# Characterisation and assessment of electrospun Poly/hydroxyapatite nanofibres together with a cell adhesive for bone repair applications

S.Y. Heo, J.W. Seol, N.S. Kim

College of Veterinary Medicine, Chonbuk National University, Jeonju, Republic of Korea

**ABSTRACT**: In this study, we fabricated Poly(lactide-co-glycolide)/hydroxyapatite (PLGA/HAp) nanofibres using electrospinning and evaluated their potential use for bone repair applications. Analysis confirmed that the PLGA nanofibres were similar to the natural extracellular matrix and included HAp particles. Further, gelatin augmented the adhesion of electrospun nanofibres in the cell adhesion test. Therefore, electrospun PLGA/HAp nanofibres together with gelatin can be utilised for bone repair applications.

**Keywords**: Poly(lactide-co-glycolide); hydroxyapatite; nanofibre; electrospinning; gelatin

Polymer/ceramic composites are used for bone repair in human medicine. Biodegradable polymers, such as poly(lactic acid) (PLA) and their random copolymer polylactic acid-co-glycolic acid (PLGA) have been evaluated for bone tissue engineering because of their non-toxic, biomimetic structure, as well as the fact that they are biodegradable in the body (Prabhakaran et al. 2009; Lao et al. 2011). Bone includes more than 60% hydroxyapatite (HAp) in the collagen matrix. Many synthetic HAp materials are in use for the repair of bone defects; however the high brittleness of HAp has been a limitation in clinical use (Chen et al. 2006; Venugopal et al. 2008; Fu et al. 2010).

In recent years, electrospun nanofibres have been used for bone repair (Fu et al. 2010). However, there is a lack of studies regarding cell adhesion on nanofibres with extracellular matrix (ECM). We fabricated PLGA/HAp nanofibres using electrospinning and evaluated the suitability of these fibres for bone repair applications. We characterised the PLGA/HAp nanofibres using a scanning electron microscope (SEM), X-ray diffraction (XRD) analyses, and thermogravimetric analysis (TGA), to assess the adhesive abilities of electrospun nanofibres.

## MATERIAL AND METHODS

**Materials**. Poly(lactide-co-glycolide) (PLGA, LA/GA 70/30, $M_W$ = 200 kDa) and gelatin (type B, from bovine skin, approximately 225 Bloom) were purchased from Sigma-Aldrich (USA). Synthetic hydroxyapatite ($Ca_3(PO_4)_2$; KT) was procured from Sigma-Aldrich (UK; Fluka cat. No. 21223) and was supplied in powder form. Methylene chloride (MC) and Dimethylformamide (DMF) (Du Pont, USA) were used as solvents without further purification. All other chemicals were used as received.

**Electrospinning**. To prepare electrospun nanofibres of PLGA/HAp, PLGA was dissolved in MC/DMF at a ratio of 80/20 to obtain a 10 wt% solution. The prepared HAp was added to the 10 wt% solutions to give a final mixture containing Hap of 0.5 wt%, 1 wt%, 1.5 wt%, and 2 wt% solids with respect to the polymer solution. Briefly, a high voltage power supply (CPS-60 K02V1, Chungpa EMT Co., Korea) capable of generating voltages up to 30 kV was used as an electric field source for spinning the nanofibres. The colloid was supplied through a plastic syringe attached to a needle tip. A copper wire originating from a positive electrode (anode) was connected

Supported by Research funds of Chonbuk National University in 2013, and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 1301001074).

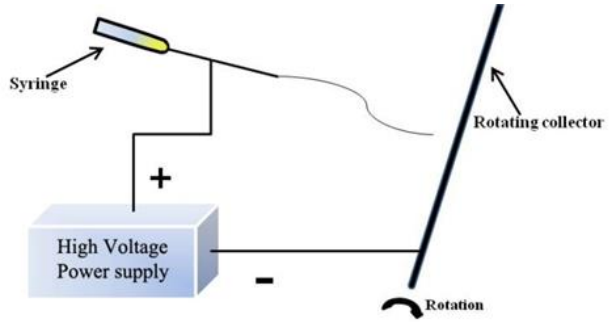


Figure 1. Schematic diagram of electrospinning apparatus

with a copper pin which was inserted into the colloidal solution and the negative electrode (cathode) was attached to a metallic collector (Figure 1). The solutions were applied to 15 kV voltages and the distance between the needle tip and the collector was 15 cm. The electrospun nanofibres were dried under vacuum for a week to remove any residual MC/DMF.

**Characterisation**. The morphology of the PLGA/HAp nanofibres was examined using a SEM (FESEM, Hitachi S-7400, Japan) operating at 20 kV. To evaluate quantitative amounts of Hap in the PLGA nanofibres, XRD analyses were performed using a Rigaku X-ray diffractometer (XRD, Rigaku Co., Japan) at Cu Kα (λ = 1.540 A°) radiation over a Bragg angle ranging from 20° to 80°. The thermal stability of the nanofibres was characterised using thermogravimetric analysis (Pyris Diamond TGA, Perkin-Elmer) under heating in a nitrogen atmosphere from 30 °C to 800 °C with a heating rate of 10 °C/min. Heating was followed by a continuous nitrogen purge of 20 ml/min.

**Cell adhesion assays**. For cell adhesion assays, we used human osteoblastoma MG63 cells. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum, and were supplemented with 2mM L-glutamine, 100 units per ml of penicillin and 100 µg/ml of streptomycin. Firstly, the nanofibres were coated by 0%, 0.2%, 1.0% and 2.0% gelatin. The cells were then seeded onto the outer surface of the nanofibres present in the six-well plates, and the cells were allowed to attach to the fibres for 24 h under static conditions. Subsequently, the cells were counterstained with haematoxylin (violet) for comparison of cellular fibre adhesion ability. The stained cell images were observed under a light microscope.

## RESULTS AND DISCUSSION

Figure 2 shows the ratio of PLGA/HAp nanofibres on SEM images. These were irregularly interconnected and had numerous porous structures. The hydroxyapatite nanoparticle was included along the grain of the PLGA nanofibres. Previous studies have observed similar forms, in which the hydroxyapatite nanoparticle is incorporated into the electrospun nanofibres (Venugopal et al. 2008; Prabhakaran et al. 2009; Lao et al. 2011). TGA analyses were performed to confirm the formation of apatite-like materials in the PLGA and PLGA/HAp composite nanofibres. The TGA results showed that the PLGA nanofibres were decomposed in a

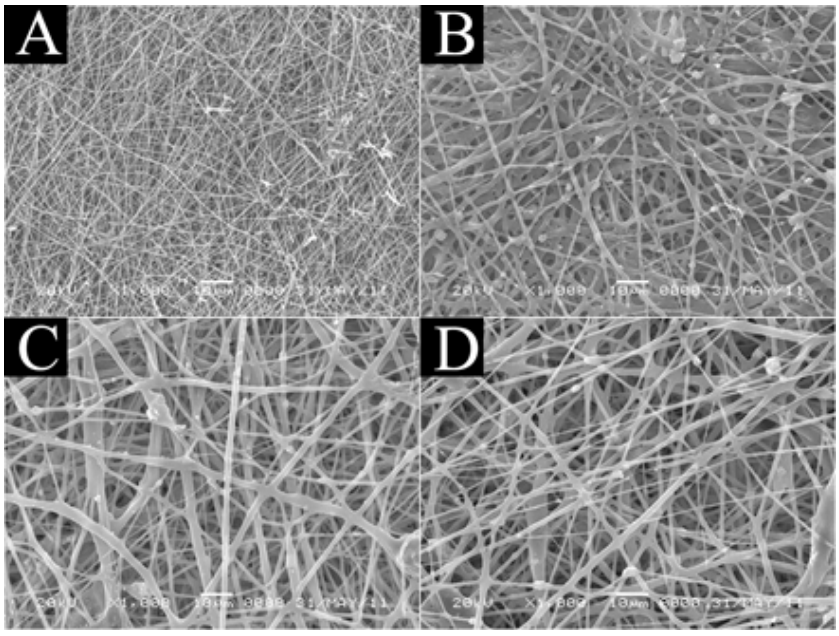


Figure 2. SEM images of electrospun PLGA/HAp nanofibres with different amounts of Hap, (A) 0.5 wt%, (B) 1 wt%, (C) 1.5 wt%, (D) 2 wt%

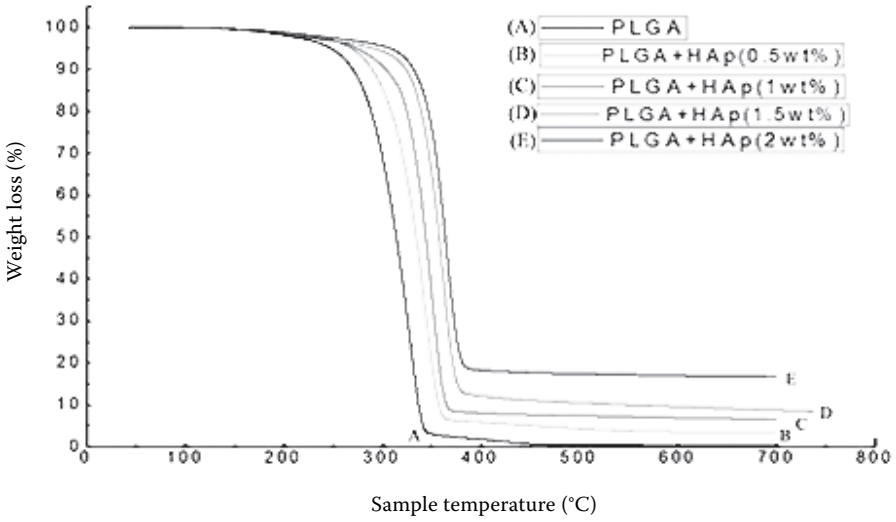


Figure 3. TGA analysis of PLGA nanofibres containing different amounts of hydroxyapatite

single step. The onset of decomposition of PLGA composite nanofibres was found to be in the range of 159–309 °C. The residual weight dramatically increased with the addition of HAp nanoparticles in the PLGA nanofibres, as shown by the solid lines in Figure 3. Fu et al. (2010) investigated nano-Hydroxyapatite/Poly(ε-caprolactone)-Poly(ethylene glycol)-Poly(ε-caprolactone) (PCEC) composite fibres for tissue engineering. According to thermal analysis, incorporation of HAp nanoparticles into the PCEC matrix enhanced the melting temperature of the fibrous mats. The reason for this might be that the addition of HAp nanoparticles into the polymer increased the interaction between HA particles and the PCEC matrix. Also, our TGA results showed a slight increase in the melting temperature from 360 to 390 °C when HAp nanoparticles increased from 0 to 2% in the PLGA nanofibres (Figure 4). This shows that the nucleation effect was increased with increasing HAp nanoparticle concentration in PLGA nanofibres. The XRD patterns of PLGA nanofibres with different HAp concentrations are shown in Figure 3. The diffraction patterns of all samples showed two clear peaks at 31.8° and 46.5°, corresponding to (211) and (222), the main reflection planes of apatite-like calcium phosphate (JCPDS No. 09-0432). The XRD data revealed that the reflection of HAp in PLGA nanofibres increased with an increasing weight percentage of HAp. Kanjwal et al. (2010) reported the XRD pattern of poly(caprolactone) nanofibres containing HAp nanoparticles. The XRD analyses performed here show that the intensities of the peaks increased in parallel with the concentration of original HAp used in the colloidal solutions. In cell adhesion assays, all samples with 1% and 2% gelatin were counterstained with haematoxylin, indicating cell adhesion on nanofibres. There was no staining in the other samples. Many studies have evaluated the effect of ECM proteins on cell adhesion. In one study it was reported that collagen type determined the adhesion and morphology of mesenchymal stem cells on a PLA film (Chen et al. 2008). Although adhesion on electrospun PLGA/HAp nanofibres with gelatin requires further study, it is clear that gelatin may be used to enhance cell adhesion on electrospun nanofibres.

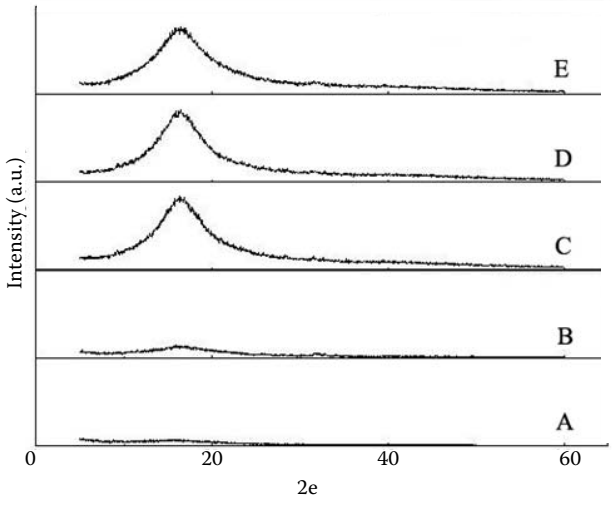


Figure 4. XRD analysis of PLGA nanofibres containing HAp. PLGA (**A**), PLGA/HAp (0.5 wt%) (**B**), PLGA/HAp (1 wt%) (**C**), PLGA/HAp (1.5 wt%) (**D**), PLGA/HAp (2 wt%) (**E**)

In conclusion, SEM images, TGA analyses, and XRD results indicated that electrospinning can be used for the fabrication of PLGA/HAp nanofibre scaffolds suitable for bone repair. The use of gelatin in cell adhesion assays shows that this agent

is suitable for augmentation of cell adhesion on electrospun nanofibres. Therefore, electrospun PLGA/HAp nanofibres with gelatin can be utilised for bone repair applications.

## REFERENCES

Chen J, Chu B, Hsiao BS (2006): Mineralization of hydroxyapatite in electrospun nanofibrous poly(l-lactic acid) scaffolds. Journal of Biomedical Materials Research, Part A 79, 307–317.

Chen Y, Cho MR, Mak AF, Li JS, Wang M, Sun S (2008): Morphology and adhesion of mesenchymal stem cells on plla, apatite and apatite/collagen surfaces. Journal of Materials Science: Materials in Medicine 19, 2563–2567.

Fu S, Wang X, Guo G, Shi S, Liang H, Luo F, Wei Y, Qian Z (2010): Preparation and characterization of nanohydroxyapatite/poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) composite fibers for tissue engineering. The Journal of Physical Chemistry C 114, 18372–18378.

Kanjwal MA, Barakat NA, Sheikh FA, Baek W-i, Khil MS, Kim HY (2010): Effects of silver content and morphology on the catalytic activity of silver-grafted titanium oxide nanostructure. Fibers and Polymers 11, 700–709.

Lao L, Wang Y, Zhu Y, Zhang Y, Gao C (2011): Poly(lactide-co-glycolide)/hydroxyapatite nanofibrous scaffolds fabricated by electrospinning for bone tissue engineering. Journal of Materials Science: Materials in Medicine 22, 1873–1884.

Prabhakaran MP, Venugopal J, Ramakrishna S (2009): Electrospun nanostructured scaffolds for bone tissue engineering. Acta Biomaterialia 5, 2884–2893.

Venugopal J, Low S, Choon A, Sampath Kumar T, Ramakrishna S (2008): Mineralization of osteoblasts with electrospun collagen/hydroxyapatite nanofibers. Journal of Materials Science: Materials in Medicine 19, 2039–2046.

Received: 2014–04–04
Acepted after corrections: 2014–11–04

Corresponding Author:

Nam Soo Kim, Chonbuk National University, College of Veterinary Medicine, Department of Surgery, Jeonju 561-756, Republic of Korea
E-mail: namsoo@chonbuk.ac.kr