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Fabrication of cellulose based scaffolds for bone regeneration application

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Abstract : Carboxymethyl cellulose, a water-soluble cellulose derivative, has been used extensively as a biomaterial for wound healing and pharmacological applications. It is a biocompatible and biodegradable polymer but its poor stability, limits its long-term application. In this study, carboxymethyl cellulose scaffolds were prepared at different concentrations (2.5 wt% and 5.0 wt%) by freeze drying method and its structural stability was induced by adding crosslinkers such as citric acid and fumaric acid. The stability of the scaffolds was assessed in phosphate buffered saline (PBS) by soaking for 24 hours. The pH of PBS remains stable for fumaric acid crosslinked scaffolds compared to citric acid crosslinked scaffolds. The cytotoxicity of the scaffolds was assessed by Saos-2 osteoblast cells. The 2.5wt% CMC crosslinked scaffolds showed better cellular activity compared to other crosslinked scaffolds. This study showed that the addition of crosslinkers has improved the stability of scaffolds without affecting its biocompatibility.
Keywords : Carboxymethyl cellulose, scaffolds, crosslinkers, freeze-drying.

1. Introduction

Biopolymers are a separate domain of biomaterials with excellent properties of biocompatibility and biodegradability. Various natural polymers like starch, cellulose, collagen, chitosan and many others have been used widely in medical applications. Despite their advantages of being cytocompatible and biodegradable, they are limited by their inadequate mechanical properties and poor stability *invivo*[1]. Hence, these polymers are either crosslinked or blended with other natural or synthetic polymers to improve their strength and stability for desired applications.

Carboxymethyl cellulose (CMC), an ether derivative of cellulose is a well-known biopolymer, synthesized by the alkali-catalyzed reaction of cellulose withchloroacetic acid[2,3]. It has an amphiphilic characteristic of having both hydrophobic and hydrophilic groups [4]. It is a very peculiar polymer among the cellulose derivatives, due to its polyelectrolytic nature and sensitivity to pH, temperature and ionic strength variation[6-8].Moreover, it is biocompatible, biodegradable, low immunogenic, non-toxic, physiologically harmless and also possesses good viscosity. It also has appreciable water-bonding capacity and rheological properties with abundant availability[2-4]. CMC can be fabricated in differentforms like membranes, wafers,

hydrogels, hydrofibres and scaffolds to meet its various applicationsas wound dressing material, drug-delivery system, trans-dermal systems, injectable polymeric systems and implants[2,3,9,10].

Like other natural polymers, the hydrophilic and water soluble property ofCMC [2,4,5]tends to decrease its mechanical strength and stability *in vivo*[11]. Therefore, it is required to improve the properties of CMC without affecting its biocompatibility in order to explore its application in bone regeneration and tissue engineering. In this study, CMC scaffolds were fabricated by freeze drying method with citric acid and fumaric acid as crosslinkers. The*in vitro* stability of the scaffolds in PBS was investigated and the biocompatibility of the scaffolds was assessed through *in vitro* cytotoxicity tests.

2. Experimental

2.1. Materials

Sodium carboxymethyl cellulose (viscosity - 1500-3000 cP), citric acid and fumaric acid were purchased from Himedia, India. Sodium chloride, potassium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate, obtained from Himedia, India, were used to prepare phosphate buffered saline (PBS). α-modified Eagle's medium (α-MEM), fetal calf serum (FCS), penicillin and streptomycin were purchased from Gibco, USA.

2.2. Fabricationof scaffolds

Scaffolds were prepared by freeze-drying method using different concentrations of CMC solution (2.5wt% and 5.0wt%) (named as C01 and C02, respectively). To stabilize the scaffolds, required amount of citric acid and fumaric acid were added to the CMC solution and scaffolds were prepared (named as C01CA, C01FA, C02CA and C02FA).

2.3. *In vitro* stability test

The stability of the scaffolds in phosphate buffered saline (PBS), pH 7.4was observed for 24 hours at 37^{o}C. The change in pH of the buffer solution was measured at different time periodsand the test was performed in triplicates.

2.4. Cytotoxicity test

Human osteoblast-like Saos-2 cells obtained from American Type Culture Collection, USA (ATCC® HTB-85™),were cultured in α-modified Eagle's medium (α-MEM) supplemented with 10% fetal calf serum (FCS) and 2% penicillin-streptomycin and maintained in an incubator at 37^{o}C with 5% carbondioxide. Cells collected from subculturing at third passage were used for the experiments.Sterilized scaffolds were seeded with a density of 2.5x10^{4}cells per scaffold after pre-wetting the sample. About 1 ml of media was added for each scaffold and incubated. The medium was changed twice a week and the test was performed in duplicates. Cell proliferation on the scaffolds were assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) assay on day 4 and 7. The MTT solution reacted with the metabolically active cells forming a purple color for which the absorbance was measured at 570 nm using an ELISA microplate reader.

3. Results and Discussion

Crosslinked and non-crosslinked polymer scaffolds are prepared by freeze-drying method. The scaffolds appear as white, soft and spongy material after the freeze-drying process.The C01CA scaffold reduced in size by 50% in two days and the other scaffolds remain unchanged in dimension during storage in an air-tight containeras shown in Figure 1. The samples were processed for further characterization.

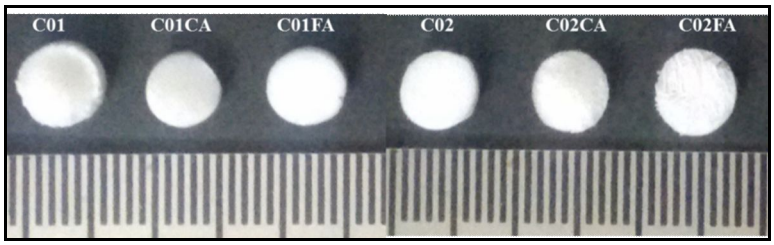

Figure.1: Photographs of polymer scaffolds prepared by freeze-drying method.

As reported, the stability of the CMC is poor*in vivo*[11],the crosslinked CMC scaffolds are tested for its stability in *in vitro* physiological fluid condition. The best possible ionic solution to consider for stability and swelling was phosphate buffered saline (PBS). Therefore, the stability of the CMC scaffolds was assessed in PBS and the change in pH was measured at different time periods. The non-crosslinked scaffolds swelled and disintegrated in PBS within the initial 3 hours whereas the crosslinked scaffolds remained stable till 24 hours in PBS. The fumaric acid crosslinked scaffolds did not react with PBS solution and hence the pH of the buffer remained stable over a period of 24 hours. The initial drop of 0.1 was measured within 30 minutes of dipping the sample in PBS, due to the surface reaction of the spongy scaffolds with PBS, as shown in Figure 2. Whereas the citric acid crosslinked scaffolds, reacted with PBS, as seen from the decrease of pH by 0.5 initially within 30 minutes and stabilizing further to pH of 6.8. This is due to the acidic environment on the surface of the scaffolds resulting from the unreacted citric acid in the scaffold. Thus, the crosslinked scaffolds prepared with improved stability are taken for the cytotoxicity study and the unstable non-crosslinked scaffolds were not used in the further study.

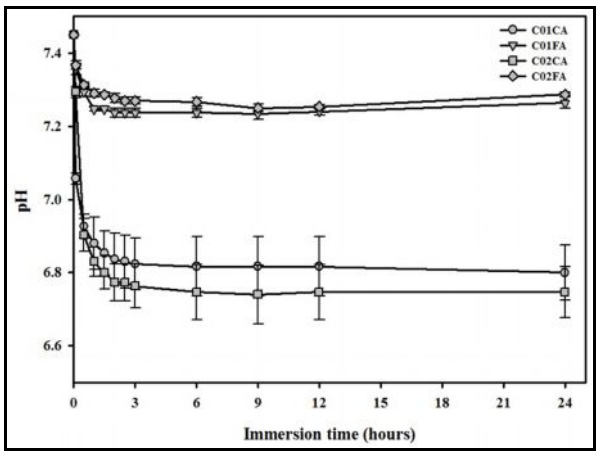

Figure.2: *In vitro* stability test in PBS and variation of pH over time.

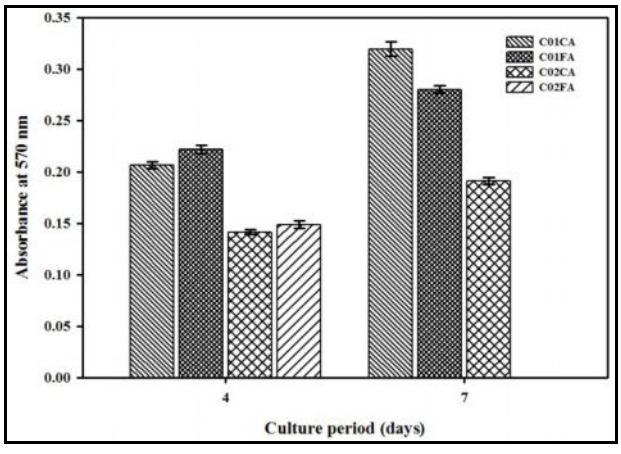

Figure.3: Absorption of active cells measured at 570 nm assessed by MTT assay in the scaffolds seeded with Saos-2 cells.

The cytotoxicity of the crosslinked scaffolds was assessed onSaos-2 cells. The cell viability was measured by MTT assay after 4 and 7 days of culture. The dark purple crystals of formazan seen on the cell-scaffold construct indicate the presence of metabolically active cells. The cells are viable and proliferating as observed by quantifying the absorbance at 570 nm as shown in Figure 3.Only the C02FA scaffold was unstable after 4 days and the other scaffolds were found to be stable and showed good cell proliferation with a higher proliferation rate on C01CA scaffold. These observations suggest that the crosslinked scaffolds are non-toxic and cytocompatible.

4. Conclusion

Cellulose-based scaffolds were successfully prepared by freeze-drying method. The study shows that the crosslinkers, citric acid and fumaric acid has increased the stability of the CMC scaffolds during storage and in PBS. These scaffolds werealso found to be non-toxic to the cells and could be further utilized for *in vivo* application.

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References

1. Reddy N., Reddy R and Jiang Q., Crosslinking biopolymers for biomedical applications,*Trends Biotechnol.*, 2015, 33;362–369.
2. Ramli NA and Wong TW., Sodium carboxymethylcellulose scaffolds and their physicochemical effects on partial thickness wound healing,*Int. J. Pharm.*, 2011, 403; 73–82.
3. Kono H., Characterization and properties of carboxymethyl cellulose hydrogels crosslinked by polyethylene glycol,*Carbohydr. Polym.*, 2014, 106; 84–93.
4. Su J-F., Huang Z., Yuan X-Y., Wang X-Y and Li M., Structure and properties of carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by Maillard reactions,*Carbohydr. Polym.*, 2010, 79; 145–153.
5. Basu P., Sharan BS., Kumar UN and Manjubala I., Polymer ceramic composite for bone regeneration application, *Int. J. ChemTech Res*., 2014, 6; 4038-4041.
6. Gorgieva S and Kokol V., Synthesis and application of new temperature-responsive hydrogels based on carboxymethyl and hydroxyethyl cellulose derivatives for the functional finishing of cotton knitwear,*Carbohydr. Polym.*, 2011, 85; 664–673.
7. Akar E., Altınışık A and Seki Y., Preparation of pH- and ionic-strength responsive biodegradable fumaric acid crosslinked carboxymethyl cellulose,*Carbohydr. Polym.*, 2012, 90; 1634–1641.
8. Sannino A., Demitri C and Madaghiele M., Biodegradable Cellulose-based Hydrogels: Design and Applications,*Materials.*, 2009, 2; 353–373.
9. Jiang L., Li Y., Wang X., Zhang L., Wen J and Gong M., Preparation and properties of nano-hydroxyapatite/chitosan/carboxymethyl cellulose composite scaffold,*Carbohydr. Polym.*, 2008, 74; 680–684.
10. Ohta S., Nishiyama T., Sakoda M., Machioka K., Fuke M., Ichimura S., Inagaki F., Shimizu A.,Hasegawa K., Kokudo N., Kaneko M., Yatomi Y and Ito T., Development of carboxymethyl cellulose nonwoven sheet as a novel hemostatic agent,*J. Biosci. Bioeng.*, 2015, 119; 718–723.
11. Varma DM., Gold GT., Taub PJ and Nicoll SB., Injectable carboxymethylcellulose hydrogels for soft tissue filler applications,*Acta Biomater.*, 2014, 10; 4996–5004.

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