Preparation of hydroxyapatite/gelatine scaffolds crosslinke by Glutaraldehyde

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Statement of Purpose: Calcified tissue, such as long bone and jaw bone is considered a biologically and chemically bonded composite between HA (HA: $Ca_{10}(PO_4)_6(OH)_2$) and type-I collagen. Gelatins are compositionally virtually identical to the collagen from which they are derived. Therefore In this study, to mimic the mineral and organic component of natural bone, hydroxapatite[HA] and gelatin[GEL] composite scaffolds were prepared using solvent-casting method combined with freeze drying process. Glutaraldehyde [GA] was used as cross linking agent in the making of gelatin-tricalcium phosphate composites rendering them no longer water soluble but since GA is cytotoxic the bisulfite sodium was used as excess GA discharger. Prepared composite scaffold of HA and gelatin is expected to show increased biodegradation together with sufficient mechanical strength.

Methods: The slurry composites were prepared using solvent casting method. Definite GEL concentration was dissolved in deionized water [DI] at temperature $45\,^{\circ}C$. Desire volumetric content (30wt%, 40wt% and 50wt%) of fine HA particles relative to gelatin were added. The reinforced slurry composite was then heat treated on magnet stirrer under constant mixing for 1h at $45\,^{\circ}C$. The slurry was deagglomerated by magnet stirring meanwhile the temperature was monitored continuously. Then to avoid air bubbles the slurry immediately was injected by using a syringe into cylindrical Teflon molds. The molds were frozen at $-70\,^{\circ}C$ and were dried in a commercial freeze-dryer for 6h for solvent (DI) removal. After that, the white composites were removed and placed in room temperature for 24 h, they were immersed in a 8% solution of GA for 3 h then. To remove the residues of GA agent, the cylinders were washed with DI for 24 h, during which time the water was changed every 6 h. Besides, the sodium bisulfite was used to discharge the excess GA.

Results / Discussion: FT-IR spectroscopy was used to estimate the conformational change of the HA/GEL composite structure. FT-IR spectrum for the cross-linked HA-GEL composite indicates chemical bond formation between carboxyl ions in GEL and HA phases. The compressive strength, Young's modulus and elongation of composites were measured with an Instron materials testing machine. The compressive modulus of HA-GEL scaffolds increased with HA content. It was found that the mechanical properties of GEL/HA with ratio of 50wt% was similar to that of trabecular bone. Water absorption of HA-GEL composites with different HA content were studied to evaluate the effect of HA content on the size and stability of material. The water absorption of composites reduced with HA content. A liquid displacement method was used to measure the porosity and density of HA scaffolds. .It was found that the addition of HA results in more dense and thicker pore walls with lower porosity The morphology and microstructure of the scaffolds were examined using SEM and light microscopy. The scaffold prepared has an open, interconnected porous structure with a pore size of 80-400 μm. The biological responses of scaffolds carried out by L929 fibroblast cell culture. Cells exhibited rather good proliferation and partially covered the composite surface after 48h.

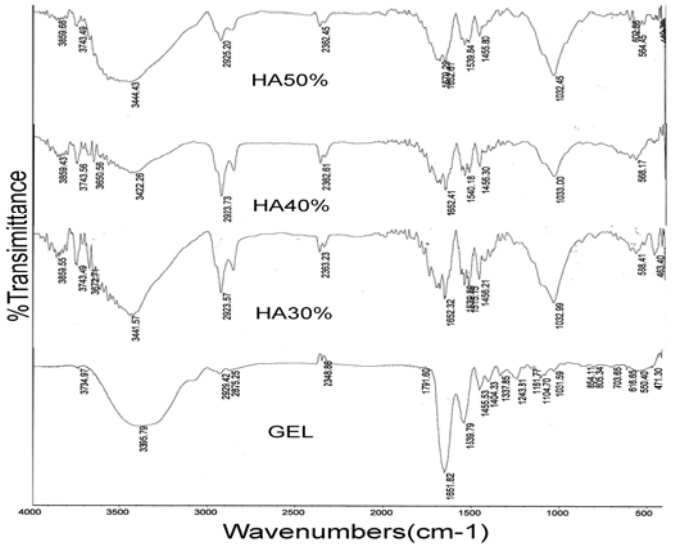

Fig. 1. FT-IR spectra for GEL and 30wt%, 40wt%, 50wt% cross-linked HA-GEL composites

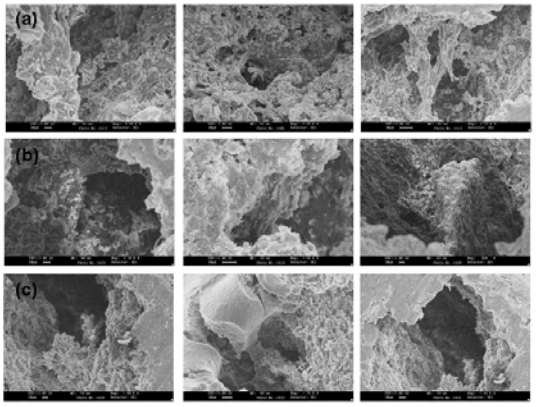

Fig.2. SEM micrographs of the pores in the cross-section of different HA-GEL scaffolds (a)HA30% (b) HA40% (c)HA50%

Conclusions: In this work a method of producing three dimensional, open-cell, composite scaffold of HA-GEL has been developed. The technique involves solvent-casting method combined with freeze drying process which has the advantages of both the methods. It was found that the properties prepared HA are close to that of trabecular bone.

References:

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Etc.