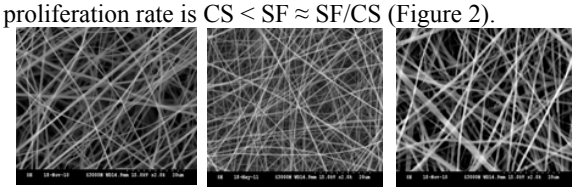
Composite Chitosan/Silk Fibroin Nanofibers for Osteogenic Differentiation of Human Mesenchymal Stem Cells

Guo-Jyun Lai, Jyh-Ping Chen.
Department of Chemical and Materials Engineering, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan, 333, ROC.

Introduction: Eletrospinning (ES) has enabled the engineering of nanostructured materials to meet current challenges in bone tissue engineering. Because of their intriguing characteristics such as large surface area and mimic the extracellular matrices (ECMs) structure of native tissue, electrospun polymeric nanofibers have been found to be a suitable scaffold for bone tissue engineering. The nanofibrous scaffolds provide a respectable substrate for the attachment and proliferation of cells. Recent findings have proposed that nanofibrous scaffolds favor the osteogenesis of bone marrow mesenchymal stem cells and stimulate bone formation. Chitosan (CS) is a natural biocompatible, biodegradable, and osteoconductive biopolymer. Recently, there is also interest in using silk fibroin (SF) in tissue engineering applications due to the unique mechanical properties, biocompatibility, and biodegradability of SF scaffolds. In order to study the effect of the CS and SF component in electrospun nanofibers on stem cells, this study prepared uniform composite electrospun nanofibers from CS and SF and studied the proliferation and osteogenic differentiation of seeded human bone marrow mesenchymal stem cells (hMSCs).

Methods: *Bombyx mori* silk fibers were treated twice with 0.5% (w/w) $NaHCO_3$ solution at 70 ^oC for 30 min and then rinsed with 70 ^oC distilled water to remove sericin. Degummed silk was dissolved in a mix solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (mole ratio, 1:2:8) at 70 ^oC for 6 h and filtered to get a SF solution. After dialysis in a cellulose dialysis tubing (MWCO = 50,000) against distilled water for 5 days with water change every 12 h, the SF solution was lyophilized to obtain regenerated SF sponges. Chitosan (MW = 1×10^5, degree of deacetylation = 98%) and SF solutions were prepared in a mixed solvent system of trifluoroacetic acid/ dichloromethane (weight ratio = 7:3) at concentrations of 8 and 12.5 wt%, respectively. CS/SF blend solutions with weight ratio (50:50) were prepared in the same solvent system at 10 wt%. The system for ES includes a glass syringe, a 22-gauge stainless-steel needle, a high-voltage power supply, and an aluminum foil as the collector. The distance between the needle tip and the collector was 15 cm. The applied voltage and flow rate were controlled at 18 kV and 0.5 ml/h, respectively. The structure and morphology of electrospun nanofibers was observed by scanning electron microscopy (SEM) after being gold-coated. The mineralization of hMSCs was determined from element percentage with energy dispersive X-ray (EDX) analysis. After seeding hMSCs at a density of 1 x 10^4 cells on nanofibrous membranes for a period of 7 and 21days in osteogenic medium, the cell/scaffold constructs were fixed in 2.5 % glutaraldehyde and analyzed with SEM/EDX at an accelerating voltage of 10 kV. The proliferation of hMSCs was determined by the MTS cell proliferation assay kit assays at 492 nm (OD_{492}).

Results: The SEM micrographs of electrospun nanofibrous membranes indicate porous, bead-free, and randomly oriented nanofibers could be formed under the well-controlled ES conditions (Figure 1). The average diameters are 317 ± 109, 399 ±184 ,and 447 ± 167 nm for CS , SF, and CS/CF nanofibers, respectively. After cultured on the nanofibers under osteogenic conditions, the elemental composition of P and Ca of hMSCs increased from day 7 to day 21, indicating continued mineral secretion from hMSCs due to osteogenic differentiation. The percentages of Ca and P are comparable for cells cultured on CS and CS/SF nanofibers but much higher than cells cultured on SF nanofibers (Table 1). In contrast, the order of cell proliferation rate is CS < SF ≈ SF/CS (Figure 2).



(a) (b) (c)

Fig. 1. SEM micrographs of (a) CS, (b) SF, and (c) CS/SF nanofibers.

Table 1. The effect of different nanofibers on the mineralization of hMSCs from atomic percentages of elements by SEM/EDX.

Culture time	Element (%)	CS	SF	CS/SF
Day 7	C	61.76	70.82	60.28
	O	36.68	28.42	37.71
	P	1.40	0.67	1.75
	Ca	0.16	0.09	0.26
Day 21	C	45.92	61.05	44.23
	O	42.47	36.45	43.05
	P	6.07	1.82	6.32
	Ca	5.53	0.68	6.40

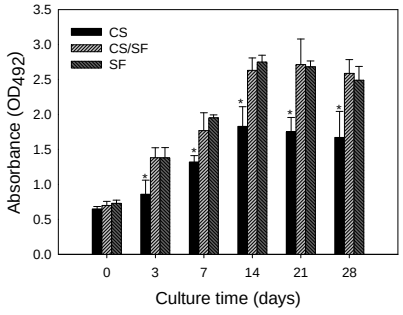


Fig. 2. The effect of different nanofibers on the proliferation of hMSCs from MTS assays.

Conclusions: The proliferation and osteogenic differentiation of hMSCs are enhanced on SF and CS nanofibers, respectively. By taking advantage of the beneficial effect of the individual component in CS/SF blend, electrospun CS/SF composite nanofibers will be a suitable scaffold for bone tissue engineering applications.