Materials Letters 166 (2016) 30–34

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

A biofunctional-modified silk fibroin scaffold with mimic reconstructed extracellular matrix of decellularized pulp/collagen/fibronectin for bone tissue engineering in alveolar bone resorption

Supaporn Sangkert [a], Suttatip Kamonmattayakul [b], Wen Lin Chai [c], Jirut Meesane [a,*]

[a] Institute of Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[b] Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[c] Department of General Dental Practice and Oral and Maxillofacial Imaging, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form
16 October 2015
Accepted 5 December 2015
Available online 8 December 2015

Keywords:
Silk fibroin
Tissue engineering
Fibronectin
Decellularized pulp
Bone tissue engineering
Alveolar bone resorption

ABSTRACT

A modified silk fibroin scaffold with mimic reconstructed extracellular matrix of decellularized pulp/collagen/fibronectin was proposed for bone tissue engineering in alveolar bone resorption. Silk fibroin scaffolds were fabricated by freeze-drying before modification by coating in a decellularized pulp/collagen/fibronectin solution. The extracellular matrix reconstruction of the decellularized pulp/collagen/fibronectin and the morphology and biofunctionalities of the modified scaffolds were evaluated. The results showed that decellularized pulp/collagen/fibronectin organized into a dense dendrite structure and adhered in the scaffold in a fibrillar network. The modified scaffold showed predominant biofunctionalities and promise for bone tissue engineering in alveolar bone resorption.

1. Introduction

Generally, alveolar bone resorption is treated by bone graft from another area, for instance the fibula, scapula, iliac crest, or radius. Nevertheless, bone grafting outcomes include donor-area morbidity and prolonged treatment [1]. Therefore, to use scaffolds for bone tissue engineering is an interesting issue to solve those problems.

Bombyx mori silk fibroin has been used for biomaterial applications for several decades [2]. To create a high performance scaffold for bone tissue engineering, silk fibroin needs enhancement of the biofunctionalities to induce bone tissue regeneration.

Some reports demonstrated that mimicking by reconstruction from the components of an extracellular matrix (ECM) was an attractive method to maintain biofunctionality [3]. Interestingly, the use of ECM from decellularized pulp was rarely reported. Therefore, to reconstruct an ECM from decellularized pulp is the focus of this research.

Collagen is used as a signal to induce cell adhesion and proliferation for bone tissue regeneration [4]. Fibronectin has the important role of inducing mineralization [5]. Due to the biofunctionalities of collagen and fibronectin, they were selected for combination with decellularized pulp and reconstruction into an ECM.

In this research modification of silk fibroin scaffolds with reconstructed ECM of decellularized pulp/collagen/fibronectin was considered. The focus was on the morphology and biological functionalities related to bone tissue engineering in alveolar bone resorption.

2. Materials and methods

2.1. Preparation of silk fibroin scaffolds

The silk fibroin solution was prepared following Chang et al. 2007. The concentration of silk fibroin solution was adjusted to 3% (w/v) [6,7]. Then, the solution was poured into 48 well plates before freeze-drying. The samples were cut into discs of 10 mm in diameter and 2 mm in thickness.

* Corresponding author.
E-mail address: jirutmeesane999@yahoo.co.uk (J. Meesane).

http://dx.doi.org/10.1016/j.matlet.2015.12.032
0167-577X/

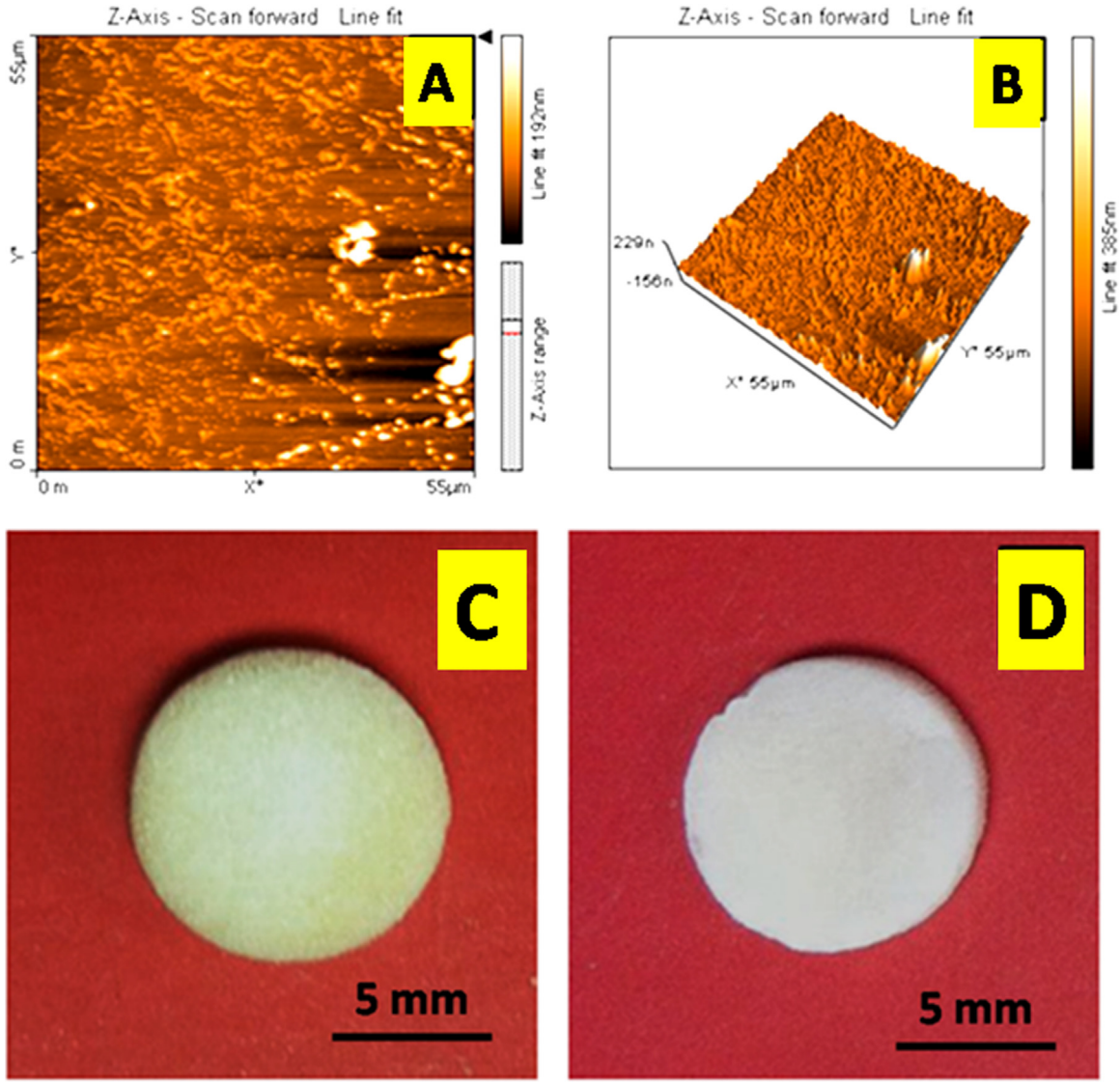

Fig. 1. AFM analysis of the combination of decellularized pulp/collagen/fibronectin fibril formation: (A) 2 dimensions and (B) 3 dimensions, (C) non-modified scaffold and (D) modified scaffold.

2.2. Preparation of type I collagen

Type I collagen was extracted from the skin of the brown banded bamboo shark (*Chiloscyllium punctatum*). Preparation of the collagen followed Kittiphattanabawon et al. [8].

2.3. Preparation of decellularized pulp

This study collected teeth pulp from children 6–10 years old. The teeth were segmented in half to harvest the dental pulp tissue. Decellularized pulp was prepared following Traphagen et al. [9].

2.4. Modification of silk fibroin scaffolds

Two groups of scaffolds were used in this study: silk scaffold and modified silk scaffold coated with decellularized pulp/collagen/fibronectin. For the modified scaffold group, the decellularized pulp powder was dissolved in a solution of 0.1% sodium hypochlorite at a concentration of 0.1 mg/ml. The collagen was dissolved in 0.1 M acetic acid at a concentration of 0.1 mg/ml and the fibronectin was dissolved in distilled deionized water at a concentration of 0.1 mg/ml. Those solutions were mixed together at a volume ratio of 1:1:1. The scaffolds were soaked in 1X PBS for 30 min and then the freeze-drying method was used.

2.5. Scanning Electron Microscopy (SEM)

A scanning electron microscope (Quanta 400, FEI, Czech Republic) was used to observe the morphology and characterization of the scaffolds that were coated with the special solution. The samples were pre-coated with gold using a gold sputter-coating machine (SPI supplies, Division of STRUCTURE PROBE Inc., Westchester, PA, USA).

2.6. Atomic Force Microscopy

A sample of the coating solution from each group was dropped onto a glass slide, smeared, and soaked in PBS for 30 min. When the slides were dry, the morphology and structure were observed by atomic force microscopy.

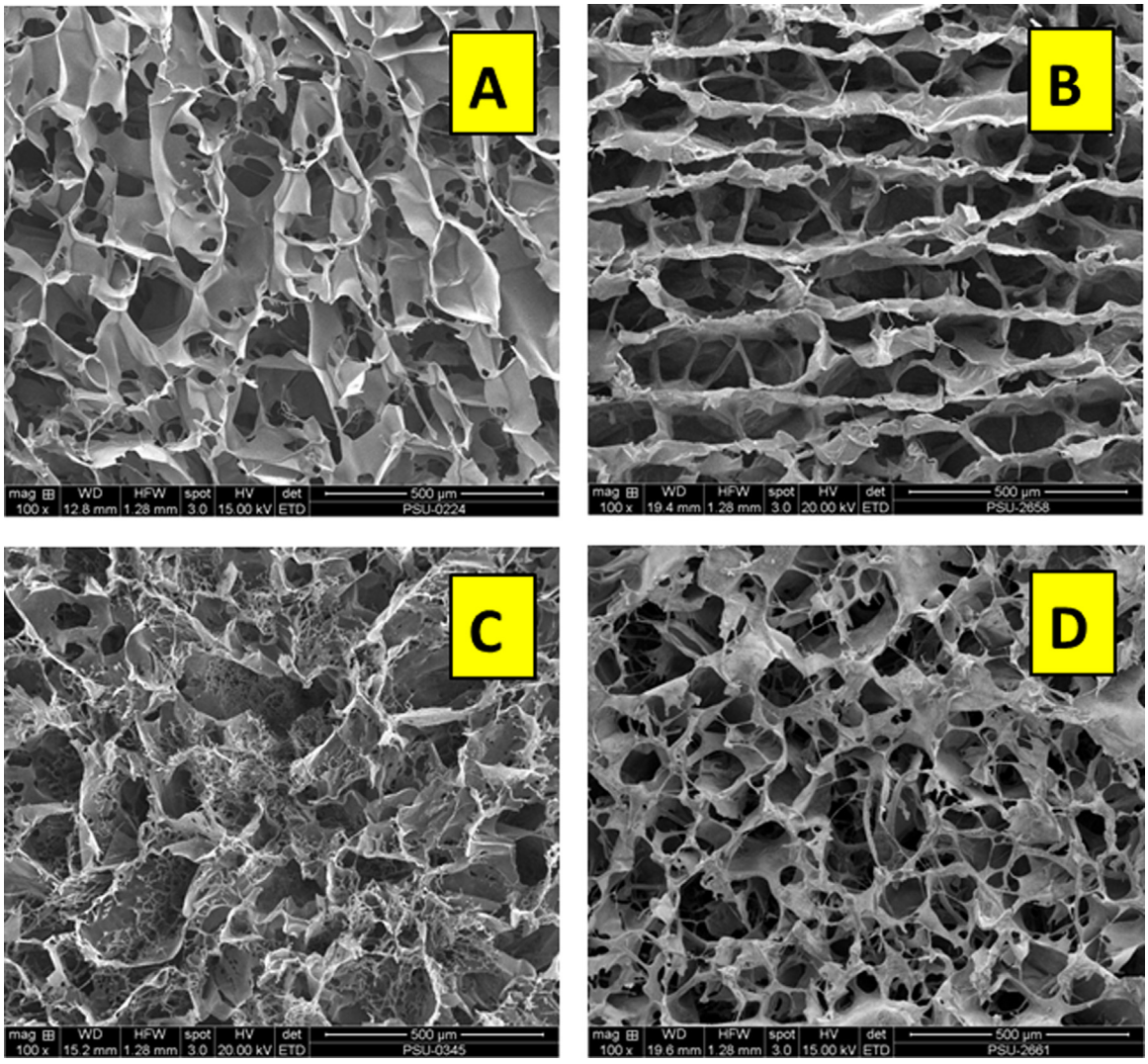

Fig. 2. SEM analysis showed the surface morphology of (A) non-modified scaffold and (C) modified scaffold. The cross-section morphology of (B) non-modified scaffold and (D) modified scaffold.

2.7. MG-63 cell culture

MG-63 cells were cultured in a 75 cm^3 flask with alpha-MEM medium (α-MEM, Gibco™, Invitrogen, Carlsbad, CA, USA) with the addition of 1% penicillin/streptomycin, 0.1% fungizone, and 10% fetal bovine serum at 37 °C in a humidified CO_2 (5%) and air (95%) incubator. The MG-63 cells were seeded at 5×10^5 per scaffold. The medium was changed every 3–4 days during the cell culture. An osteogenic medium (OS; 10 mM b-glycerophosphate, 50 mg=mL ascorbic acid, and 100 nM dexamethasone; Sigma-Aldrich) was used for osteoblast differentiation of the MG-63 cells [10].

2.8. Alkaline phosphatase activity analysis

After cell culture at days 7, 14, and 21, the scaffolds were washed twice with PBS. Then, lysing of the cells followed Hong Ji [11]. The cell lysate solutions were used for the ALP activity according to the manufacturer's instructions (Abcam®, Cambridge, UK).

2.9. Calcium content analysis

The cell lysate solution from paragraph 2.8 was used to analyze the calcium content.

The calcium value in extracellular calcification was measured with calcium colorimetric assays (Calcium colorimetric assay, Biovision).

2.10. Alizarin Red staining for mineralized matrix

The cells were fixed with 70% ethanol for 1 h and removed to −20 °C. Alizarin red S (40 mM, pH at 4.2; Sigma-Aldrich) was used to stain the calcium nodule deposition with a 10 min incubation period [12].

2.11. Von Kossa staining

After cell culture on day 14, the scaffolds were transferred to 48 well plates and washed twice with PBS and 4% formaldehyde was used for cell fixation at 4 °C for 24 h. The scaffolds were immersed in paraffin. The paraffin sections were cut at 5 µ and placed on a

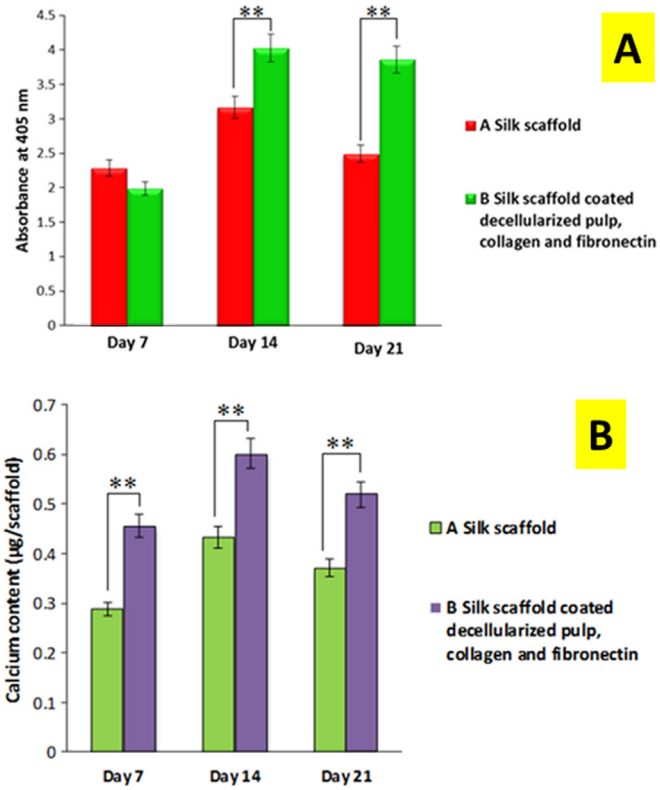

Fig. 3. (A) ALP activity among different groups of scaffold at day 7, 14, and 21. (B) Calcium deposits on the different groups of scaffold at day 7, 14, and 21. The symbol (*) represents significant changes in ALP and calcium activity of the MG-63 osteoblasts ($P < 0.05$), (**) ($P < 0.01$).

glass slide, deparaffinized, and hydrated in distilled water. The sample slides were stained with von Kossa reagent to investigate the deposits of calcium synthesized from the MG-63 osteoblast cells [13].

2.12. Statistical analysis

All data were shown as mean ± standard deviation. The samples ($n=5$) were measured and statistically compared by one-way ANOVA (SPSS 16.0 software package). A P value < 0.05 was accepted as statistically significant.

3. Results and discussion

3.1. Reconstructed ECM formation and morphological structure of scaffolds

AFM and SEM was used to observe reconstructed ECM and morphology of scaffolds, respectively. The decellularized pulp/collagen/fibronectin organized into a dense structure of dendrites (Fig. 1A, B). The modified scaffold appeared whiter than the non-modified scaffold (Fig. 1C, D). The morphology of the non-modified scaffold showed pores that had a smooth surface (Fig. 2A, B). The pores of the modified scaffold had a fibrillar network (Fig. 2C, D) as a native ECM structure [14].

3.2. Alkaline phosphatase activity and calcium content analysis

The ALP and calcium content was used to analyze the calcium synthesis from osteoblast. The ALP assay was used for early MG-63 osteoblast differentiation on culture days 7, 14, and 21 (Fig. 3A). The ALP activity in the modified scaffold increased from day 7 to 21. The non-modified scaffold started to increase during days 7 to 14 and decreased at day 21. The modified scaffold showed predominant ALP activity of the MG-63 osteoblasts. The calcium deposition ratio in both groups increased between days 7 and 14 and went down on day 21 (Fig. 3B). At days 7 and 14, the modified scaffold had a higher calcium value than the non-modified scaffold.

3.3. Calcium deposition and mineralization analysis

Alizarin red staining was used to detect the calcium on the scaffold on day 14 of osteogenic induction. The modified scaffold indicated more calcium deposition compared with the silk scaffold (Fig. 4B). The results demonstrated that the reconstructed ECM had the ability to promote calcium synthesis of the osteoblasts. Von Kossa staining performed on day 14 of osteogenic induction revealed mineral matrix secretion of the MG-63 osteoblast cells (red arrows) were attached to the scaffold (blue arrows).The modified scaffold (Fig. 4D) showed more mineral matrix (yellow arrows) compared with the non-modified scaffold (Fig. 4C). The results showed that the reconstructed ECM was suitable to induce bone regeneration in alveolar bone resorption [15].

4. Conclusion

A modified scaffold was proposed for use in bone tissue engineering in alveolar bone resorption The morphology and biofunctionalities of the modified scaffold were evaluated. The results demonstrated that decellularized pulp/collagen/fibronectin formed a reconstructed ECM into a fibrillar network. This reconstructed

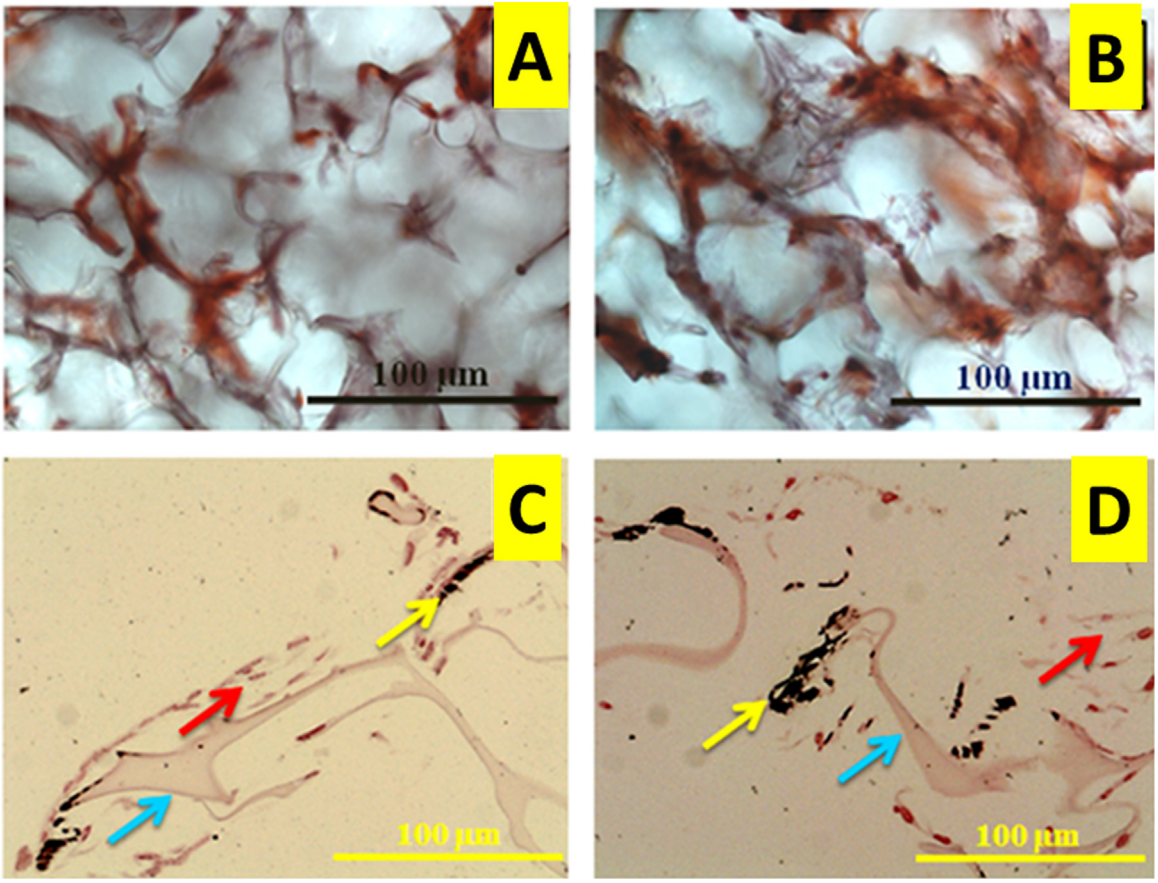

Fig. 4. Alizarin red staining of scaffold on day 7: (A) non-modified scaffold and (B) modified scaffold. Histological sections of scaffold structures stained with von Kossa after day 14: (C) non-modified scaffold and (D) modified scaffold.

ECM could enhance biofunctionalities of the scaffold. The results demonstrated that the modified scaffold showed promise for alveolar bone resorption treatment.

Acknowledgments

This work was financially supported by Grant no. EC 50-042-25-2-3 from the Faculty of Medicine, Prince of Songkla University. Many thanks to the Biological Materials for Medicine (BMM) Research Unit and Queen Sirikit Sericulture Centre, Narathiwat, for supply of the silk.

References

[1] Swapan Kumar Sarkar, Byong Taek Lee, Hard tissue regeneration using bone substitutes: an update on innovations in materials, Korean J. Intern. Med. 30 (2015) 279–293.
[2] Derya Aytemiz, Tetsuo Asakura, Application of Bombyx mori Silk Fibroin as a biomaterial for vascular grafts, Biol.-Inspired Syst. 5 (2014) 69–85.
[3] Junmin Zhu, Roger E. Marchant, Design properties of hydrogel tissue-engineering scaffolds, Expert Rev. Med. Devices 8 (2012) 607–626.
[4] B.P. Chan, K.W. Leong, Scaffolding in tissue engineering: general approaches and tissue-specific considerations, Eur. Spine J. 17 (2008) S467–S479.
[5] Nileshkumar Dubey, Ricardo Bentini, Intekhab Islam, Tong Cao, Antonio Helio, Castro Neto, Vinicius Rosa, Graphene: a versatile carbon-based material for bone tissue engineering, Stem Cells Int. 2015 (2015) 1–12.
[6] Akihide Kamegai, Toshi-ichiro Tanabe, Masahiko Mori, Laminin and fibronectin in bone formation induced by bone morphogenetic protein (BMP) in mouse muscle tissue, J. Bone Miner. Metab. 12 (1994) 31–41.
[7] G. Chang, H.-J. Kim, D. Kaplan, G. Vunjak-Novakovic, R.A. Kandel, Porous silk scaffolds can be used for tissue engineering annulus fibrosus, Eur. Spine J. 16 (2007) 1848–1857.
[8] Phanat Kittiphattanabawon, Soottawat Benjakul, Wonnop Visessanguan, Hideki Kishimura, Fereidoon Shahidi, Isolation and characterisation of collagen from the skin of brownbanded bamboo shark (Chiloscyllium punctatum), Food Chem. 119 (2010) 1519–1526.
[9] Samantha B. Traphagen, Nikos Fourligas, Joanna Xylas, Sejuti Sengupta, David Kaplan, Irene Georgakoudi, Pamela C. Yelick, Characterization of natural, decellularized and reseeded porcine tooth bud matrices, Biomaterials 33 (2012) 5287–5296.
[10] Beom-Su Kim, Hyo Jin Kang, Jun Lee, Improvement of the compressive strength of a cuttlefish bone-derived porous hydroxyapatite scaffold via polycaprolactone coating, J. Biomed. Mater. Res. Part B: Appl. Biomater. 101 (2013) 1302–1309.
[11] Hong Ji, Lysis of cultured cells for immunoprecipitation, Cold Spring Harb. Lab. Press 2010 (2010) 1–5.
[12] Yu-Hsiung Wang, Yaling Liu, Peter Maye, David W. Rowe, Examination of mineralized nodule formation in living osteoblastic cultures using fluorescent dyes, Biotechnol. Prog. 22 (2006) 1697–1701.
[13] Lin Sun, Sara T. Parker, Daisuke Syoji, Xiuli Wang, Jennifer A. Lewis, David L. Kaplan, Direct-write assembly of 3D silk/hydroxyapatite scaffolds for bone co-cultures, Adv. Healthc. Mater., 1, (2012) 729–735.
[14] Martijn de Wild, Wim Pomp, Gijsje H. Koenderink, Thermal memory in self-assembled collagen fibril networks, Biophys. J. 105 (2013) 200–210.
[15] Hee-Young Yang, Joseph Kwon, Min-Suk Kook, Seong Soo Kang, Se Eun Kim, Sungoh Sohn, Seunggon Jung, Sang-Oh Kwon, Hyungseok Kim, Jae Hyuk Lee, Tae Hun Lee, Proteomic analysis of gingival tissue and alveolar bone during alveolar bone healing, Mol. Cell. Proteom. 12 (2013) 2674–2688.