, M. 1999 Transplantation Proceedings 31 2818-2822
[8] Zhang, L., Wang, J., Ni, C., Zhang, Y., Shi, G. 2016 Materials Science and Engineering C, 58 724 – 729
[9] Baxter, A., Dillon, M., Taylor, K. D. A., Roberts, G. A. F., 1992 International Journal of Biological Macromolecules 14 166 – 169
[10] Kasaa, M. R. 2008 Carbohydrate Polymers71 497 – 508
[11] Rodríguez-Vázquez, M., Vega-Ruiz, B., Ramos-Zuniga, R., Saldana-Koppel, D. A., Quinones-Overa, L. F. 2015 BioMed Research International 2015 821279
[12] Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Arguelles-Monal, W., Desbrieres, J., Rinaudo, M 2001 Polymer 42 3569 – 3580
[13] Sun, T., Xu, P. X., Liu, Q., Xue, J. A., Xie, W. 2003 European Polymer Journal 39 189-192

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