Biomaterials Science: Processing, Properties and Applications III.
Edited by Susmita Bose, Amit Bandyopadhyay and Roger Narayan.

SURFACE COATING OF POLY-D-L-LACTIDE/NANO-HYDROXYAPATITE COMPOSITE SCAFFOLDS FOR DEXAMETHASONE-RELEASING FUNCTION AND WETTABILITY ENHANCEMENT

Ling CHEN[1], Chak Yin TANG[1], Harry Siu-lung KU[2], Da Zhu CHEN[3], Chi Pong TSUI[1]

[1]Department of Industrial and Systems Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China
[2]Faculty of Engineering and Surveying and Centre of Excellence in Engineered Fibre Composites, University of Southern Queensland, West Street, Toowoomba, QLD 4350, Australia
[3]College of Materials Science and Engineering, Shenzhen University, Shenzhen, Guangdong, China

ABSTRACT

Biodegradable polymeric nano-composite scaffolds have recently attracted considerable attention in curing bone deficiencies resulting from fractures or traumas. In this article, the latest progress in fabrication and characterization of poly-D-L-lactide/nano-hydroxyapatite (PDLLA/nano-HA) composite scaffolds, with poly (ethylene glycol) (PEG)-coating and dexamethasone (Dex)-releasing for bone tissue engineering is presented. For the design of drug release scaffolds, some aspects related to the choice of polymer matrix, bioactive inclusions, drug release coatings, and fabrication techniques are firstly reviewed. A PEG-based coating method was subsequently developed to modify a biodegradable polymeric nano-composite scaffold in providing a drug release function with improved wettability. The changes of water contact angle of the scaffolds before and after PEG/Dex coating were measured and the drug release function of the PEG/Dex coating was studied. The experimental findings showed that the proposed coating method was effective in enhancing the wettability and in controlling the early stage of drug release of the PDLLA/nano-HA composite scaffold.

INTRODUCTION

With increasing aging populations in recent years, bone tissue engineering has been regarded as an ultimate medical treatment to reconstruct the loss or malfunction of tissue. Three dimensional porous scaffolds, designed with the required architecture, surface properties, mechanical properties and biocompatibility, play an important role in bone tissue engineering for bone cell attachment, proliferation, differentiation and tissue regeneration[1-3]. Scaffolds, used as drug carriers in some delivery systems, can deliver biologically active molecules at a desired rate for an appropriate period, and can thus reduce the risk of side-effects and optimize the drug delivery rate to improve the therapeutic efficiency and safety of the drugs[4].

Great progress has been made in bone tissue engineering with the rapid development of biomaterial science and engineering in the last decade. Synthetic biodegradable polymers, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers poly(DL-lactic-co-glycolic acid) (PLGA), have attracted much attention in regard to the fabrication of 3D porous scaffold implants. As an implant undergoing degradation in a human body can be gradually replaced by the natural tissue, tissue recovery time can be reduced and secondary surgery is not required to remove the implant[5-7]. However, the low mechanical strength and lack of bioactive function for implants made of biodegradable polymers only can limit their applications in the field of bone tissue engineering. In order to solve these problems, bioactive inorganic particles such as hydroxyapatite (HA), tricalcium phosphates (BCP, TCP), Bioglass®, have been incorporated into biodegradable polymers for developing polymer/inorganic composite scaffolds with better osteoconductive properties and improved

mechanical strength[8, 9]. HA, $Ca_{10}(PO_4)_6(OH)_2$, the major inorganic component (69 wt%) of human natural bone, has been extensively investigated for bone tissue engineering for a few decades because it possesses excellent biocompatibility and promotes faster bone regeneration[10]. Polymer/HA composites, therefore, are attractive because the polymer and HA can be combined to meet the physiological and mechanical demands of the bone tissue.

In order to satisfy the requirements for bone regeneration, scaffolds should possess optimum pore size, porosity and an interconnected pore structure. A variety of fabrication technologies, including fiber boding, solvent casting/particulate leaching, gas foaming, phase separation/emulsification, and solid freeform fabrication[2, 3, 11], have been developed for processing biodegradable polymers into highly porous scaffolds. Solvent casting/particulate leaching has probably been the best known and most widely used method for the preparation of bone scaffolds because it is easier to operate and can control the porosity and the pore size of the scaffolds independently by varying the amount and the size of leachable particles, respectively. Three-dimensional scaffolds produced by this technique have been assessed for bone tissue engineering purpose[12, 13].

Biodegradable poly-D-L-Lactide (PDLLA) shows a strength of approximately 1.9 GPa, and a degradation time of 12-16 months[6]. It was investigated as a biomedical orthopedic coating material because it could optimize the interface interaction between the implant and tissue[14]. This suggested that PDLLA is an ideal candidate as a drug carrier and low load-bearing scaffolds for bone tissue engineering. In this study, PDLLA was chosen as a polymer matrix, while nano-HA was incorporated into the polymer matrix to mimic the natural bone structure (organic-inorganic hybrid) and enhance the mechanical properties and osteoconductivity of the PDLLA matrix. A technique combining polymer coagulation, cold compression moulding, and salt leaching, developed in a previous study[15], was adopted to fabricate PDLLA/nano-HA composite scaffolds. However, PDLLA is hydrophobic in nature[16], and Lee et.al.[17] reported that the surface wettability of the scaffold is critical because cells have difficulty to attach, proliferate and differentiate on a hydrophobic surface. Poly (ethylene glycol) (PEG) was blended with the PDLLA to fabricate electrospun PDLLA/PEG fibrous scaffolds with a hydrophilic surface[16]. PEG was also reported as a drug coating material to enhance the surface wettability and provide the scaffolds with a drug-release function[18]. In this study, PEG together with a drug model, dexamethasone (Dex), were coated onto porous PDLLA/nano-HA scaffolds to fabricate Dex-releasing composite scaffolds for improving the wettability of the scaffolds. The morphology, water contact angles, and drug release profiles of the unfilled PDLLA scaffolds, the PDLLA/nano-HA scaffolds, and the PEG/Dex coated PDLLA/nano-HA scaffolds were investigated and compared.

MATERIALS AND METHODS

Materials

PDLLA ($\overline{M_\eta}$=75,000) and nano-HA particles (20-30 nm) were purchased from the Jinan Daigang Bio-Technology Co., Ltd. (China) and Berkeley Advanced Biomaterials, Inc. (USA), respectively. Dex (CAS 50-02-2, 98% purity) was supplied by Sigma Aldrich Co., Ltd., to serve as the drug model. PEG ($\overline{M_w}$ = 6,000) and sodium chloride (NaCl) were supplied by the Tianjin Reagent Chemical Co., Ltd. (China). Chloroform was purchased from the Shanghai Shenxiang Chemical Reagent Co., Ltd. (China). All chemicals and reagents were of analytical grade or better, and used without further purification.

Fabrication of PEG/Dex coated porous PDLLA/nano-HAp scaffolds

The porous PDLLA/nano-HA scaffolds with PEG/Dex coating were fabricated using a new technique recently developed by the authors [15]. 20 wt.% of nano-HA was incorporated into

the PDLLA matrix to produce PDLLA/nano-HA composite scaffolds. This method, combining polymer coagulation, cold compression moulding, salt leaching and drug coating, was adopted in this study for producing composite scaffolds with improved wettability and controllable pore size, porosity and drug release rate. The porosity of the PDLLA/nano-HA scaffolds was approximate 80%, controlled by a PDLLA/NaCl weight ratio of 1:8. For comparison, unfilled PDLLA scaffolds were fabricated as well. The NaCl particles were sieved to a pore size in the range of 150 to 300 μm, similar to that of bone structures, and stored in a cool and dry condition. The PDLLA was dissolved in chloroform, with stirring. Subsequently, the nano-HA and the sieved NaCl particles were added to the solution of the PDLLA and chloroform under vigorous stirring to obtain a uniform dispersion of the particles. A PDLLA/NaCl/nano-HA mixture formed into a gel paste when ethanol was added to the solution. This gel paste, put into a cylindrical die of 10 mm in diameter, was compressed at a pressure of 10 MPa, at room temperature, for 2 min. After salt leaching and vacuum drying of the molded composites, porous PDLLA/nano-HA scaffolds were fabricated. Afterward, a PEG/Dex film was coated onto the fabricated composite scaffolds by immersing them in a hydrophilic pre-prepared PEG/Dex solution under vacuum for 24 hours. PEG/Dex coated porous PDLLA/nano-HA scaffolds were finally fabricated.

Morphology observations

Photographs of the PDLLA/nano-HA scaffold before and after the salt leaching process were taken by a digital camera. The microstructure of the unfilled PDLLA scaffolds, PDLLA/nano-HA scaffolds, and PEG/Dex coated PDLLA/nano-HA scaffolds were characterized using scanning electron microscopy (SEM, JEOL JSM-6490). A gold coating was applied to the samples to improve the conductivity of the fabricated scaffolds.

Water contact angle measurements

Water-in-air contact angles of the unfilled PDLLA, nano-HA filled PDLLA, and PEG/Dex coated PDLLA/nano-HA scaffolds, each of which was in contact with a droplet of distilled water (5 μl), were determined by a digital contact angle meter (Digidrop, Model R&D, GBX Company). The water contact angles were measured every 30 seconds for the nano-HA filled and unfilled PDLLA scaffolds for 15 min after the distilled water had been in contact with the scaffolds, while the water contact angles of the PEG/Dex coated PDLLA/nano-HA scaffolds were measured every 10 seconds for 2 minutes.

Weight gains of the scaffolds after PEG/Dex coating

Weight gains of the unfilled PDLLA scaffolds and PEG/Dex coated PDLLA/nano-HA scaffolds were measured by an analytical balance (AL204, Mettler Toledo) to estimate the initial Dex loading ability.

Drug release studies

The PEG/Dex coated PDLLA scaffolds, with and without nano-HA addition, were separately placed in closed vials with 10 ml of 10 mM phosphate buffered saline solution (PBS, pH 7.4). Each vial was placed in a constant temperature water bath at 37°C to undergo the drug release process for each scaffold for 35 days. The mediums with released Dex were collected and replaced with an equal amount of new PBS at specified intervals. The mediums were analyzed by a UV/vis spectrophotometer (UV1102, Techcomp Ltd.) at a wavelength of 242.5 nm to determine the amounts of the released Dex. The total amounts of drug release were determined as the cumulative amounts of the Dex released from the scaffolds within 35 days.

RESULTS AND DISCUSSION

Morphology of the scaffolds

Photographs of the PDLLA/nano-HA composite before and after salt leaching are shown in Figure 1. It can be clearly observed that several pores appeared on the PDLLA/nano-HA composite after the salt leaching process, while there were no pores in the composite before the salt leaching. The shapes of the composite before and after the salt leaching are almost the same.

In order to investigate the microstructures of the fabricated scaffolds, SEM images of the unfilled PDLLA, nano-HA filled PDLLA, and PEG/Dex coated PDLLA/nano-HA scaffolds were capatured, as shown in Figure 2. From Figure 2, it can be seen that the pores are uniformly distributed in all three kinds of scaffolds. It is well know that the minimum requirement of pore size in a bone scaffold is 100 μm[1]. The pore sizes of the scaffolds, as shown in Figure 2, range from 200 to 350 μm, meeting the minimum requirement for a bone scaffold. Some pores larger than 300 μm will benefit vascularization and new bone regeneration[1]. This suggests that NaCl particles of size 150 to 300 μm can be good space holding agents. Moreover, compared to the unfilled PDLLA scaffold, the surface of the pore walls in the PDLLA/nano-HA scaffolds was rougher. This was because the incorporated nano-HA particles throughout the PDLLA matrix were attached to the surfaces of the pore walls, as shown in Figure 2 (a) & (b). After the PEG/Dex coating, the surface roughness of the pore walls in the PDLLA/nano-HA scaffold was reduced [Figure 2 (c)]. This indicated that PEG/Dex was successfully coated on the pore walls of the PDLLA/nano-HA scaffold.

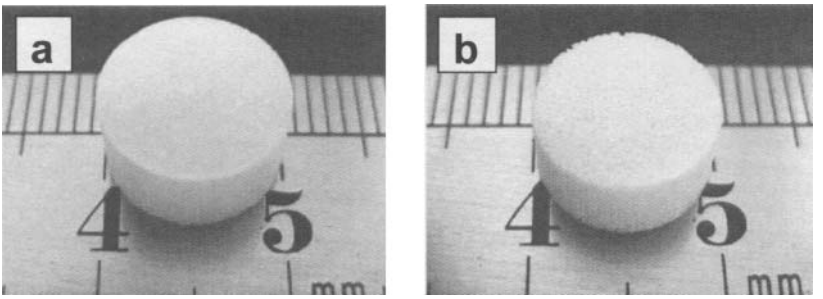
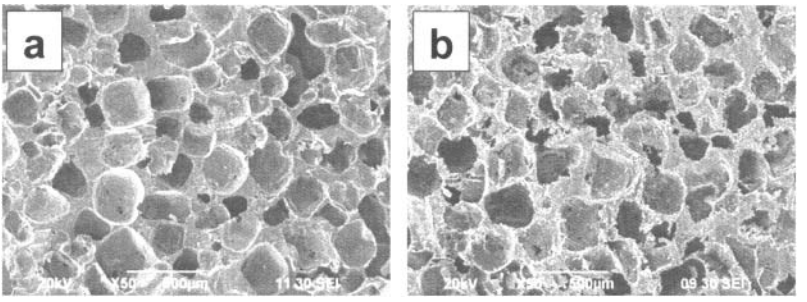


Figure 1. Photographs of the PDLLA/nano-HA composite (cylinder: Ø10×5 mm) (a) before and (b) after salt leaching process.



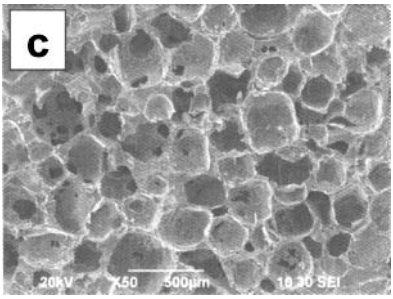


Figure 2. SEM micrographs of (a) unfilled PDLLA, (b) nano-HA filled PDLLA, and (c) PEG/Dex coated PDLLA/nano-HA scaffold.

Water contact angles

Wettability is one of the important factors affecting the cell attachment and growth on the bone scaffolds. Figure 3 shows the dynamic water sessile drop deformation with time on the surface of the unfilled porous PDLLA scaffold, PDLLA/nano-HA scaffold and PEG/Dex coated PDLLA/nano-HA scaffold. The corresponding water contact angles are summarized in Figure 4. The water drop was deformed more slowly on the unfilled PDLLA scaffold [Figure 3 (a)] than on the nano-HA filled one [Figure 3 (b)]. Unlike the previous two scaffolds, the water drop on the PEG/Dex coated PDLLA/nano-HA scaffold deformed quickly and finally penetrated into the porous scaffold [Figure 3 (c)]. From Fig. 4, the water contact angle decreased with time, for all these three kinds of scaffolds. The water contact angle of the PEG/Dex coated PDLLA/nano-HA scaffold decreased from 42° to 8° within only 2 minutes. The decreasing rate of this coated scaffold was 17°/min as compared to 1°/min for the uncoated ones. The reduction of the contact angles suggested a significant improvement in surface hydrophilicity. Furthermore, the initial water contact angle of the PDLLA/nano-HA scaffold, without the PEG/Dex coating, was twice as large as that of the coated one. The unfilled PDLLA scaffold had a larger water contact angle than the filled one. This implied that the surface hydrophilicity of the PDLLA scaffold was improved by the nano-HA filling and PEG/Dex coating. The PEG/Dex coating enabled the conversion of the scaffold into a hydrophilic state and increased the water spread speed on the scaffold, leading to enhancement of cellular attachment, proliferation, differentiation and growth on the coated scaffold.

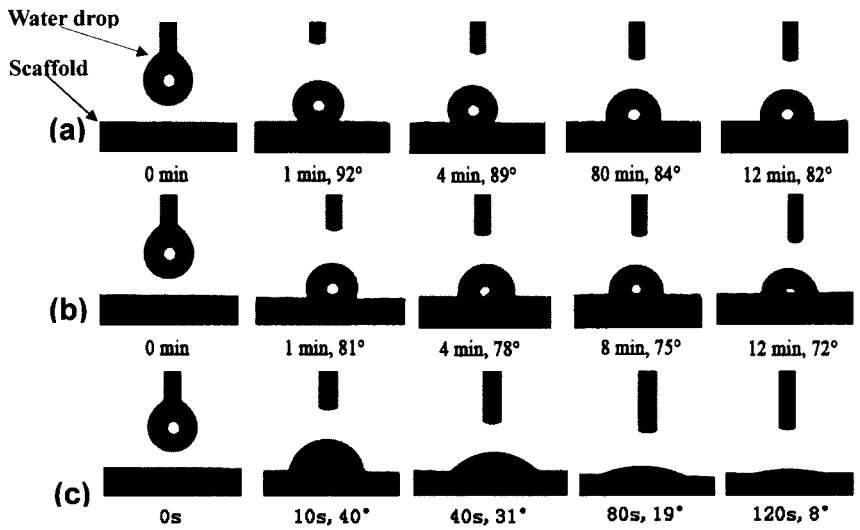


Figure 3. Sequence images of dynamic water sessile droplet on the surface of (a) unfilled porous PDLLA scaffold, (b) porous PDLLA/nano-HA scaffold, and (c) PEG/Dex coated porous PDLLA/nano-HA scaffold.

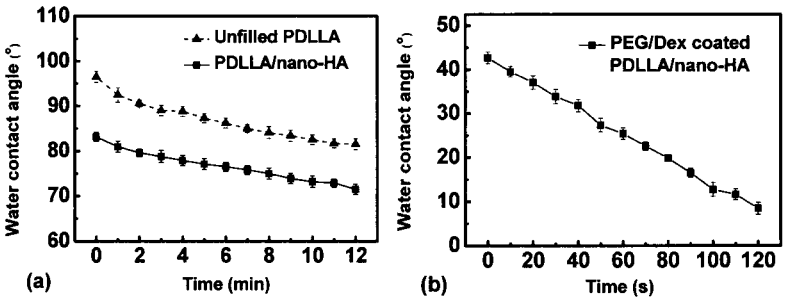


Figure 4. Water contact angle versus time curves (a) unfilled PDLLA and PDLLA/nano-HAp scaffolds without PEG/Dex coating, (b) PEG/Dex coated PDLLA/nano-HAp scaffolds.

Drug loading ability

The weight gains of the nano-HA filled and unfilled PDLLA scaffolds after the PEG/Dex coating are summarized in Table I. The scaffolds with nano-HA addition gained more weight than the unfilled ones. This suggests that incorporation of the nano-HA into the PDLLA could increase the initial drug loading by improving the wettability of the PDLLA scaffolds. Moreover,the hydrophilic surfaces of the PDLLA scaffold would benefit in the subsequent PEG/Dex coating.

The total drug release amounts of the nano-HA filled and unfilled PDLLA scaffolds were

determined as the cumulative Dex release amounts within 35 days and are summarized in Table I[15]. The PDLLA scaffolds with nano-HA addition resulted in a higher Dex release amount. This also indicated that incorporation of the nano-HA into the PDLLA scaffolds could increase the drug loading amounts.

Table I. Weight gains of scaffolds after drug coating

Scaffold	Weight Gain (mg)	Total Drug Release Amount (mg)
PDLLA	48.8±1.7	1.67±0.06
PDLLA/nano-HA	58.0±2.3	2.22±0.08

Drug release studies

Figure 5 shows the cumulative percentage of the Dex release amounts of the nano-HA filled and unfilled PDLLA scaffolds over time. An initial burst effect is shown in both curves where nearly 50% of the Dex was released from the scaffolds within 1 day. The remaining Dex was released in a relative sustained manner in the following 34 days. Compared with the unfilled PDLLA scaffolds, the PDLLA/nano-HA scaffolds resulted in slower drug release rates from the 5^{th} to 10^{th} day and faster rates after the 10^{th} day. These results indicated that incorporation of the nano-HA is one of the factors influencing the drug release rate. It can control the drug release rate to a certain extent.

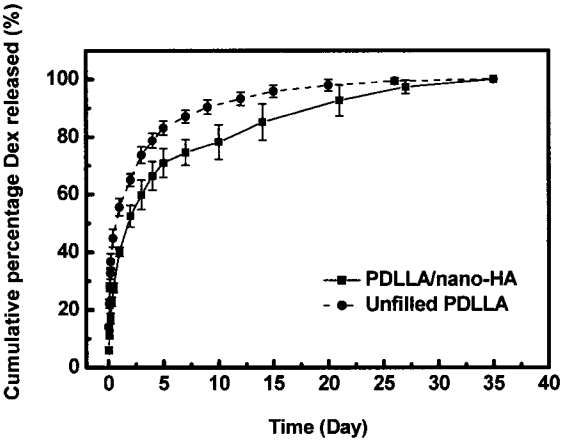


Figure 5. Cumulative release of Dex from the unfilled PDLLA scaffold and nano-HA filled PDLLA scaffold as a function of time

CONCLUSION

Porous PDLLA/nano-HA composite scaffolds with a PEG coating and a Dex releasing agent were fabricated by a novel leaching method combined with polymer coagulation, cold compression moulding and drug coating. The surface wettability of the PDLLA/nano-HA scaffolds were significantly improved by the PEG-based coating and nano-HA incorporation. As PEG/Dex has been effectively coated on the PDLLA/nano-HA scaffolds, the Dex release behaviour was influenced by the nano-HA addition. With the proposed method, these porous PDLLA/nano-HA scaffolds with an inter-connected pore structure, a hydrophilic surface, and a Dex releasing function, and therefore have high potential for tissue engineering applications.

REFERENCES

[1]V. Karageorgiou, D. Kaplan, Porosity of 3D biornaterial scaffolds and osteogenesis, Biomaterials, 26, 5474-91 (2005).

[2]C. Liu, Z. Xia, J.T. Czernuszka, Design and development of three-dimensional scaffolds for tissue engineering, Chem. Eng. Res. Des., 85, 1051-64 (2007).

[3]D.W. Hutmacher, Scaffold design and fabrication technologies for engineering tissues - state of the art and future perspectives, J. Biomat. Sci-Polym. E., 12, 107-24 (2001).

[4]R. Langer, Drug delivery and targeting, Nature, 392, 5-10 (1998).

[5]P Gunatillake, R Mayadunne, R Adhikari. Recent developments in biodegradable synthetic polymers, Biotechnol. Annu. Rev., 12, 301-47 (2006).

[6]L.S. Nair, C.T. Laurencin, Biodegradable polymers as biomaterials, Progress in Polymer Science (Oxford), 32, 762-98 (2007).

[7]M.I. Sabir, X.X. Xu, L. Li, A review on biodegradable polymeric materials for bone tissue engineering applications, J. Mater. Sci., 44, 5713-24 (2009).

[8]K. Rezwan, Q.Z. Chen, J.J. Blaker, A.R. Boccaccini, Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering, Biomaterials, 27, 3413-31 (2006).

[9]I. Armentano, M. Dottori, E. Fortunati, S. Mattioli, J.M. Kenny, Biodegradable polymer matrix nanocomposites for tissue engineering: A review, Polym. Degrad. Stab., 95, 2126-46 (2010).

[10]I. Sopyan, M. Mel, S. Ramesh, K.A. Khalid, Porous hydroxyapatite for artificial bone applications, Sci. Technol. Adv. Mat., 8, 116-23 (2007).

[11]K.F. Leong, C.M. Cheah, C.K. Chua, Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs, Biomaterials, 24, 2363-78 (2003).

[12]K. Ochi, G. Chen, T. Ushida, S. Gojo, K. Segawa, H. Tai, K. Ueno, H. Ohkawa, T. Mori, Use of isolated mature osteoblasts in abundance acts as desired-shaped bone regeneration in combination with a modified poly-DL-lactic-co-glycolic acid (PLGA)-collagen sponge, J. Cell. Physiol., 194, 45-53 (2003).

[13]L. Xu, Z.C. Xiong, D.J. Yang, L.F. Zhang, J. Chang, C.D. Xiong, Preparation and in vitro degradation of novel bioactive Polylactide/Wollastonite scaffolds, J. Appl. Polym. Sci., 114, 3396-406 (2009).

[14]H. Gollwitzer, P. Thomas, P. Diehl, E. Steinhauser, B. Summer, S. Barnstorf, L. Gerdesmeyer, W. Mittelmeier, A Stemberger, Biomechanical and allergological characteristics of a biodegradable poly(D, L-lactic acid) coating for orthopaedic implants. J. Orthop. Res., 23, 802-9 (2005).

[15]L. Chen, C.Y. Tang, D.Z. Chen, C.T. Wong, C.P. Tsui, Fabrication and characterization of poly-D-L-lactide/nano-hydroxyapatite composite scaffolds with poly (ethylene glycol) coating and dexamethasone releasing, Compos. Sci. Technol., 71, 1842-9 (2011).

[16]W.G. Cui, X.L. Zhu, Y. Yang, X.H. Li, Y. Jin, Evaluation of electrospun fibrous scaffolds of poly(DL-lactide) and poly(ethylene glycol) for skin tissue engineering, Mat. Sci. Eng. C-Mater., 29, 1869-76 (2009).

[17]Y.J. Lee, S.J. Park, W.K. Lee, J.S. Ko, H.M. Kim, MG63 osteoblastic cell adhesion to the hydrophobic surface precoated with recombinant osteopontin fragments, Biomaterials, 24, 1059-66 (2003).

[18]L.F. Zhang, R. Sun, L. Xu, J. Du, Z.C. Xiong, H.C. Chen, C.D. Xiong, Hydrophilic poly (ethylene glycol) coating on PDLLA/BCP bone scaffold for drug delivery and cell culture, Mat. Sci. Eng. C-Mater., 28, 141-9 (2008).