

Seminar Title : Development of a sodium alginate/silk fibroin based three-dimensional printed nano- composite scaffold for bone tissue regeneration

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Abstract : The present work aims to develop a sodium alginate (SA) and silk fibroin (SF) based polymeric nano-composite scaffold with appropriate design and properties mimicking the natural extracellular bone matrix by 3D printing technique. SA/SF polymer blends prepared with different volume ratios were printed by a 3D printer, thereby 3D microfibrillar open pore structures with definite pore size range and pore interconnectivity were fabricated. Fourier transform infrared (FTIR) spectroscopic study disclosed the substantial intermolecular hydrogen bonding between SA and SF, favorable for cell adhesion and proliferation. The amorphous, and hydrophilic nature of the scaffolds were proven by X-ray diffraction (XRD) analysis and measured contact angles. Among them, SA₇₀/SF₃₀ scaffold showed higher tensile strength and protein adsorption ability, desired swelling and degradation behavior than the other scaffolds. *In-vitro* biomineralization study confirmed their bioactivity with comparatively higher apatite deposition shown by SA₇₀/SF₃₀ and SA₆₀/SF₄₀. However, SA₇₀/SF₃₀ scaffold exhibited superior cell supportive characteristics exhibiting higher human fibroblast-like osteosarcoma cells (MG-63) adhesion, proliferation and viability and hence was selected for further study.

The mechanical strength and biological properties of the SA₇₀/SF₃₀ scaffold were improved by adding 2-5%(w/v) polyvinyl alcohol(PVA). The fabricated SA₇₀/SF₃₀/PVA scaffold was structurally stable with distinct individual fiber formation and intact layer-by-layer fiber deposition possessing pore size ranging 818-830 μ m, amorphous, and hydrophilic. An enhanced protein adsorption and apatite layer synthesis represented their higher cell adhesion and bone tissue regeneration abilities. The scaffolds promoted cell attachment, viability and proliferation. However, SA₇₀/SF₃₀ scaffold with 3wt% PVA (SA₇₀/SF₃₀/PVA₃) showed superior hydrophilicity, controlled swelling, degradation, protein adsorption and cellular activities than other scaffolds and thus, was proven to be more potential for bone tissue regeneration.

The osteogenic property of the SA₇₀/SF₃₀/PVA₃ scaffold was further improved by reinforcing nano-hydroxyapatite (n-HA) in different concentration (0.5%-1.5 %w/v) into the polymeric network. The resulting composite bioinks were used to fabricate 3D printed SA₇₀/SF₃₀/PVA₃/n-HA scaffolds with desired pore size range of 321-454 μ m with pore interconnectivity. The scaffolds were amorphous and hydrophilic in nature. Among the scaffolds, SA₇₀/SF₃₀/PVA₃ containing 1.5%(w/v)n-HA representing as SA₇₀/SF₃₀/PVA₃/n-HA_{1.5} have controlled swelling and degradation, and enhanced protein adsorption. The compressive strength was enhanced with increase of n-HA measuring in the range 0.083 -0.193 MPa, the highest strength was shown by SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}. The scaffold is cytocompatible as confirmed by *in vitro* cell study using bone osteosarcoma cells (HOS). The osteogenic property was proven by alkaline phosphatase (ALP) activity. Thus SA₇₀/SF₃₀/PVA₃/n-HA_{1.5} was proven to be a suitable substrate for BTE.

The mechanical and osteogenic properties of the SA₇₀/SF₃₀/PVA₃/n-HA_{1.5} scaffold was further enhanced by reinforcing 0.5wt% and 1 wt% magnesium oxide nano-particles (n-MgO) with particle size 44-75 nm. The fabricated scaffolds were designated as SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO_{0.5} and SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO₁ possessing microfibrillar porous network with desired pore size ranging 444.86 \pm 42.01 μ m - 496.30 \pm 95.09 μ m, hydrophilic and amorphous. The scaffolds exhibited controlled swelling and degradation rate. The tensile strength measuring 4.09 \pm 1.13 MPa and 5.08 \pm 1.14 MPa for SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO_{0.5} and SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO₁ was remarkably enhanced upon reinforcing n-MgO. The protein adsorption, and biomineralisation study confirmed their cell adhesion and bioactivity. However, SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO₁ possess superior physico-chemical and mechanical properties than SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO_{0.5} scaffold. *in vitro* cell study have shown its cytocompatible and cell adhesion properties. The upregulation of ALP activity indicated that the scaffold may support osteogenic differentiation compared. Overall, the study demonstrated that the developed 3D printed SA₇₀/SF₃₀/PVA₃/n-HA_{1.5}/n-MgO₁ scaffold may serve as a prospective template for bone tissue regeneration in future.

Keywords: Scaffold Bone tissue engineering, Sodium alginate Silk fibroin Polyvinylalcohol Nano-hydroxyapatite Nano-magnesium oxide 3D printing.