1 Introduction
Electrospinning is the most famous technique for the production of high aspect ratio nanofibers and microfibers. Electrospinning of biologically significant polymers have dramatically increased since the electrospun membranes were identified as a candidate for tissue engineering constructs [1]. In particular, polyamide-6 is commercially important, and one of the prominent members of the polyamides which has polymorphic, biodegradable, biocompatible and synthetic polymeric material with good mechanical and physical properties [2]. Recently, the feasibility of incorporating non-electrospinnable inorganic nanoparticles into polymer to form composite nanofibers has revealed electrospinning as an attractive technique to meet several specific functional applications [3-7]. The physical and biological properties of the nanofibrous scaffolds are strongly determined by the materials chemical composition.

Lecithin is a natural mixture of phospholipids and neutral lipids, which is a significant constituent of nervous tissue and brain substance [8-11]. Phospholipids possess a positively charged head group and a hydrocarbon tail that contain various amounts of unsaturation. However, to date, there is no report dealing with polyamide-6/lecithin blended nanofibers by electrospinning to exploit their features to be utilized in osteoblastic cell proliferation process.

In this work, we describe one step synthesis of polyamide-6/lecithin homogenously blended nanofibers via electrospinning for osteoblastic cell culture applications. The cytocompatibility of the polyamide-6/lecithin nanofibrous scaffold with different amount of lecithin was studied by using MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide) test and lactate dehydrogenase (LDH) measurements. The new class of composite material, which has never been documented before, will allow the design of new material formulations to be used for many biological applications such as guided bone regeneration.

2 Experimental
Polyamide-6 with different wt% of lecithin (Sigma-Aldrich, USA) with 0, 1, 3 and 5 wt% were used to prepare the composite nanofiber mats. Polyamide-6/lecithin nanofibers were electrospun from 18 wt% concentration of polyamide-6 in 85 % formic acid (analytical grade, Showa, Japan). Polyamide-6 (KN120 grade, Kolon Industries, South Korea) was used in making the solution. A high voltage power supply (CPS-60 K02V1, Chungpa EMT, South Korea) of 22 kV to the syringe micro-tip was supplied to electrospin the nanofibers. The tip-to-collector distance was 15 cm. Polymer solution was fed to the 5 ml syringe with plastic micro-tip with a
The morphology of the as-spun polyamide-6/lecithin nanofibers was observed by using scanning electron microscopy (SEM, S-7400, Hitachi, Japan). Structural characterization was carried out by X-ray diffraction (XRD, Rigaku, Japan) operated with Cu-Kα radiation (λ = 1.540 Å).

Dulbecco’s modified eagle’s medium nutrient mixture F-12 HAM (DMEM-F12 HAM) media were purchased from Sigma (St. Louis, MO). Fetal calf serum was acquired from Gibco (Grand Island, NY). Human osteoblast (HOB) cells were purchased from ATCC (No. CRL-11372). The HOB cell line was grown on 50 mL tissue culture flasks in DMEM-F12 HAM media; this was supplemented with 10% fetal calf serum, 5 mM L-glutamine, 50 U/mL of penicillin, and 50 µg/mL of streptomycin in a humidified 5% CO₂-95% air environment at 37 °C. In order to observe cell attachment manner on composite nanofibers, chemical fixation of cells was carried out in each sample. After 3 days of incubation the scaffolds was rinsed twice with phosphate buffer saline (PBS) and subsequently fixed in 2.5% glutaraldehyde for 1 h. After that, a sample was rinsed with distilled water and then dehydrated with graded concentration of ethanol, for 10 min each. And then the cell morphology was analyzed by SEM.

3 Results and Discussion

The crystalline structures of as electrospun polyamide-6/lecithin nanofibers were characterized by XRD, and the result was compared with that acquired from the pristine. The XRD pattern of the pristine and blended polyamide-6/lecithin nanofibers are shown in Fig. 1. The crystalline form of lecithin and polyamide-6 nanofibers was mainly composed of one broad peak appeared at 2θ = 20 and 22°, respectively. As shown in Fig. 1, the XRD data of blended polyamide-6/lecithin nanofibers were composed of their respective characteristic peaks. The intensity was slightly increased with increasing lecithin content in the blended nanofibers. These results indicated that the successful blending of lecithin in polyamide-6 nanofibers via electrospinning process.

Fig. 1.XRD patterns of electrospun polyamide-6/lecithin nanofibers with different lecithin concentration of 0, 1, 3 and 5 wt%.

To confirm the findings of cell viability, the morphological appearance of cells on composite nanofiber mats were obtained after 3 days of culture. Fig. 2 shows the SEM images of osteoblast attachment manner on polyamide-6/lecithin blended nanofibers. The cells spread over the scaffold fibers, linked with fibers by cytoplasmic extensions. It was observed that osteoblast cells were incorporated into composite nanofibers. From this data, one can clearly see the cell attachment and cell spreading in the nanofiber matrix.
Fig. 2. SEM image of the cell growth on electrospun polyamide-6/lecithin nanofibers containing different concentration of lecithin with (a) 0, (b) 1, (c) 3 and (d) 5 wt%.

Fig. 3 shows the quantitative cell viability test results. In the control HOB cell culture, cellular proliferation gradually increased until day 3. The adhesion and proliferation of HOB cells on polyamide-6/lecithin composite scaffolds were examined, and the results are shown in Fig. 2. SEM observations indicated that the cell growth pattern of lecithin incorporated polyamide-6 nanofibers was far higher than that of the controls [Fig. 3(a)]. In order to determine whether lecithin incorporated polyamide-6 composite nanofibers exerts any cytotoxic effects on HOB cells, LDH assays were performed by measuring the level of pyruvic acid with a spectrophotometer.

As shown in Fig. 3(b), LDH activities were slightly increased as a result of incorporation with lecithin in these cells. The electrospun polyamide/lecithin composite nanofibers are known to be involved in bone metabolism. As a result of this study, the composite nanofiber was determined to induce cell proliferation. We observed that increased cell viability in HOB cells as the result of lecithin incorporated polyamide-6 blended nanofibers.

It can be concluded from Fig. 8 that the polyamide-6/lecithin composite nanofibers can obviously improve the cell growth behaviors. However, the MTT level appeared to be slightly reduced with increasing lecithin concentration, and this was considered to be partially attributable to the density of nanofibers. On the other hand, the MTT level was observed to be higher than that of the negative control (tissue culture polystyrene plated cells), which demonstrates that the non-toxic behavior of lecithin. This result is in good agreement with the LDH data as shown in Fig. 3(b). In view of the above results it is reasonable to expect that physical and chemical properties are equally important in yielding a suitable environment for cell growth. In this context, the prime factor is considered to be crucial in determining the cell attachment and cell proliferation properties of the surface. The important factor is the surface morphology, which has been demonstrated from FE-SEM, and the existence of an optimally dispersed mesh-like morphology in the polyamide-6/lecithin blended nanofibers. The results also indicated that the absence of a cytotoxicity response of the blended nanofibers. The cells tended to grow on the optimally dispersed hard segments of polyamide-6/lecithin, especially on the surface uniformly in all directions.
accordance with LDH and MTT test. The viability of control cells was set at 100%, and viability relative to the control was expressed. The experiments were conducted at least in triplicate. *P < 0.01 vs. control. **P < 0.005 vs. control.

4 Conclusions

Lecithin blended in polyamide-6 nanofibers with high aspect ratio structure were successfully produced by electrospinning technique. XRD pattern of the electrospun polyamide-6/lecithin nanofibers exhibited a broad peak which was attributed to the formation of blended nanofibrous structure. The MTT level appeared to be slightly reduced with increasing lecithin concentration, and this was attributable to the density of nanofibers. However, the MTT level of all the samples was observed to be higher than that of the negative control. The in vitro cytotoxicity evaluation of the polyamide-6/lecithin blended nanofibers indicated that this scaffold material was non-toxic for the osteoblast cell culture.

References


