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Biomimetic Nanofibrous Gelatin/Apatite Composite Scaffolds for Bone Tissue Engineering

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Abstract

Mimicking certain features (e.g. nanoscale topography and biological cues) of natural extracellular matrix (ECM) is advantageous for the successful regeneration of damaged tissue. In this study, nanofibrous gelatin/apatite (NF-gelatin/apatite) composite scaffolds have been fabricated to mimic both the physical architecture and chemical composition of natural bone ECM. A thermally induced phase separation (TIPS) technique was developed to prepare nanofibrous gelatin (NF-gelatin) matrix. The NF-gelatin matrix mimicked natural collagen fibers and had an average fiber diameter of about 150 nm. By integrating the TIPS method with porogen leaching, three-dimensional NF-gelatin scaffolds with well-defined macropores were fabricated. In comparison to Gelfoam® (a commercial gelatin foam) with similar pore size and porosity, the NF-gelatin scaffolds exhibited a much higher surface area and mechanical strength. The surface area and compressive modulus of NF-gelatin scaffolds were more than 700 times and 10 times higher than that of Gelfoam®, respectively. The NF-gelatin scaffolds also showed excellent biocompatibility and mechanical stability. To further enhance pre-osteoblast cell differentiation as well as improving mechanical strength, bone-like apatite particles (< 2 µm) were incorporated onto the surface of NF-gelatin scaffolds via a simulated body fluid (SBF) incubation process. The NF-gelatin/apatite scaffolds showed significantly higher mechanical strength than NF-gelatin scaffolds 5 days after SBF treatment. Furthermore, the incorporated apatite in the NF-gelatin/apatite composite scaffold enhanced the osteogenic differentiation. The expression of BSP and OCN in the osteoblast-(NF-gelatin/apatite composite) constructs was about 5 times and 2 times higher than in the osteoblast-(NF-gelatin) constructs 4 week after cell culture. The biomimetic NF-gelatin/apatite scaffolds are, therefore, excellent for bone tissue engineering.

Introduction

In tissue engineering, scaffolds are designed to serve as a temporary, artificial extracellular matrix (ECM) in order to support cell attachment and guide three-dimensional (3D) tissue formation [1–4]. Therefore, an ideal scaffold should mimic the advantageous characteristics of the natural ECM [1,2]. Most components of the natural ECM have structural features in the

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nanometer dimensions, and the organization of cells and the corresponding tissue properties are found to be highly dependent on the architecture of the ECM [3–5]. For example, collagen (type I) is the most abundant extracellular protein of bone and is composed of nanoscale fibrillar structure *in vivo* [6,7]. This nanoscale fibrillar structure has been shown to be important for cell attachment, proliferation, and differentiation [8,9]. Several techniques have been developed to fabricate biomimetic nanofibrous matrix by using different synthetic and natural materials [10–13].

Gelatin is a natural material derived from collagen by hydrolysis and has almost identical composition as that of collagen [14,15]. Since gelatin is a denatured biopolymer, the selection of gelatin as a scaffolding material can circumvent the concerns of immunogenicity and pathogen transmission associated with collagen. Porous gelatin scaffold can be fabricated by using the freeze-drying method [16]. However, the gelatin scaffold obtained by freeze-drying cannot mimic the nanofibrous structure of natural collagen. When dissolved in some specific solvents (1,1,1,3,3,3 hexafluoro-2-propanol and 2,2,2-trifluoroethanol), gelatin nanofibers could be fabricated by using the electrospinning technique [17,18]. However, the electrospinning technique typically forms two-dimensional (2D) sheets and is incapable of making 3D nanofibrous scaffolds with well-defined pore size and pore geometry. In tissue engineering strategy, the 3D architecture of a scaffold is believed to contribute significantly to the development of biological functions in tissues [19]. To engineer functional tissues and organs successfully, the scaffolds have to be designed to facilitate cell distribution and guide tissue regeneration in three dimensions.

On the other hand, natural bone ECM is a composite mainly composed of nanofibrous type I collagen and partially carbonated hydroxyapatite (HAP). HAP is orderly deposited within nanofibrous collagen matrix. HAP has shown good osteoconductivity and bone bonding ability [20–22]. However, the use of HAP alone is limited due to its brittleness and difficulty to process into complex shapes. The combination of HAP and gelatin as composite scaffolds, however, would offer the promise to have favorable properties of both HAP and gelatin.

Here, we report the fabrication of 3D NF-gelatin/apatite composite scaffolds, which mimic both nanoscale architecture and chemical composition of natural bone ECM. 3D nanofibrous gelatin scaffolds with well-defined macropores were first prepared by using a new thermally induced phase separation (TIPS) and porogen leaching technique. The biocompatibility and mechanical stability of NF-gelatin scaffolds were compared with Gelfoam® (commercial gelatin foam), which has smooth surfaces on the pore walls. In order to further mimic the composition of natural bone ECM, we incorporated partially carbonated HAP (the bone-like apatite) onto the complex pore surface of NF-gelatin scaffolds through an *in situ* simulated body fluid incubation technique. Thus formed composite scaffolds integrate the advanced structural characteristics of both NF gelatin and nanosized apatite. We hypothesized that such NF-gelatin/nanoapatite composite scaffold would provide not only excellent biocompatibility but also advantageous scaffolding properties, such as enhanced cell adhesion, proliferation, and osteoblastic differentiation, as well as enhanced mechanical properties.

Materials and Methods

Materials

Gelatin (type B, from bovine skin, approximately 225 Bloom) was purchased from Sigma Chemical Co. (St. Louis MO). Gelfoam® was purchased from Pharmacia & Upjohn Company (Kalamazoo, MI). N-hydroxy-succinimide (97%) (NHS), (2-(N-morpholino) ethanesulfonic acid) hydrate (MES) were from Aldrich Chemical (Milwaukee, WI). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) was purchased from Pierce Biotechnology (Rockford, IL). Fetal bovine serum, α -minimum essential medium (α -MEM), penicillin-

streptomycin, and trypsin-EDTA were purchased from Gibco BRL Products, Life Technologies (Grand Island, NY). Ascorbic acid-free α -MEM (Formula 94–5049EL) was purchased from Quality Biological (Gaithersburg, MD). Ascorbic acid was purchased from Fisher Scientific (Pittsburgh, PA) and ethylene oxide was purchased from H.W. Anderson Products (Chapel Hill, NC).

Preparation of 3D nanofibrous gelatin scaffolds

Three-dimensional NF-gelatin scaffolds were fabricated by combining thermally induced phase separation with porogen leaching techniques. Paraffin spheres were chosen as porogen. Paraffin spheres (0.4 g) of selected size (diameter range: 150–250 μ m, 250–420 μ m, or 420–600 μ m) were added to Teflon molds and the top surface was leveled. The molds were then preheated at 37°C for 20 min (or 40, 80, 200 min) to ensure that paraffin spheres are interconnected. Gelatin (1.0 g) was dissolved in a water (10 mL) and ethanol (10 mL) mixture at 45°C. A gelatin solution (0.35 mL) was cast onto paraffin sphere assemblies and the gelatin/paraffin composite was immediately transferred into a freezer at –76°C to induce phase separation for at least 4 h.

The gelatin/paraffin composite was then soaked in 50 mL –18°C cold ethanol for 24 h. At which time, the composite was transferred into 50 mL 1,4-dioxane for solvent exchange for 24 h with fresh 1,4-dioxane replaced every 8 h. The composite was then kept in a freezer at –18°C for 12 h to be completely frozen. The frozen composite was freeze-dried in a salt-ice bath for 4 days and then vacuum dried at room temperature for another 3 days.

The gelatin/paraffin composite was cut to samples with 2.0 mm thickness. The composite was soaked in 50 mL hexane to leach out paraffin spheres. Hexane was changed approximately 6 times every 12 h. To accelerate the dissolution of paraffin spheres, the process can be carried out in an oven at 37°C. Cyclohexane was used for solvent exchange. The gelatin scaffold was frozen at –18°C for 12 h, freeze-dried at between –10°C and –5°C in a salt-ice bath for 4 days and vacuum dried at room temperature for 3 days.

Chemical crosslinking of 3D nanofibrous gelatin scaffolds

Chemical crosslinking of 3D gelatin scaffold with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) and N-hydroxy-succinimide (NHS) was carried out in (2-(N-morpholino) ethanesulfonic acid) hydrate (MES) buffer (pH 5.3, 0.05 M) at 4°C for 24 h. To maintain the microstructure of gelatin matrices and prevent the swelling of gelatin matrices in water, 90/10 (v/v) dioxane/water (or 90/10 (v/v) acetone/water) solvent mixture was chosen instead of water. The scaffolds were washed with distilled water for 3 times at 37°C and was frozen at –18°C for at least 12 h. The crosslinked scaffolds were freeze-dried for 4 days and then vacuum dried at room temperature for 3 days. The dried gelatin foam was then stored in a desiccator for later use.

NF-gelatin scaffold incubation in simulated body fluid (SBF)

The SBF was prepared as previously reported [23]. NF-gelatin scaffolds were immersed in 100 mL 1.5×SBF in a glass bottle maintained at 37°C. The SBF was changed every 24 h. After being incubated for various periods of time, the scaffolds were removed from the fluid and immersed overnight in 400 mL deionized water to remove the soluble inorganic ions.

Scaffold Characterizations

The surface morphology of the scaffolds was observed by SEM (Philips XL30 FEG). The scaffolds were coated with gold using a sputter coater (DeskII, Denton vacuum Inc). During

the process of gold coating, the gas pressure was kept at 50 mtorr, and the current was 40 mA. The coating time was 200 s. Samples were analyzed at 10 kV.

The surface area was measured by N₂ adsorption experiments at liquid nitrogen temperature on a Belsorp-Mini (Bel Japan, Osaka, Japan), after evacuating samples at 25°C for 10 h (<7×10⁻³ Torr). Porosity ε was calculated as: $\varepsilon = 1 - D_p/D_0$. Where D_p is the skeletal density of gelatin foam, and D_0 is the density of gelatin. D_p was determined by:

$$D_p = \frac{4m}{\pi d^2 h}$$

Where m was the mass, d was the diameter, and h was the thickness of the foam. For the gelatin used (Type B: from calf skin, Approx. 225 Bloom), $D_0 = 1.35 \text{ g/cm}^3$.

Compressive modulus of scaffolds was measured using an MTS Synergie 200 mechanical tester (MTS Systems Corporation, Eden Prairie, MN). All samples were circular discs (16 mm in diameter and 2 mm in thickness). Six specimens were tested for each sample. The averages and standard deviations were reported.

Cell seeding on scaffolds

The thawed MC3T3-E1 osteoblasts (clone 26) were cultured in ascorbic acid-free α -MEM supplemented with 10% fetal calf serum (FBS), 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin in a humidified incubator at 37°C with 5% CO₂. The medium was changed every other day.

The scaffolds were sterilized with ethylene oxide for 24 h. The scaffolds were soaked in PBS for 1 h. Afterwards, the scaffolds were washed with a complete medium (α -MEM, 10% FBS, 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin) twice for 2 h each time on an orbital shaker (3520, Lab-Line Instruments, INC), and 2×10⁶ cells suspended were seeded on each scaffold (diameter of 7.2 mm and thickness of 2.0 mm). Media was changed every 12 h while in the Teflon seeding trays. After 48 h, cell-scaffold constructs were removed from the Teflon seeding trays, and transferred into 6-well tissue culture plates containing 3 mL of complete media. The constructs were cultured on the orbital shaker at 100 rpm in the humidified incubator at 37 °C with 5% CO₂. After 7 d, the complete medium was supplemented with 50 mg/mL ascorbic acid and 10 mm β -glycerol phosphate. The medium was changed every other day.

Histology assay

After cultured *in vitro* for varying time intervals, the cell-polymer constructs were fixed in 10% neutral-buffered formalin, dried through an ethanol gradient, and embedded in paraffin. Paraffin-embedded disk specimens were cut into 5 μm cross sections and stained with hematoxylin and eosin for histological analysis.

Gene expression (Real time RT-PCR)

Total RNA was isolated using an RNeasy Mini Kit (Qiagen) with Rnase-Free DNase set (Qiagen, Valencia, California) according to the manufacturer's protocol after scaffolds were mechanically homogenized with a Tissue-Tearor. The cDNA was made using a Geneamp PCR (Applied Biosystems, Foster City, California) with TaqMan (Applied Biosystems) reverse transcription reagents and 10 min incubation at 25 °C, 30 min reverse transcription at 48 °C, and 5 min inactivation at 95 °C. Real-time PCR was set up using TaqMan Universal PCR Master mix and specific primer sequence for OCN (5'-CCGGGAGCAGTGTGAGCTTA-3' and 5'-TAGATGCGTTTGTAGGCGGTC-3') and BSP (5'-

CAGAGGAGGCAAGCGTCACT-3' and 5'-CTGTCTGGGTGCCAACACTG-3'), with 2 min incubation at 50°C, a 10 min Taq Activation at 95 °C, and 50 cycles of denaturation for 15 s at 95 °C followed by an extension for 1 min at 72 °C on an ABI Prism 7500 Real-Time PCR System (Applied Biosystems). Target genes were normalized against Beta Actin (Applied Biosystems).

Statistical analysis

All data were presented as means $\pm$ standard deviation (SD). To test the significance of observed differences between the study groups, an unpaired Student's t-test was applied. A value of $p < 0.05$ was considered to be statistically significant.

Results

NF-gelatin scaffolds with well-defined macropores were fabricated by combining the thermally induced phase separation and porogen leaching techniques (Figure 1a–1c). Paraffin spheres were used as a template to create macropores. The pore wall of the NF-gelatin scaffold was composed of gelatin nanofibers (Figure 1b). The fiber diameter ranged from 50 nm to 500 nm, which is on the same scale as natural collagen fibers, with the average fiber diameter being about 150 nm (Figure 1c). The average fiber diameter did not statistically change with alterations in gelatin concentration. In contrast, Gelfoam® had an irregular macropore structure (Figure 1d). The pore wall of Gelfoam® was composed of sheet-like smooth surfaces (Figure 1e).

The NF-gelatin scaffolds had a low density and a high porosity. For example, the NF-gelatin scaffolds prepared with 7.5% (wt/v) gelatin solution and with a pore size of 250–420 μm had a porosity of 97.51%. These NF-gelatin scaffolds had similar porosity and pore size distribution to that of Gelfoam®, and were selected to compare with Gelfoam® in the following studies.

The surface area measurement showed that the surface area of NF-gelatin scaffolds was more than 700 times higher than that of Gelfoam® (Figure 2). Furthermore, the mechanical strength of NF-gelatin scaffolds was much better than that of Gelfoam®. The compressive modulus of NF-gelatin scaffolds was 801 ± 108 kPa, while it was only 80 ± 8 kPa for Gelfoam® (Figure 2).

The same amount of MC3T3-E1 osteoblast cells were seeded on both NF-gelatin and Gelfoam® scaffolds. More cells were attached on NF-gelatin scaffolds than on Gelfoam® 1 day after cell seeding (Figure 3). After 14 days, cells proliferated on both NF-gelatin and Gelfoam® scaffolds. However, the cell number on NF-gelatin scaffolds remained significantly higher than that on Gelfoam® scaffolds. More importantly, the diameter of the disk-shaped cell/NF-gelatin construct maintained its size, while the cell/Gelfoam® construct shrunk to almost half of its original diameter after 14 days of cell culture (Figure 4).

Cell distribution and neo-tissue formation in the scaffolds was examined with histology. After 14 days, cells were distributed throughout the entirety of both the Gelfoam® and NF-gelatin scaffolds (Figure 5). However, the cell mass was denser in the areas closest to the surface of Gelfoam® compared to the center of the scaffold (Figure 5a). Due to its shrinkage, a thick layer of cell/matrix formed on the outside of the Gelfoam®. This layer could severely obstruct the diffusion of nutrients and thus endanger cellular survival inside the scaffolds. In contrast, the outside pores of NF-gelatin scaffold were still open after 14 days cell culture, and cells were almost uniformly distributed throughout the entire scaffold (Figure 5b).

In order to further improve the mechanical strength of the NF-gelatin scaffolding and enhance osteoblasts differentiation, bone-like apatite was incorporated onto the surface of NF-gelatin scaffolds *in situ* via a simulated body fluid (SBF) technique. Only scattered and small

microparticles were observed on the surface of NF-gelatin pore walls after 1 day of incubation (Figure 6a, 6b). After 7 days of incubation, substantial amount of apatite microparticles with a diameter up to 2 μm were formed on the surface of the pore walls throughout the NF-gelatin scaffold (Figure 6c, 6d). After 21 days of incubation, the whole inner pore wall surfaces of NF-gelatin scaffold was covered by a layer of apatite, and the underlying nanofibers was not observable (Figure 6e, 6f). A longer incubation time of the NF-gelatin scaffold led to more apatite formation (Figure 7). However, the interconnected macroporous structure of the scaffolds was still maintained, which is important for cell migration and mass transport when used for tissue regeneration.

The compressive modulus had a slightly increase after the NF-gelatin scaffold was incubated in SBF for 3 days (Figure 8). After 5 and 7 days, the compressive modulus was significantly higher than that of the initial NF-gelatin scaffold. The compressive modulus of the NF-gelatin/apatite composite scaffold could further increase with incubation time. However, with longer incubation times, the composite scaffold surfaces would be entirely covered by a thick layer of apatite, and the composite scaffold became brittle. Therefore, the gelatin composite scaffold incubated in SBF for 7 days was chosen for the following *in vitro* study.

MC3T3-E1 osteoblast cells were seeded onto both NF-gelatin scaffolds and NF-gelatin/apatite scaffolds and cultured *in vitro* for 4 weeks. Bone sialoprotein (BSP) and osteocalcin (OCN), two well-known late osteogenic differentiation markers, were used to exam osteoblastic cell differentiation. After 1 week of cell culture, the expression of BSP and OCN in both the osteoblast-(NF-gelatin/apatite composite) constructs and osteoblast-(NF-gelatin) constructs was low, which was consistent with the facts of BSP and OCN as late osteogenic differentiation markers (Figure 9). After 4 weeks of cell culture, the expression of BSP in the osteoblast-(NF-gelatin/apatite composite) constructs was about 5 times higher than in the osteoblast-(NF-gelatin) constructs, while the expression of OCN in the osteoblast-(NF-gelatin/apatite composite) constructs was approximately 2 times higher than in the osteoblast-(NF-gelatin) constructs. These results indicated that the incorporated apatite in the composite scaffold enhances the osteogenic differentiation.

Discussion

In order to mimic both the physical architecture and chemical composition of natural bone ECM, we have developed a method to fabricate NF-gelatin/apatite composite scaffolds in this study. We first developed a TIPS process to prepare NF-gelatin matrix which mimics both the nanofibrous structure and chemical composition of natural collagen matrix. TIPS process was previously utilized to prepare synthetic nanofibrous poly(L-lactic acid) scaffolds. However, this method could not be used for water-soluble natural materials. To prepare NF-gelatin matrix, a new TIPS process was developed in this work. Water and ethanol solvent mixture was used to dissolve gelatin. The addition of ethanol to the aqueous gelatin solution was a critical step in creating the nanofibrous structure. When gelatin was dissolved in water alone, it could only form smooth surface structure after phase separation. The addition of certain amount of ethanol in gelatin aqueous solution resulted in the formation of gelatin nanofibers after phase separation (Figure 1). Typically, the nanofibrous structure could be created when the ethanol/water ratio in gelatin solution ranged from 20/80 (v/v) to 50/50 (v/v). Further increasing the amount of ethanol in the solvent mixture resulted in poor solubility of gelatin.

While electrospinning technique has been widely used to fabricate nanofibrous materials, this technique typically forms 2D sheets instead of 3D scaffolds. In contrast to the electrospinning technique, the TIPS technique described here can fabricate 3D NF-gelatin scaffolds with a well-defined pore network when combined with porogen leaching technique. A typical 3D NF-gelatin scaffold with spherical macropores was shown in Figure 1a. Various important

architectural parameters of scaffolds (e.g. porosity, pore size, and interconnectivity) can precisely be manipulated by tailoring the design and fabrication process. The pore interconnectivity was controlled by varying heat-treatment time of paraffin spheres. Longer heat-treatment times formed larger bonding areas between the paraffin spheres resulting in larger openings between the macropores of the scaffold. High interconnectivities between pores are desirable for uniform cell seeding distribution as well as for the diffusion of nutrients to and metabolites from the cell/scaffold constructs.

The pore size of the scaffolds was determined by the size of paraffin spheres used as a porogen. The NF-gelatin scaffolds had a high porosity. A porosity of up to 98% could be achieved when gelatin concentration was 5.0% (wt/v). High scaffold porosity improves the quality of the regenerated tissue through enhanced cell seeding, migration and function throughout the cell/scaffold construct. The NF-gelatin scaffolds had a much higher surface area in comparison to gelatin scaffolds with smooth surfaces (solid-walled gelatin scaffolds), such as commercial product Gelfoam®. The surface area measurement showed that the surface area of NF-gelatin was 32.02 m²/g, while the surface area of Gelfoam® was only 0.046 m²/g. The high surface area of nanofibrous structure could provide for better protein adhesion and thus may be beneficial to cell adhesion.

In tissue engineering, a basic requirement for a scaffold is that the scaffold should have adequate mechanical strength to maintain the spaces required for cell ingrowth and matrix production until later neo-tissue formation. Gelfoam® has a long history of use as a hemostatic agent [24]. Gelfoam® has also been tested in bone repair [25,26]. However, its poor mechanical strength (the compressive modulus was 80±8 kPa) has greatly limited its application for bone tissue engineering. In contrast, our NF-gelatin scaffolds with well-defined spherical pores could provide good mechanical support for cell growth and tissue formation. The compressive modulus of the NF-gelatin scaffold was 801±108 kPa, which was 10 times higher than that of Gelfoam® (Figure 2). The *in vitro* experiment also showed that the NF-gelatin possessed sufficient mechanical strength to maintain its size after 2 weeks of MC3T3-E1 pre-osteoblast cell culture, while the diameter of Gelfoam® shrunk to almost half of its original size (Figure 4).

The *in vitro* experiment also indicated that the NF-gelatin scaffolds enhanced cell adhesion and proliferation (Figure 3). More cells attached on NF-gelatin scaffolds than on Gelfoam® 1 day after cell seeding. After 14 days, the cell number on NF-gelatin scaffolds was still significantly higher than that on Gelfoam®. Histological slides showed the shrinking of the cell/Gelfoam® contract, and a thick layer of cell/matrix formed on the outside surface of the Gelfoam®. This thick layer severely obstructed the diffusion of nutrients and thus endangered cellular survival inside the scaffold. Very few cells were distributed in the centre of the Gelfoam®. In contrast, cells were distributed evenly throughout the NF-gelatin scaffolds. All the above results demonstrate that the NF-gelatin scaffold is better than Gelfoam® for bone tissue engineering.

To further improve the mechanical strength of the scaffolding and enhance osteoblast differentiation, we incorporated bonelike apatite onto the surface of NF-gelatin scaffolds *in situ* via a SBF technique. Previously, our group developed the simulated body fluid method to prepare biomimetic poly(L-lactic acid)/apatite composite scaffolds [23]. Deposition of a biomimetic apatite layer throughout the porous structures of 3D scaffolds is an effective method of controlling surface topography and chemistry within large, complex structures. Here we introduced this method to fabricate biomimetic NF-gelatin/apatite composite scaffolds. The particle number and size in the scaffold were controlled by the incubation time and ionic concentration of the SBF. The average particle number and size increased with incubation time. After 7 days of incubation in SBF, the compressive modulus was more than 75% higher than

that of the initial NF-gelatin scaffolds (Figure 8). The compressive modulus of the NF-gelatin/apatite composite scaffold could further increase with incubation time. Therefore, The SBF process is an effective way to increase the mechanical strength of the NF-gelatin scaffolds.

The deposition of apatite on NF-gelatin scaffolds also enhanced osteoblastic cell differentiation. The expression of BSP and OCN on NF-gelatin/apatite scaffolds was significantly higher than that on NF-gelatin scaffolds 4 weeks after cell seeding.

Conclusions

We fabricated NF-gelatin/apatite composite scaffolds to mimic both physical architecture and chemical composition of natural bone ECM. We first prepared 3D NF-gelatin scaffolds with a well-defined pore structure via thermally induced phase separation and porogen leaching techniques. The NF-gelatin scaffolds exhibited high porosity, interconnectivity and good mechanical strength. The NF-gelatin scaffolds also showed excellent biocompatibility and mechanical stability. We further incorporated bone-like apatite onto the surface of NF-gelatin scaffolds via a SBF incubation method. The addition of apatite enhanced osteoblastic cell differentiation as well as mechanical strength of the scaffold. Such biomimetic NF-gelatin/apatite scaffolds are, therefore, excellent scaffolds for bone tissue engineering.

Acknowledgements

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Figure 1. SEM micrographs of NF-gelatin scaffold and Gelfoam®. (a) NF-gelatin scaffold (porogen diameter: 150–250 μm), overview; (b) Pore-wall structure of NF-gelatin scaffold (higher magnification image of (a)); (c) Higher magnification image of (b); (d) Gelfoam®, overview; (e) Pore-wall structure of Gelfoam®.

Figure 2.

Surface area and compressive modulus comparisons between Gelfoam® and NF-gelatin scaffolds. The NF-gelatin scaffolds (prepared with 7.5% (wt/v) gelatin solution) had a pore size of 250–420 μm and a porosity of 97.51%.

Figure 3. The adhesion and proliferation of MC3T3-E1 osteoblasts cultured on Gelfoam® and NF-gelatin scaffolds. 2×10^6 cells were seeded on each scaffold. (*) represents statistically significant differences ($p < 0.05$).

Figure 4.
The size variation of NF-gelatin and Gelfoam® scaffolds after culturing MC3T3-E1 osteoblasts for 14 days.

Figure 5. H&E staining of Gelfoam® and NF-gelatin scaffolds after culturing MC3T3-E1 osteoblasts for 14 days. (a) Gelfoam®, 4x; (b) NF-gelatin, 4x.

Figure 6.

SEM micrographs of NF-gelatin/apatite scaffolds incubated in 1.5×SBF for varying times. (a) 1 day, overview; (b) 1 day, pore-wall structure; (c) 7 days, overview; (d) 7 days, pore-wall structure; (e) 21 days, overview; (f) 21 days, pore-wall structure.

Figure 7.
Mass increase of NF-gelatin scaffolds over incubation time in 1.5xSBF.

Figure 8. Compressive modulus of NF-gelatin scaffolds over incubation time in 1.5×SBF. (*) represents statistically significant differences ($p<0.05$).

Figure 9. Quantitative RT-PCR results of bone sialoprotein (BSP) and osteocalcin (OCN) gene expression. MC3T3-E1 cells were cultured on NF-gelatin and NF-gelatin/apatite scaffolds for 1 and 4 weeks. The NF-gelatin/apatite composite scaffold was obtained by immersing NF-gelatin scaffolds in 1.5×SBF for 7 days. The Y-axis of the figure is the gene expression results normalized by beta actin. Data are means ± standard deviations from 3 to 4 specimens. (*) represents statistically significant differences ($p < 0.05$).