In vitro and in vivo evaluation of electrospun PCL/PMMA fibrous scaffolds for bone regeneration

So-Ra Son¹, Nguyen-Thuy Ba Linh¹, Hun-Mo Yang² and Byong-Taek Lee¹

¹Department of Biomedical Engineering and Materials, College of Medicine, Soonchunhyang University, 366-1, Ssangyong-dong, Cheonan, Chungnam 330-090, Republic of Korea
²Department of Physiology, College of Medicine, Soonchunhyang University, 366-1, Ssangyong-dong, Cheonan, Chungnam 330-090, Republic of Korea

E-mail: lbt@sch.ac.kr

Received 20 June 2102
Accepted for publication 24 November 2012
Published 7 March 2013
Online at stacks.iop.org/STAM/14/015009

Abstract
Scaffolds were fabricated by electrospinning using polycaprolactone (PCL) blended with poly(methyl methacrylate) (PMMA) in ratios of 10/0, 7/3, 5/5 and 3/7. The PCL/PMMA ratio affected the fiber diameter, contact angle, tensile strength and biological in vitro and in vivo properties of the scaffolds, and the 7/3 ratio resulted in a higher mechanical strength than 5/5 and 3/7. In vitro cytotoxicity and proliferation of MG-63 osteoblast cells on these blended scaffolds were examined by MTT assay, and it was found that PCL/PMMA blends are suitable for osteoblast cell proliferation. Confocal images and expression of proliferating cell nuclear antigen confirmed the good proliferation and expression of cells on the 7/3 PCL/PMMA fibrous scaffolds. In vivo bone formation was examined using rat models, and bone formation was observed on the 7/3 PCL/PMMA scaffold within 2 months. In vitro and in vivo results suggest that 7/3 PCL/PMMA scaffolds can be used for bone tissue regeneration.

Keywords: PMMA, PCL, electrospinning, bone regeneration

1. Introduction
Artificial bone scaffolds are used in bone tissue engineering to provide support in the repair and regeneration of bone defects. The scaffolds are used to carry cells to the sites of interest. An ideal scaffold should be able to mimic the mechanical and biological properties of natural bone tissue. Biopolymers have distinct advantages over ceramic materials for bone engineering. Polymers have received increasing attention and have been used for bone tissue engineering because of their biodegradability and biocompatibility. Bone tissue engineering involves the fabrication of three-dimensional (3D) scaffolds that can support cellular in-growth and proliferation [1]. Some tissue engineering scaffolds have biomimetic structure and exhibit biological functions that are similar to those of native extracellular matrix (ECM) [2, 3]. Artificial ECM provides cells with the mechanical support needed, a broad range of chemical signals that control cell functions [2], and a suitable environment for cell attachment because of physical dimensions similar to those of natural ECM [4–6].

In this study, ECM-like scaffolds composed of thin fibers were fabricated by electrospinning. Electrospinning generates connected 3D mats with high porosity and high surface area that can mimic ECM structure and are suitable for tissue engineering applications [3]. It also produces non-woven meshes containing fibers with diameters ranging from tens of microns to tens of nanometers. Fibrous scaffolds have a high surface-to-volume ratio that enhances cell adhesion [4].
Cell adhesion, in turn, affects cell migration and proliferation and other functions. Therefore, fibrous scaffolds can provide a better environment for cell attachment and proliferation than non-fibrous scaffolds. Tissue engineering materials should be biocompatible and biodegradable, as the scaffold should be gradually replaced with newly regenerated tissues. Hydrophobic/hydrophobic polymer blends have been widely used in fabricating scaffolds for biomedical applications [5], as drug-carrying materials [6] and in tissue engineering [7].

Polycaprolactone (PCL) is synthetic polyester that is compatible with many types of polymers. It has several advantages, including biocompatibility, low cost and processability. PCL is being considered for soft-tissue and hard-tissue biomaterials [8] for uses such as bone tissue engineering [9–11], nerve tissue engineering [12] and drug delivery systems [13]. However, PCL-only scaffolds might not be optimal for bone tissue engineering, as several research groups have reported their slow degradation rate in a physiologic environment and hydrophobicity [14].

Polymethyl methacrylate (PMMA) has good mechanical properties and low toxicity. It is being tested as a scaffold material to achieve long-term mechanical stability after implantation [15]. It has a wide range of biomaterial applications, such as bone cement [16], lenses [17], bone substitutes [18, 19] and drug delivery systems [20, 21]. Although PMMA is a popular material for prosthetic hip-joint transplantation due to its inertness, it exhibits a very slow degradation. Therefore, alternatives to PMMA have been suggested by biomedical and regenerative scientists.

In this study, we report the fabrication of PCL/PMMA blends for bone tissue engineering. After optimizing the mixing ratio for mechanical properties and biocompatibility, we tested selected PCL/PMMA scaffolds in vitro and in vivo. There have been a few reports on PMMA and PCL blends fabricated for biomedical applications by a supercritical antisolvent precipitation process [22], to improve the mechanical properties of a scaffold for bone imitation [23]. These polymers have also been blended by synthetic hydrolysis, and the blended microstructure might have a potential effect on bone cell proliferation and attachment, and eventually improve tissue regeneration in the area of the implanted scaffold.

2. Materials and methods

2.1. Materials

PCL ($M_n = 70000–90000$) was obtained from Sigma (USA), and PMMA and acetone were purchased from LG Chemical (Korea). Human osteoblast-like MG-63 cells derived from human osteosarcoma were purchased from the Korean Cell Line Bank for in vitro studies. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Hyclone (USA). Phosphate buffered saline (PBS), fetal bovine serum (FBS) and penicillin–streptomycin (PS) were purchased from Sigma-Aldrich (USA). Dimethylsulfoxide (DMSO) and xylene were obtained from Dae-jung (Korea), and 4,6-diamidino-2-phenylindole (DAPI) was purchased from Invitrogen (USA).

2.2. Preparation PCL/PMMA solution

PCL (10% w/v) and PMMA (20% w/v) were separately dissolved in acetone by ultrasonication for 2 h. The solution was stirred for 1 h, and PCL and PMMA were mixed in ratios of 10/0, 7/3, 5/5 and 3/7. Mixed PCL/PMMA solutions were stirred for 2 h to fabricate PCL/PMMA blends.

2.3. Fabrication of scaffolds by electrospinning

The prepared PCL/PMMA solutions (10/0, 7/3, 5/5 and 3/7) were placed in a 10 ml syringe with a 25G needle (0.25 mm in diameter). The solution was pumped out of the syringe (KDS100, NanoNC, Korea) at a rate of 1.5 ml h$^{-1}$. A cylindrical metal collector covered with aluminum foil was used to collect the fiber released from the syringe. A voltage of 25 kV was applied to the syringe. All experiments were carried out at room temperature and the samples were air-dried and stored in desiccators.

2.4. Characterization of PCL/PMMA scaffolds

2.4.1. Morphology analysis. The surface of the PCL/PMMA (10/0, 7/3, 5/5 and 3/7) scaffolds were sputter-coated with platinum (Cressington 108 Auto, UK) and observed in a scanning electron microscope (SEM, JSM-6701F, JEOL, Japan) at an accelerating voltage of 10 kV. Diameters of the PCL/PMMA fibers were measured in 30 areas in each sample and the average value was reported with the standard deviation.

2.4.2. Contact angle. Contact angles were measured at room temperature using a drop shape analysis system (Krüss GmbH Inc., Hamburg). Deionized water was used as liquid media, and the droplet size was set at 0.5 µl. The contact angle of the specimen surface was measured 10 s after dropping the liquid, and the values were expressed as the average of five trials with standard deviation.

2.4.3. Tensile strength. The tensile properties of the PCL/PMMA scaffolds (10/0, 7/3, 5/5 and 3/7) were tested using a universal testing machine (UTM, R&B UNITECH-T, Korea). Samples were cut into $20 \times 3 \times 0.1$ mm$^3$ blocks from the electrospun fibrous scaffolds. Tensile strength was calculated by breaking the material using a 500 g load at a rate of 1 cm min$^{-1}$.

2.5. In vitro study

2.5.1. Cell culture. Cell viability of the PCL/PMMA scaffolds (10/0, 7/3, 5/5 and 3/7) was evaluated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma) assay. MG-63 osteoblast cells were maintained and suspended in a humidified incubator at 37°C in a 5% CO$_2$ atmosphere (Incubator, ASTEC, Japan) in DMEM supplemented with 10% FBS and 1% PS solution.
2.5.2. Cell viability assay. PCL/PMMA scaffolds (10/0, 7/3, 5/5 and 3/7) were sterilized by UV light for 3 h, soaked and washed with PBS solution. Extract solutions were prepared by immersing the sterilized PCL/PMMA scaffolds in DMEM containing 10% heat-inactivated FBS and 1% PS, followed by incubation at 37 °C. In the preparation we followed the protocol outlined in the ISO 10993 standard [24].

The PCL/PMMA extract solutions were filtered with 0.20 mm disposable sterile filters (DISMIC, Japan), and several dilutions were made with fresh DMEM media (0, 12.5, 25, 50 and 100%). The MG-63 cells (1 × 10^4 cells per well) were incubated in a 96-well plate for 24 h. Then extract solution was added and the plate was placed in a CO2 incubator at 37 °C. After 1 day, the MG-63 cells were cultured with the PCL/PMMA extract solution for 3 days. Then 20 µl of MTT solution was added to the cell culture media, followed by further incubation for 4 h. The dark blue formazan crystals formed in the reaction were dissolved in 200 µl of DMSO. The optical density (OD) of the solution was measured at a wavelength of 595 nm using an absorbance plate reader (Infinite-F50, Tecan, Austria) to assess the cell viability of each sample. The result was compared to the values of untreated control.

2.5.3. Cell proliferation. To evaluate the cell proliferation, MG-63 cells were seeded at 1 × 10^4 cells per well and incubated on the PCL/PMMA (10/0 and 7/3) fibrous scaffolds for 1, 5 and 7 days. After each incubation period, the samples were carefully moved from the original culture plate, placed in a new 24-well tissue culture plate containing 1 ml of fresh media and 100 µl of MTT solution per well, and then incubated for 4 h. The media and MTT solution in the wells were removed and replaced with DMSO. The optical density of each culture sample was measured using an absorbance plate reader at 595 nm.

2.5.4. Confocal laser scanning microscopy. The MG-63 cells were seeded on PCL/PMMA (10/0 and 7/3) fibrous scaffolds at 1 × 10^4 cells per well and incubated for 1, 5 and 7 days. The cultured cells were washed with PBS solution and then fixed with 4% paraformaldehyde and permeabilized in 0.5% Triton X-100. After blocking with 2.5% BSA dilutions, the membranes were stained by phalloidin, and labeled with fluorescein isothiocyanate (FITC, Sigma, USA) overnight at 4 °C. The samples were stained with 4,6-diamidino-2-phenylindole (DAPI) for 5 min for cell nuclei staining. Fluorescence of cells was observed using an Olympus Fluoview FV10i confocal microscope.

2.5.5. Western blot analysis. The protein expression was evaluated by western blot analysis. In the prepared PCL/PMMA (10/0 and 7/3) extract solutions, MG-63 cells were cultured for 1, 5 and 7 days. The MG-63 cell pellets were mixed with 1X RIPA lysis buffer (Millipore, USA), and 30 µg of protein was loaded onto 12% sodium dodecyl sulfate polyacrylamide electrophoresis gel. Separated proteins were electrophoretically transferred from gels to polyvinylidine fluoride (PVDF) membranes (Bio-Rad, USA). Blots were blocked for 1 h in a tris-buffered saline Tween 20 (TBST, 10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20) buffer with skim milk. Membranes were separately incubated at 4 °C overnight with the proliferating cell nuclear antigen antibody (PCNA, Anaspec Inc.) and anti-beta-actin antibody as a secondary antibody treated for 1 h. After washing, immunopositive bands were visualized by enhanced chemiluminescence (ECL, Bio-Rad, USA) solution using an image analysis system (Chemi-Doc, Bio-Rad, USA).

2.6. In vivo study

2.6.1. Implantation of the 7/3 PCL/PMMA scaffold in rat skull. A total of 12 male Sprague Dawley (300 g) rats were used in this study (three rats for each period), observing the protocol approved by the Animal Ethical Committee of Soochunhyang University for the care and use of laboratory animals. The rats were anesthetized by diethyl ether (Daejung, South Korea) and the hair on the skull was shaved. Antisepsis was provided with povidone iodine. After exposure of the parietal skull, defects on the left side of the rat skull (5 mm diameter) were made using a trephine drill. One defect was grafted with a 7/3 PCL/PMMA scaffold and another defect was sutured without sample as negative control. The subcutaneous tissue and skin incisions were sutured. The rats were sacrificed 1 and 2 months after implantation.

The samples and the surrounding bone tissues were fixed in a 10% formalin solution for micro-computed tomography (Micro-CT, Skyscan 1076, Skyscan, Belgium) analysis and histopathology study 1 and 2 months after implantation. Micro-CT was used to observe the new bone formation in the defected skull site. Each sample was fixed on the object stage, and imaging was performed on the sample for 360° of rotation with an exposure time of 20 min. Micro-CT images were reconstructed over the region of interest (ROI) using CTAn (Skyscan) and CTVol (Skyscan) to make 3D images. The bone volume (BV/TV, %) was calculated from the total bone (TV) and bone volume (BV) to evaluate the new bone quantity, and the bone surface density (BS/TV, %) was calculated from the bone surface (BS) and TV as a measure of the bone surface density.

2.6.2. Histomorphometry. After the micro-CT analysis, the implanted samples, including the surrounding bone and the bone without implanted samples, were fixed in a 10% formalin solution and dehydrated by ethanol (70, 80, 90% and 100%), xylene solutions to remove the alcohol, and then embedded in paraffin wax. The samples were cut into sections of 5 ± 2 µm thickness by a microtome (Thermo-scientific, USA), and the sections were stained by hematoxylin and eosin (H&E) and Masson’s trichrome methods. Tissue sections were viewed with an Olympus BX53 light microscope and photographed with an Olympus DP72 camera. Images were analyzed using the accompanying Cellsens software.
2.7. Statistical analysis

All the statistical data were calculated as the mean ± standard error and analyzed by ANOVA to determine the statistical significance between the two mean values.

3. Results and discussion

PCL/PMMA is a potential bone regeneration candidate material because both PCL and PMMA are non-toxic and cost-effective materials, and the absence of immunogenensis allows cell adherence and proliferation on their surface.

3.1. Morphology of scaffolds

A biomaterial such as an artificial scaffold to be applied in tissue engineering should be biocompatible and non-toxic. This study focuses on electrospun PCL/PMMA for the production of fibrous scaffolds and assesses the cell behavior as an indicator of the scaffolds’ potential in bone tissue engineering. The diameters of fibers formed by electrospinning depend on the polymer and the process conditions. Poly(lactic acid), poly(glycolic acid), polycaprolactone and their copolymers are the most widely studied fibrous systems used in bone tissue engineering. One of the most important qualities related to electrospinning is the fiber morphology [25].

In this study, PCL/PMMA ratios of 10/0, 7/3, 5/5 and 3/7 were selected for further investigation. The total concentrations of PMMA and PCL were optimized to produce uniform bead-free fibers [26]. The PCL/PMMA scaffolds consisted of randomly arranged fibers, and their surface morphology varied with the PCL/PMMA ratio. As shown in figure 1, the average diameters of electrospun fibers were 1.98 ± 0.06 µm (10/0), 2.24 ± 0.05 µm (7/3), 2.38 ± 0.03 µm (5/5) and 2.40 ± 0.04 µm (3/7)—the fiber diameter increases with decreasing PCL/PMMA ratio. The high concentration of PMMA (20% w/v) resulted in a high viscosity of the solution [28]. Thus, a relatively high voltage was needed to initiate Taylor cones and fiber jets [29]. The electrostatic repulsion forces of the charged jet were reduced, resulting in wider fibers. The fibers were uniform, and the space between them increased with increasing PMMA concentration. At higher polymer concentrations, faster solidification of the jet was realized, because the amount of solvent necessary to dissolve the polymers has been reached earlier in the process [30], resulting in uniform fibers.

3.2. Contact angle

Water contact angles were averaged over at least five different positions on every sample. The wettability of the PCL/PMMA (10/0, 7/3, 5/5 and 3/7) scaffolds was determined by dropping distilled water and measuring the contact angle. The contact angle of the PCL scaffold was 110.0° ± 0.3°, which indicates hydrophobic behavior caused by the CH₃ groups of PCL. However, with increasing PMMA content, the contact
angle decreased to $95.0 \pm 0.4^\circ$ for the 3/7 PCL/PMMA scaffold as shown in figure 2. This decrease can be associated with high water absorption by the cotton-like structure of the PCL/PMMA fibrous scaffold.

3.3. Tensile strength

A common method for measuring the tensile strength of thin scaffolds was applied for the analysis of the mechanical properties [27]. Figure 3 shows the stress and strain values obtained. The tensile strength of the PCL/PMMA scaffolds increases with PCL concentration, which can be attributed to the decrease in fiber diameter.

3.4. In vitro studies

The ability of cells to adhere to the fibrous scaffold, mimicking the structure of ECM, is an important property for tissue engineering applications. MG-63 osteoblast-like cells are commonly used in in vitro studies to assess the scaffolds’ potential as bone tissue engineering materials. MG-63 osteoblast-like cells were cultured in the extract solution of PCL/PMMA (10/0, 7/3, 5/5 and 3/7) scaffold sections for 3 days, and the viable cell densities in the specimens were estimated by the MTT assay, as shown in figure 4. MTT is metabolized into a purple formazan salt by mitochondrial enzymes in living cells. The extract media were prepared from the PCL/PMMA (10/0, 7/3, 5/5 and 3/7) scaffolds. The cell viability was estimated as $\sim 80\%$ for the culture of 10/0 scaffold in a 100% extract solution, and as $\sim 86\%$ for the culture of 3/7 scaffold in a 100% extract solution.

To evaluate the cell adhesion and spreading behaviors on the PCL/PMMA (10/0 and 7/3) scaffolds, the osteoblast-like MG-63 cells were incubated for 1, 5 and 7 days. Figure 5 shows the analysis results of MG-63 cell proliferation measured by the MTT assay. The OD values of 10/0 and 7/3 scaffolds increase with culture time. After 1 day of culture, the OD value is slightly higher for the 7/3 than 10/0 scaffold, but the difference becomes much larger after 7 days of culture.
Figure 6. Confocal microscopy images of 7/3 PCL/PMMA scaffolds (a-1) and (b-1) and MG-63 cells attached after 1 day of culture: low magnification (a-2) and (a-3) and high magnification (b-2) and (b-3). Phalloidin (green) was applied for staining membrane and DAPI (red) for staining nuclei.

Figure 7. Confocal microscopy images of MG-63 cells proliferated on the 10/0 (a-1), (a-2) and (a-3) and 7/3 PCL/PMMA scaffolds (b-1), (b-2) and (b-3) cultured for 1 day (a-1) and (b-1), 5 days (a-2) and (b-2) and 7 days (a-3) and (b-3).

FITC-conjugated phalloidin was used to visualize the filamentous actin (F-actin) cytoskeleton of MG-63 cells cultured on the 7/3 PCL/PMMA fibers. The proliferating nuclei stained with DAPI are indicated by yellow arrows in figure 6. This figure shows that MG-63 cells were attached to the 7/3 PCL/PMMA scaffold after 1 day. The cells attached and dispersed within the scaffolds following the fibers, as shown in the merged low-magnification and high-magnification figures 6(a-3) and (b-3), respectively.

Figure 8. Identification of proliferating cell nuclear antigen (PCNA) on the PCL/PMMA (10/0 and 7/3) scaffolds for 1, 5 and 7 days of incubation obtained by western blot analysis.

Figure 7 shows confocal micrographs of MG-63 cells on the PCL/PMMA (10/0 and 7/3) scaffolds after 1, 5 and 7 days of culture. After 1 day of incubation, the cells were well
attached to the PCL/PMMA (10/0 and 7/3) fibers, as shown in figures 7(a-1) and (b-1), respectively. Comparison with cell proliferation data revealed that a higher number of cells were attached to the 7/3 than 10/0 scaffold. Figures 7(a-2) and (b-2) show a uniform cytoskeleton of MG-63 cell network interconnecting the PCL/PMMA (10/0 and 7/3) scaffolds, respectively. Cell spreading is observed with the attached cells showing spindle-like processes. For day 7 and onward, figures 7(a-3) and (b-3) shows a dense cellular mass of MG-63 cells with multiple layers. Generally, the 7/3 PCL/PMMA scaffold showed better cell proliferation than 10/0 scaffold. Results of MTT assay, microscopy images and surface contact areas of cells revealed higher proliferation of MG-63 cells on the 7/3 PCL/PMMA scaffolds.

In the western blot analysis, PCNA expression showed a periodic behavior in accordance with the cell cycle used as a physiological or pathological marker protein of proliferating cells [29]. The western blot analysis of the MG-63 cell proliferation ability in the PCL/PMMA (10/0 and 7/3) scaffolds was carried out by PCNA. PCNA is used as a marker for the S phase (synthesis phase) of the cell cycle and as a marker for cell proliferation [30]. After 1, 5 and 7 days of incubation, the proliferation activity on the 10/0 and 7/3 scaffolds indicated similar expressions, as shown in figure 8. When the culture time was increased from 1 to 7 days, the protein expression of the 7/3 scaffold remarkably showed better proliferation compared to the 10/0 scaffold. Hydrophobic scaffolds in tissue culture can influence the initial cell adhesion and cell migration [31–33].

3.5. In vivo study

Osteoconductivity, biocompatibility and stability during implantation are important requirements for bone regeneration. The 7/3 PCL/PMMA composition was selected for the in vivo test, because previous experiments demonstrated that the combination of bioactive PCL and PMMA could provide materials with good mechanical properties and can be an alternative to traditional extrudable polymers used in tissue regeneration [34]. After implantation of the 7/3 PCL/PMMA scaffold and the creation of a defect without scaffolds as control, rats were euthanized at 1 and 2 months, and the defected skull areas were extracted to evaluate the bone regeneration, as shown in figure 9. The 7/3 PCL/PMMA scaffold was fabricated for bone tissue engineering, and its biodegradability was evaluated by micro-CT observation. Figure 9 shows the micro-CT 3D image of the bone defect 1 and 2 month after implantation without (a-1) and (a-2) and with a 7/3 PCL/PMMA scaffold (b-1) and (b-2), respectively. The bone volume (BV/TV) and bone surface density (BS/TV) are shown in figure 9(c). The BV/TV and BS/TV values are significantly larger (p < 0.001) for the 7/3 PCL/PMMA scaffold than control, 1 or 2 months after implantation, with BV/TV values of 7.0 ± 0.4% and 8.3 ± 0.6% for the control and 14.6 ± 0.5% and 27.5 ± 0.8% for the 7/3 PCL/PMMA scaffold, 1 and 2 months after implantation, respectively. The corresponding BS/TV values are 0.32 ± 0.03% and 0.70 ± 0.04% for the control and 1.50 ± 0.04% and 2.40 ± 0.06% for the 7/3 scaffold. The micro-CT results clearly show new bone formation and the effect of the 7/3 PCL/PMMA scaffold. The biodegradability of the scaffolds and the new bone formation were observed 2 months after implantation. Figures 10–12 show the histological sections of rat skull defect implantation with and without scaffold for 1 and 2 months. The 7/3 fibrous scaffold-implanted areas showed bone regeneration that originated from the edge of the skull defect, which possibly contributed to the regeneration of defects, including periosteum and adjacent bone [35]. The 7/3 PCL/PMMA-implanted area showed more evidence of new bone tissue and undifferentiated cells along the scaffold fiber, with new bone formation occurring along the scaffold. The scaffold-implanted area did not show foreign body reaction (figures 10 and 11). Thus the PCL/PMMA biopolymer blend showed obvious osteoconductivity and
superior bone regeneration. Recently we have reported that osteoconductivity of biopolymers can be enhanced by bioceramics. No ceramic material was used in this study, yet the PCL/PMMA scaffold possessed superior osteogenesis than that of PCL/gel blends [36]. Therefore, a further improvement of the newly fabricated PCL/PMMA...
biocomposite is possible by adding bioactive additives or bioceramics. In a more recent study, a functional composite material was prepared by electrospinning a gelatin-apatite-poly(lactide-co-caprolactone) blend [37]. It was demonstrated that small amounts of gelatin and apatite were necessary for osteogenic differentiation and guided tissue regeneration. Other authors reported that bioinert materials such as PMMA induce bioactivity, providing a barrier to direct fracture healing without inflammation [38–40]. On the other hand, without an implanted sample, the defected area showed only periosteum formation 1 month after implantation, and insignificant bone formation was observed 2 months after implantation (figures 10(a-1), (a-2) and 11(a-1), (b-1)). The purple color in figures 11(b-1) and (b-2) shows that the 7/3 PCL/PMMA fiber remained, and bone formation was progressing. Masson’s trichrome-stained tissue slides were used to identify new bone formation in the defect healing area (figure 12). Collagen fiber was stained blue and new bone, residual scaffold and cytoplasm were stained red. The area implanted with a 7/3 PCL/PMMA scaffold shows successful bone recovery (figures 12(b-1) and (b-2)), and the area without implanted scaffold showed minimal initial growth with perimetric natural bone (figures 12(a-1) and (a-2)). These observations reveal that the 7/3 PCL/PMMA scaffold has good biocompatibility and osteoconductivity. All of the obtained results clearly suggest that PCL/PMMA fibrous scaffolds were non-toxic and enhance the osteoconductivity of these PCL and PMMA composites for bone regeneration.

The fabricated scaffolds showed favorable mechanical properties and the potential for new bone formation in rats.

4. Conclusions

Biopolymer composites with different ratios of PCL/PMMA were fabricated by electrospinning. The average diameter of the PCL/PMMA fibers slightly increased with increasing concentration of PMMA. PMMA improved the wettability of the scaffolds, while PCL enhanced the tensile strength. The 7/3 PCL/PMMA scaffold was suitable for cell growth in vitro and for new bone formation in vivo. This study suggests that PCL/PMMA scaffold can be used for biopolymer composite scaffolds in bone tissue engineering. Other potential biopolymer scaffolds may combine PCL/PMMA blends and ceramics or proteins.

Acknowledgments

This work was supported by the Mid-Career Research Program through an NRF grant funded by the MEST (NO 2009–0092808), Republic of Korea. The authors acknowledge the contributions of Mr Shin-Woo Kim and Alexander Sadiasa to the in vivo experiments.
References

[1] Langer R and Vacanti J 1993 Science 260 920
[2] Voytik-Harbin S L 2001 Methods in Cell Biology (Academic: New York) pp 561–81
[3] Sill T J and von Recum H A 2008 Biomaterials 29 1989
[4] Elsdale T and Bard J 1972 J. Cell Biol. 54 626
[5] Han J, Branford-White C J and Zhu L-M 2010 Carbohydr. Polym. 79 214
[6] Han J et al 2009 Int. J. Pharm. 382 215
[7] Hiep N and Lee B-T 2010 J. Mater. Sci., Mater. Med. 21 1969
[8] Yoshimoto H et al 2003 Biomaterials 24 2077
[9] Oh S H et al 2007 Biomaterials 28 1664
[10] Salerno A et al 2010 Compos. Sci. Technol. 70 1838
[11] Pan L et al 2012 Colloids Surf. B 93 226
[12] Ghasemi-Mobarakeh L et al 2010 Mater. Sci. Eng. C 30 1129
[13] Kim H-W, Knowles J C and Kim H-E 2004 Biomaterials 25 467
[14] Baldwin S P and Saltzman W M 1998 Adv. Drug Deliv. Rev. 33 71
[15] Downes S, Archer R S, Kayser M V, Patel M P and Braden M 1994 J. Mater. Sci., Mater. Med. 5 85
[16] Zivic P M et al 2012 J. Mech. Behav. Biomater. 5 129
[17] Zhang L et al 2009 Appl. Surf. Sci. 255
[18] Socol G et al 2010 Mater. Sci. Eng. B 169 159
[19] Han J, Ma G and Nie J 2011 Mater. Sci. Eng. C 31 1278
[20] Elvira C et al 2004 J. Control. Release 99 231
[21] Tao S L, Lubeley M W and Desai T A 2003 J. Control. Release 88 215
[22] Vega-González A et al 2008 Eur. Polym. J. 44 1081
[23] Kim Y H and Lee B T 2011 Sci. Technol. Adv. Mater. 12 035002
[24] American National Standard 1999 Biological evaluation of medical devices — Part 5: tests for cytotoxicity in vitro methods: 8.2 Tests on extracts AAMI Guidance Document ANSI/AAMI/ISO 10995–5
[25] Huang Z M, Zhang Y Z, Kotaki M and Ramakrishna S 2003 Compos. Sci. Technol. 63 2223
[26] Uyar T et al 2009 Polymer 50 475
[27] Linh N T B et al 2010 J. Biomed. Mater. Res. 95 184
[28] De Vrieze S, Westbroek P, van Camp T and de Clerck K 2010 J. Appl. Polym. Sci. 115 837
[29] Hall P A et al 1990 J. Pathol. 162 285
[30] Linh N T and Lee B-T 2011 J. Biomed. Appl. 27 255
[31] Baker S C et al 2009 Biomaterials 30 1321
[32] Oh S et al 2006 Biomaterials 27 1936
[33] Shi X et al 2011 Colloids Surf. B 85 73
[34] Abraham G A, Gallardo A, Motta A, Migliaresi C and San Román J 2000 Macromol. Mater. Eng. 282 44
[35] Özerdem O R et al 2003 J. Craniofac. Surg. 14 393
[36] Linh N T B, Min Y K and Lee B-T 2012 J. Biomater. Sci. Polym. DOI: 10.1080/09205063.2012.697696
[37] Jegal S-H et al 2011 Acta Biomater. 7 1609
[38] Cho S B, Kim S B, Cho K J, Kim I Y, Ohtsuki C and Kamitakahara M 2005 Key Eng. Mater. 284 286
[39] Shinzato S et al 2000 J. Biomed. Mater. Res. 51 258
[40] Hayashi K, Inadome T, Tsumura H, Mashima T and Sugioka Y 1993 Biomaterials 14 1173