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In vivo bone regeneration using a bioactive nanocomposite scaffold and human mesenchymal stem cells

Nazanin Andalib · Mousa Kehtari · Ehsan Seyedjafari · Nassrin Motamed · Maryam M. Matin

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Abstract Due to the osteoconductive role of bioceramics, use of these bioactive nanocomposite scaffolds that can maintain their structural integrity during bone tissue repair is one of the major goals of tissue engineering. Herein, a nanofibrous poly-L-lactic acid (PLLA) scaffold was fabricated by electrospinning and then gelatin and hydroxyapatite nanoparticles (nHA) were coated over the surface of the scaffold. Osteoconductivity of the fabricated nano-composite scaffolds was then studied while grafted on the rat calvarial defects. Our results indicated that the coating of PLLA scaffold with nHA and gelatin increased the adhesion and growth of the human bone marrow derived mesenchymal stem cells (BM-MSCs) and also significantly increased the level of mineralization over a week culture period. The results of radiographic and histological studies showed that the newly created bone tissue at the defect site was significantly higher in animals treated with nanocomposite scaffolds than the empty scaffolds and control groups. This increase in the defect reconstruction was significantly increased after culturing BM-MSCs on the scaffolds, especially nanocomposite scaffolds. It can be concluded that the combination of nanocomposite scaffolds and BM-MSCs could be a very good candidate for treatment of bone lesions and could be considered as a bony bioimplant.

N. Andalib · M. M. Matin (✉)
Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
e-mail: matin@um.ac.ir

M. Kehtari (✉)
Department of Stem Cell Biology, Stem Cell Technology Research Center, Tehran, Iran
e-mail: mousakehtari@gmail.com

E. Seyedjafari
Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran

N. Motamed
Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran

M. M. Matin
Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

M. M. Matin
Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran

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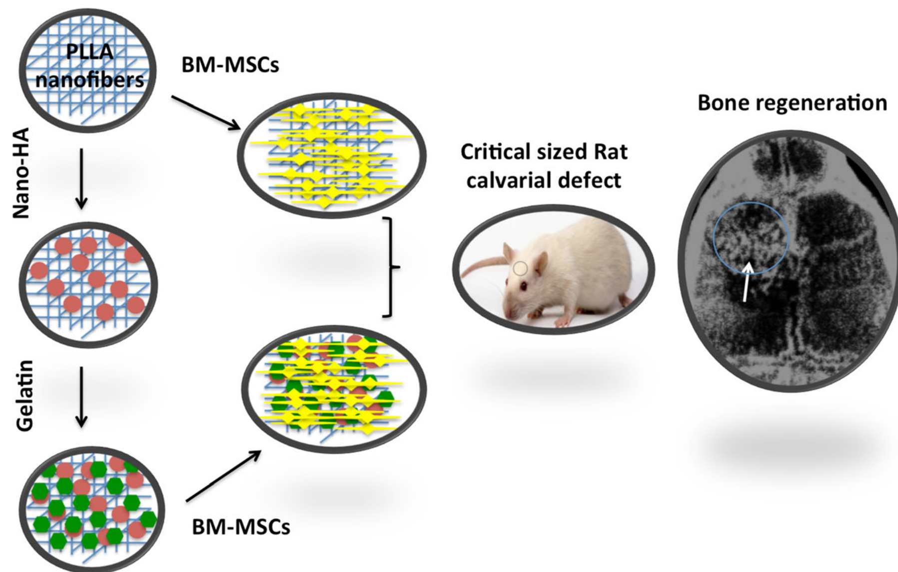
Graphic abstract



Keywords Hydroxyapatite · Gelatin · Nanofibrous scaffold · Critical-sized rat calvarial defect · Bone tissue engineering

Introduction

Bone lesions are a common problem worldwide and their prevalence increases with age in different populations. In addition, bone diseases and injuries from accidents and traumas are common in most societies, and in some cases, broken bones are unable to heal (Pawelec 2019). Today, bone implantation is performed in two ways: allograft, and autograft as a commonly available method (Conway 2010; Shibuya and Jupiter 2015). Although autograft is the gold standard method of treatment, it also suffers from some drawbacks such as body-source shortage and the burden of additional surgery. In allograft transplants, there are also serious problems such as disease transmission and rejection (Betz 2002). Further efforts to develop new therapies have led to numerous researches in the field of tissue engineering as alternative therapies for different goals including damaged-tissue regeneration and cancer treatment as two major human being problems (Shafiee 2020; Wu et al. 2014).

In tissue engineering, specialized cells along with a set of bioactive molecules and engineered scaffolds can be used to reconstruct the desired tissue (Henkel 2013). Engineered scaffolds have some advantages such as failure to produce an immune response, providing a suitable structure for cell proliferation, biocompatibility and biodegradability, and also mimicking the filamentous and complex structure of the natural extracellular matrix (ECM) (Dahlin et al. 2011; Holzwarth and Ma 2011). A suitable scaffold should have a proper hydrophilicity to facilitate cell attachment. The scaffold, on the other hand, should create a network of internally connected pores to facilitate cell migration and food entry and removal of waste (Amani et al. 2019). Today, many synthetic polymers are used to make scaffolds, since they have better mechanical properties and easier manipulability than natural polymers (Moradi et al. 2018). These include polyhydric acid polymers such as poly-L-lactic acid (PLLA), which is widely used in medical devices and tissue engineering due to its biocompatibility and intrinsic biodegradability (Jing et al. 2016 Mahboudi et al. 2020; Santoro et al. 2016). There are various ways to improve the structural properties of the scaffolds to better mimic the underlying ECM. One of these methods is polymer-ceramic composite that has a special value in bone tissue engineering

(Bageshlooyafshar 2019; Huang et al. 2018; Pesaraklou et al. 2019). Studies have shown that the use of ceramics, especially calcium phosphate ceramics such as hydroxyapatite (HA), along with polymeric materials, in addition to achieving better mechanical properties, will significantly increase the degree of biocompatibility and bone mineralization of the implant (Venkatesan and Kim 2014; Zhou and Lee 2011). Other complementary methods include the use of proteins that are naturally part of the cellular matrix in the scaffold composition. Another important issue is the method used for manufacturing the scaffold. One of the easiest and most affordable methods that has been widely used in recent decades is electrospinning to produce nanofibers (Cui et al. 2010). The use of nanofibers in scaffold construction is an advantage over other scaffolds due to the increased surface area to volume ratio and consequently increased cellular interactions with the scaffolds.

In recent years, there has been a growing trend towards the use of stem cells alone or as a component of tissue-engineering constructs for treatment of various diseases (Campbell 2015). One group of these stem cells that is currently under extensive study constitutes mesenchymal stem cells (MSCs). MSCs participate in the repair of tissues of mesenchymal origin, such as bone, cartilage, muscle, tendon, and adipose tissue and, of course, are also supporting hematopoiesis in the bone marrow (BM) (Kehtari 2019; Mousaei Ghasroldasht 2019; Shafieian 2017). In addition, their immunomodulatory properties are also known when used in conjunction with organ transplantation. These cells make up less than 0.1% of the BM-mononuclear cells, and have the ability to proliferate in vitro as fibroblast-like adherent cells at the bottom of plastic containers (Seshi et al. 2000).

In the present study, a PLLA nanofibrous scaffold was prepared by electrospinning and then the surface of the fabricated scaffold was modified first by plasma treatment and then coated with HA nanoparticles (nHA) and gelatin. After morphological examination and non-toxicity confirmation of the fabricated composite scaffolds, their osteoconductive properties were investigated by implantation at a critical-sized rat calvarial defect with and without BM derived MSCs (BM-MSCs).

Materials and methods

Electrospinning

Electrospinning was applied for PLLA nanofiber fabrication according to a previously reported protocol (Izadpanahi et al. 2018). Polymer solution was prepared by dissolving PLLA powder in chloroform and dimethylformamide (DMF) (all from Merck, Germany) while stirring at 37 °C for 4 h. Electrospinning was performed at room temperature, 55% humidity and 18.3 kV. The polymer solution was transferred into a 5 ml syringe that its needle tip was blunted and placed at 15 cm distance from a collector. The solution flow rate was 0.5 ml/h.

Scaffold surface modification

For surface modification, at first, scaffolds were placed under UV at room temperature for 1 h, and then rinsed in 70% ethanol for 30 min. After that, fabricated PLLA scaffold was placed in an oxygen plasma reactor (Diener Electronics, Germany), which worked with a frequency of 44 GHz, and cylindrical quartz while oxygen was poured for 5 min on the surface of the scaffold with a pressure of 0.4 mbar. Plasma treated scaffold was rinsed for 12 h in the prepared Gelatin (0.1% w/v, cell culture grade, Sigma-Aldrich, USA), nHA (0.1% w/v, Nikceram, Iran), and nHA/Gelatin (0.1% w/v) solutions/mixtures in deionized water, which were properly dispersed ultrasonically for 22 min. Finally, surface modified scaffolds were washed gently with deionized water and then kept at room temperature to dry. Fabricated scaffolds were categorized into two groups including PLLA and PLLA-nHA-Gelatin (PLLA/NP) for biocompatibility and bone regeneration evaluations.

Scanning electron microscopy

Scanning electron microscopy (SEM) was applied to characterize surface morphology of the fabricated scaffolds. Cell seeded scaffolds (BM-MSCs with a density of 5×10^3 cells/cm^2) were fixed with 2.5% glutaraldehyde at room temperature for 2 h and then samples were dehydrated by rinsing in graded ethanol solutions from 50% to 100%. Fixed seeded and non-seeded scaffolds were sputtered by a thin layer of gold and then placed in a scanning electron microscope

(SEM, LEO AIS2100, Seron Technology, Korea) for further analysis.

Viability assay

For non-toxicity confirmation of the fabricated scaffolds, previously characterized human BM-MSCs (Mahboudi et al. 2018) with a density of 5×10^3 cells/cm^2 were cultured on the surface of the scaffolds in various groups and cell viability was studied using 3-(4,5 -dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, Germany) assay on days 1, 3, and 7 after cell seeding. At specified time points, MTT (5 mg/ml) solution was added to each sample and incubated for 3.5 h at 37 °C, under 5% CO_2. After that, solution was removed and dimethyl sulfoxide (DMSO, Gibco, USA) was added to the wells for dissolving formazan crystals. Finally, the absorbance of the solutions was read using an automatic plate reader (Awareness Technology Inc, USA) at 570 nm. It should be noted that human BM-MSCs seeded on conventional tissue culture plates (TCPs) were considered as a control group.

Animal surgery

In present study, 24 male Wistar rats were purchased from Razi Institute (Karaj, Iran) with an average weight of 200 ± 10 g. Animals were kept at 22 ± 3 °C temperature and a 12/12 h light/dark cycle for two weeks. Animal's maintenance and surgery were performed according to the Ethical Guidelines for Research on Animals developed by the Iranian Ministry of Health and Medical Education. A critical-sized rat calvarial defect was made to investigate osteoconductivity of the fabricated scaffolds. Dexamethasone (1.6 mg/kg, i.p.) was daily injected to all animals for two weeks and cyclosporine-A was also added to their drinking water (210 mg/l). First, animals were anesthetized by ketamine (20 mg/kg) and xylazine (2 mg/kg) solution through intra peritoneal injection. After that, the dorsal surface of head was shaved and sagittal plane across the cranium was cut and then with elevating the skin and periosteum, the calvarial bone was exposed. Then, a critical-sized circular defect with 8 mm diameter was produced with a saline-cooled trephine drill. Experiments were performed in five groups including, control without any filling, while in the other four groups defects were filled with PLLA, human BM-MSC-seeded PLLA, PLLA-nHA-Gelatin (PLLA/NP) and human BM-MSC-seeded PLLA/NP. At the end of surgery, skin was properly sutured at the defect site for animals in all groups.

Radiographic analysis (RG)

After eight weeks, animals were euthanized and their craniums were removed and transferred to a 10% formalin solution for fixation. Then radiographic (RG) assessment was performed using a direct digital mammography equipment (Konica Minolta, Regius model 110HQ).

Histological investigations

For histological examination, samples were decalcified via inserting in ethylenediaminetetraacetic acid/hydrochloric acid (HCl) and then embedded in paraffin, tissue sections were then prepared with 35-μm thickness and finally samples were stained with hematoxylin and eosin (H&E). The new-formed bone at the defect site was quantified using a computer-assisted Image-Pro Plus System (Media Cybernetics, Silver Springs, MD) from prepared images by a light microscope.

Statistical analysis

Viability assays were repeated three times for each sample and animal studies were performed on 8 rats in each group. IBM SPSS Statistics software version 22 (IBM, USA) and GraphPad Prism version 6 (GraphPad Software, USA) were applied for statistical analyses. Shapiro–Wilk normality test was applied for normalization of the acquired data. One/two-way analyses of variance (ANOVA) and Tukey's multiple comparison tests were applied for calculating significant statistical differences between various groups based on the mean ± S.D. (Standard deviation) and probability values less than 0.05 were considered as significant, and are indicated by a star (*).

Results

Scaffold characterization

Morphological evaluation

Fabricated PLLA and PLLA/NP were morphologically characterized using SEM and results demonstrated that PLLA has fibers in nanometer size, which were bead-free, smooth and porous with interconnected pores (Fig. 1a). These characteristics were similar in PLLA/NP scaffolds, although accumulation of gelatin and HA nanoparticles made it difficult to observe fibers properly (Fig. 1b).

Biocompatibility

For non-toxicity and biocompatibility assessment of fabricated scaffolds, frozen human BM-MSCs at passage two were thawed (Fig. 1c) and cultured on the surface of the scaffolds as well as TCPs as a control. Quantitative and qualitative assays by MTT assay and SEM imaging were performed, respectively to evaluate biocompatibility of the scaffolds. MTT results demonstrated that on days 3 and 7 after cell seeding, human BM-MSC proliferation was significantly increased when cultured on the surface of the scaffolds compared to the control group (Fig. 1d). It should be mentioned that cells cultured on PLLA/NP showed a significantly higher proliferation rate compared with cells cultured on PLLA at both time intervals.

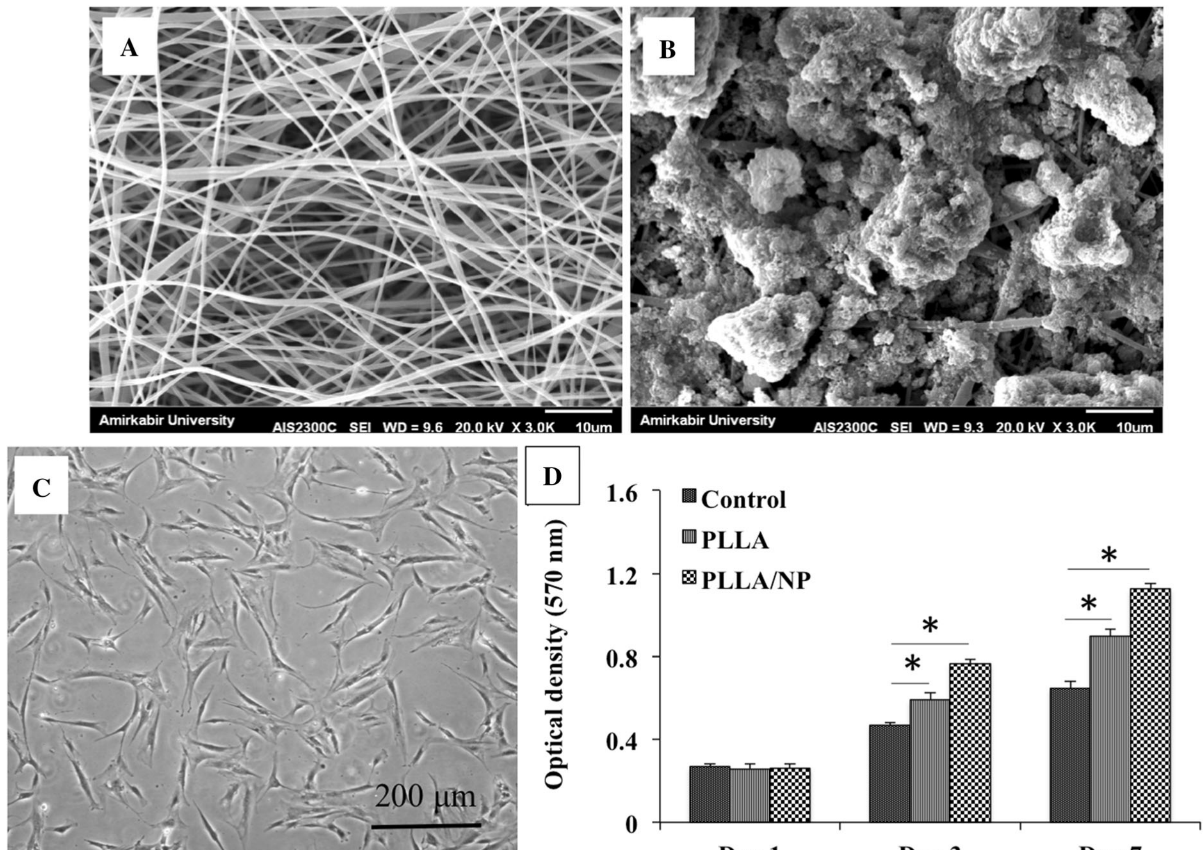


Fig. 1 Scanning electron microscopic images of PLLA **a** and gelatin-HA-coated PLLA scaffolds (PLLA/NP) **b**; Human bone marrow derived mesenchymal stem cells (BM-MSCs) at passage two **c** and viability assay of human BM-MSCs cultured on PLLA and PLLA/NP scaffolds and tissue culture plates (TCPs) on days 1, 3 and 7 after cell seeding. The significant difference ($P < 0.05$) between the groups is indicated by asterisks

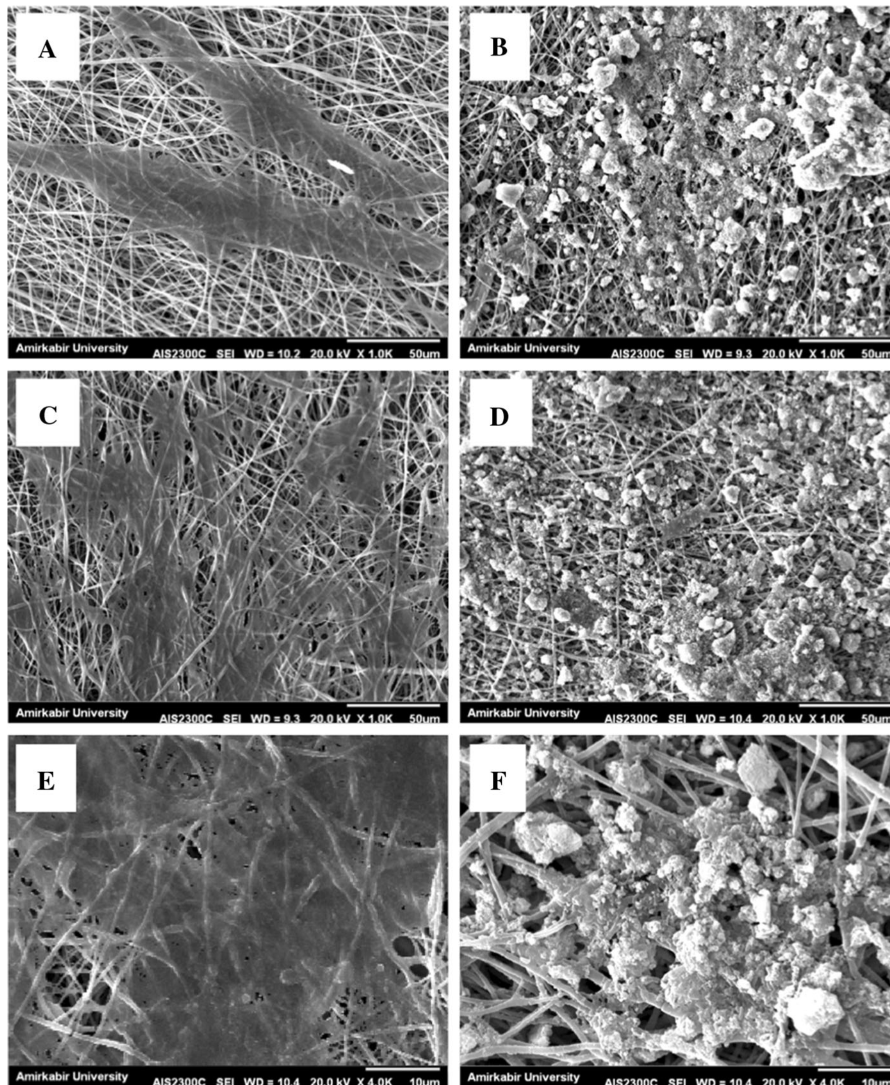


Fig. 2 Scanning electron microscopic images of human bone marrow derived mesenchymal stem cells (BM-MSCs) cultured on PLLA and gelatin-HA-coated PLLA scaffolds (PLLA/NP) on day 3 **a**, **b** and day 7 **c**, **d**, respectively. Human BM-MSCs cultured on PLLA and PLLA/NP scaffolds on day 7 with higher magnification **e**, **f**, respectively

For qualitative biocompatibility evaluation of the fabricated scaffolds, human BM-MSCs cultured on PLLA and PLLA/NP were examined by SEM and results demonstrated that cells properly attached, grew and expanded on the surface of the PLLA (Fig. 2a, c) and PLLA/NP (Fig. 2b, d) on days 3 and 7, respectively. However, due to presence of gelatin and HA on the surface of PLLA/NP, mineralization on PLLA/NP scaffold was obviously detected (Fig. 2f) compared to the PLLA scaffold (Fig. 2e).

In vivo evaluations

Macroscopic observations

Critical-sized rat calvarial defect (Fig. 3a) was created and then scaffolds with and without human BM-MSCs were implanted at the defect site (Fig. 3b). Eight weeks after implantation, skin was opened and the defect site was visualized macroscopically. No signs of inflammation, infection or bleeding were observed and all implanted scaffolds were well integrated into

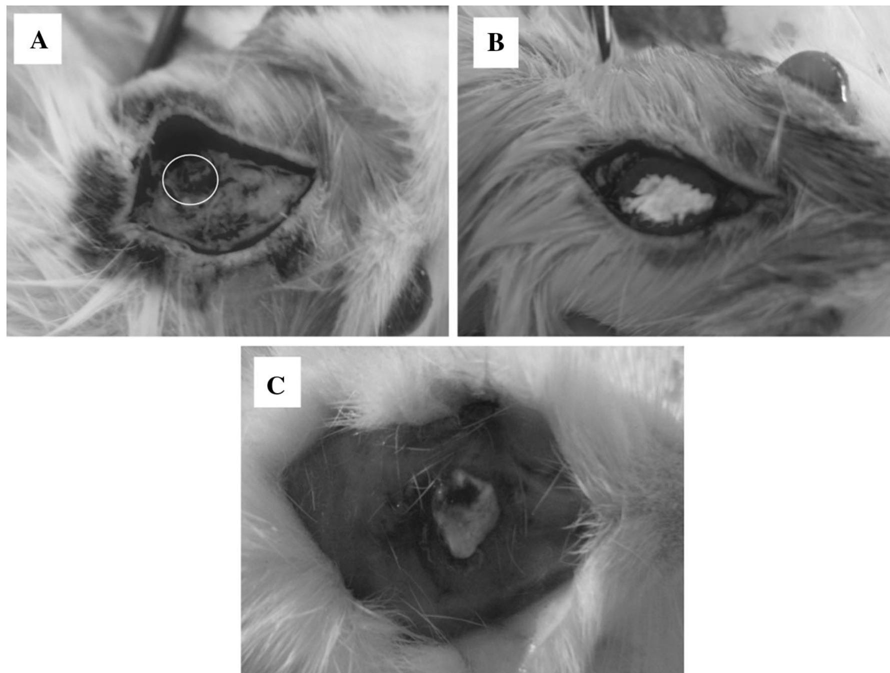


Fig. 3 Macroscopic visualization of critical-sized calvarial defect created in rats before **a** and after **b** implantation, and eight weeks after implantation with a nanofibrous scaffold **c**

the bone at the defect site with no sign of encapsulation or noticeable foreign body reaction (Fig. 3c).

RG assessment

The newly formed bone at the defect site was evaluated by digital mammography on the fixed calvarium specimens eight weeks after surgery (Fig. 4). Quantifying radiologic images (Fig. 4f) demonstrated that the highest reconstruction of the calvarial defects was detected in animals treated with human BM-MSCs cultured on PLLA/NP (Fig. 4e), although treating animals with PLLA/NP also demonstrated significantly higher bone regeneration among other groups (Fig. 4d). In addition, newly bone formation in animals treated with human BM-MSCs cultured on PLLA (Fig. 4c) was also significantly higher than animals treated with empty PLLA (Fig. 4b) and it was also significantly higher than control group (Fig. 4a).

Histological staining

Finally, a histological evaluation was carried out to compare the regeneration of the bone at the defect site 8 weeks after surgery (Fig. 5). The area of regenerated bone is reported quantitatively in Fig. 5f while measured using Image-J software. The highest amount of bone regeneration was observed in the rats treated with human BM-MSCs cultured on PLLA/NP (Fig. 5e). As shown, newly formed bone has penetrated into the scaffold structure and used it as a suitable matrix for tissue formation. However, bone regeneration in animals treated with PLLA/NP (Fig. 5d) was also significantly higher than those animals treated with cells cultured on PLLA (Fig. 5c). Futhermore, bone regeneration in animals treated with PLLA (Fig. 5b) was higher than control group who did not received any implantation (Fig. 5a).

Discussion

In tissue engineering, selection of suitable biomaterials for making scaffolds that are biocompatible, biodegradable and bioactive is very important (Cheung et al. 2007; Rezwan et al. 2006). The scaffold is important and responsible for shaping final structure of engineered tissue and its function. Several studies showed that PLLA has many of these properties and

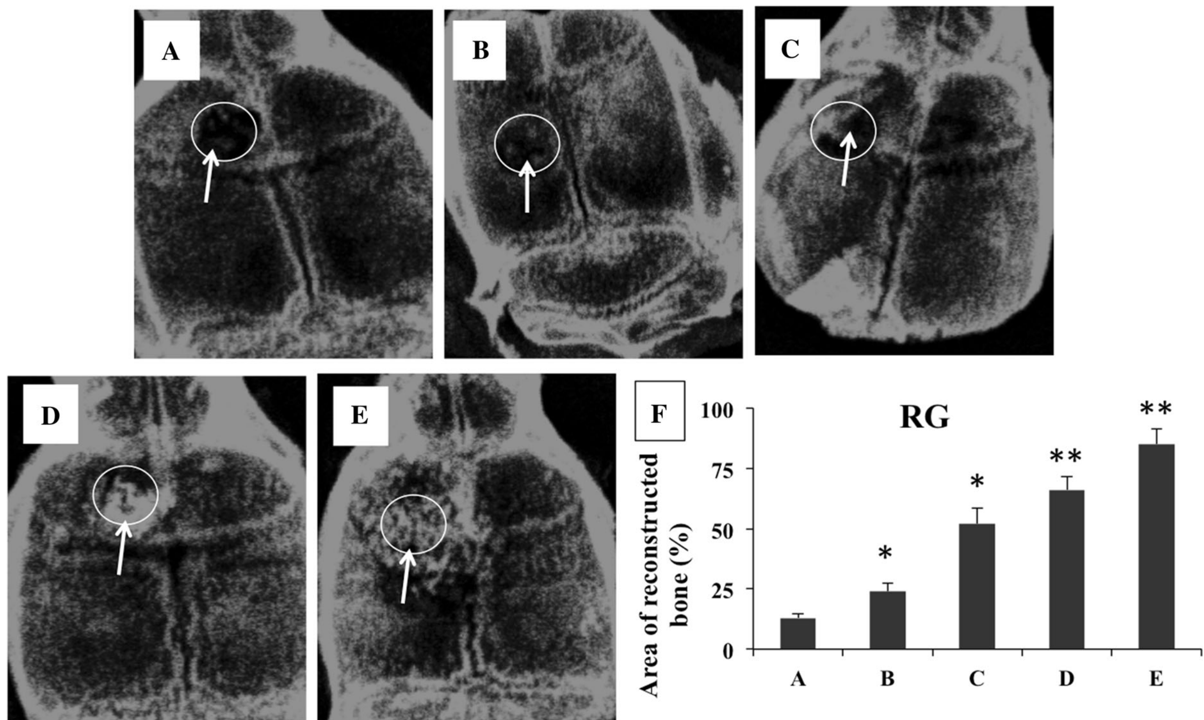


Fig. 4 Radiographic (RG) assessment of rat's calvaria eight weeks after surgery: untreated control group **a**, PLLA **b**, human bone marrow derived mesenchymal stem cell (BM-MSC)-cultured PLLA **c**, gelatin-HA-coated PLLA scaffolds (PLLA/NP) **d**, human BM-MSC-cultured PLLA/NP **e**. Reconstructed bone area obtained from quantification of RG data **f**. Significant differences ($P < 0.05$ and 0.01) between groups are indicated by one and two asterisks, respectively

is especially effective in bone tissue regeneration (Fattahi et al. 2019). On the other hand, despite poor mechanical properties hydroxyapatite is a bone-conducting bioactive material that is suitable for use as a composite in bone tissue engineering (Kattimani et al. 2016). Gelatin is also a protein that is mainly derived from collagen processing, which is the main building block of skin, tendons, bones, and vertebrates, and therefore its presence in scaffolds can greatly increase the biocompatibility and bioactivity of the scaffolds, especially to help the initial attachment of cells to the scaffold (Monroy et al. 2018).

In the present study, the biocompatibility of the composite scaffolds cultured with human BM-MSCs was investigated by SEM and MTT assays. According to the SEM images, it can be concluded that the fiber size was in the range of 850 ± 250 nm and scaffolds were smooth and bead-free. In addition, cultured cells had normal growth and expansion, and interacted with adjacent cells. The results also showed that cells not only had the ability to bind and proliferate on gelatin-HA-coated PLLA scaffolds (PLLA/NP) but also had a natural morphology. In fact, the presence of HA and gelatin nanoparticles on the scaffold increases the uptake of macromolecules such as fibronectin from the serum and this enhances their integrin binding to the cell membrane and rearrangement of F-actin fibers enhances cell proliferation and differentiation signalings (Dolatshahi-Pirouz et al. 2009; Kechagia et al. 2019). Quantitative evaluation of biocompatibility performed by MTT assay revealed that at different time points the growth of human BM-MSCs on the scaffold containing HA nanoparticles and gelatin was higher than the control scaffold. The results indicate a more favorable biological condition of the cells on the surface of the composite scaffolds containing HA and gelatin nanoparticles than the control and PLLA groups, which might be due to the improved surface specificity for attachment of more cells to the scaffold (Zandi et al. 2010). In our previous study, we also demonstrated that ALP activity and bone-related gene expression in the BM-MSCs cultured on the PLLA/NP nanofibers were significantly increased compared to

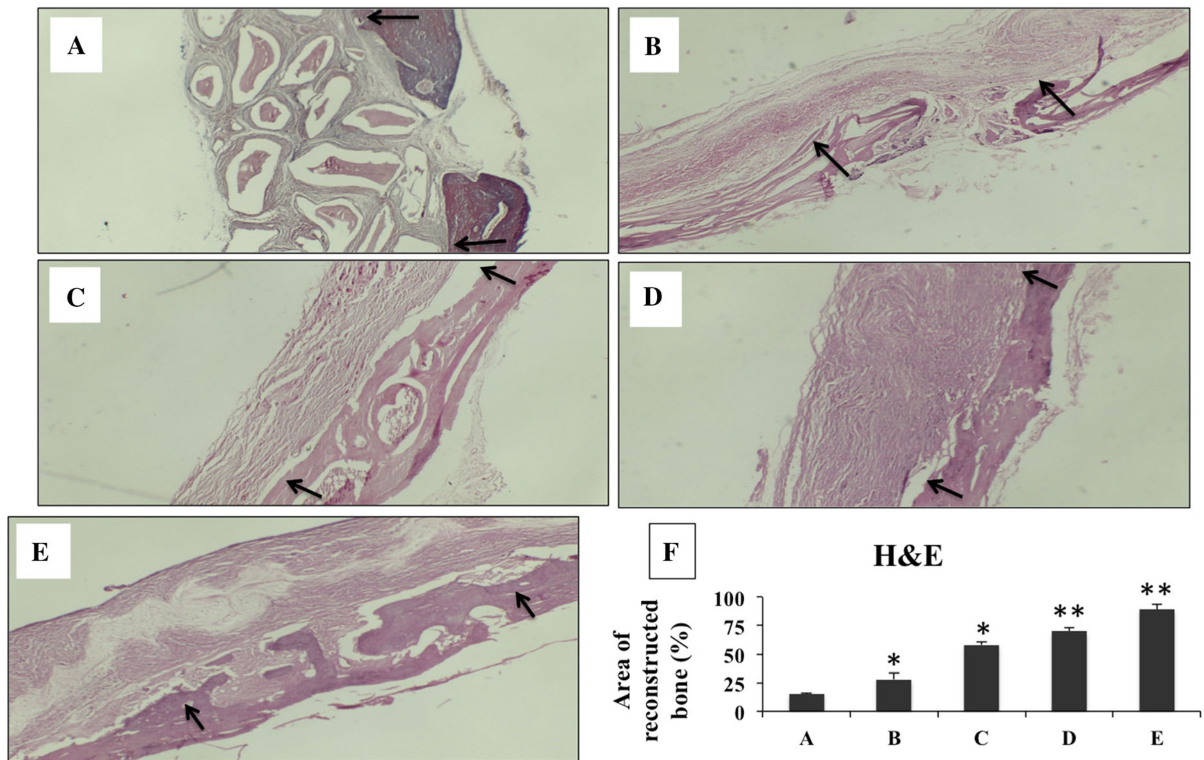


Fig. 5 H&E staining of rat's calvaria eight weeks after surgery: untreated control group **a**, PLLA **b**, human bone marrow derived mesenchymal stem cell (BM-MSC)-cultured PLLA **c**, gelatin-HA-coated PLLA scaffolds (PLLA/NP) **d**, human BM-MSC-cultured PLLA/NP **e**. Reconstructed bone area from quantification of H&E data **f**. Significant differences ($P < 0.05$ and 0.01) between groups are indicated by one and two asterisks, respectively

the BM-MSCs cultured on the PLLA nanofibers (Andalib et al. 2020).

After evaluating the biocompatibility of the fabricated scaffolds, the osteoconductivity of PLLA and PLLA/NP scaffolds with and without cells was evaluated in a critical-sized rat calvarial defect model. The macroscopic studies were performed through objective observation of the defect site eight weeks after surgery immediately after the surgical site was opened; and no complications such as inflammation, infection or bleeding were observed. Radiographic (RG) assessment was also performed using direct digital mammography and results demonstrated that the highest new bone tissue formation was detected in animals treated with human BM-MSC-seeded PLLA/NP scaffold compared to the animals treated with other scaffolds or control group. Overall, bone regeneration in animals treated with cell-seeded scaffolds was significantly better than those treated with empty scaffolds. After that, microscopic studies were performed using histological techniques and H&E staining. As observed, the highest bone healing was observed in the group containing composite scaffolds containing gelatin and HA nanoparticles when mesenchymal stem cells were cultured on them. In fact, better results in animals treated with human BM-MSC-seeded PLLA/NP scaffold might be due to the presence of factors such as HA as an important compound in bone structure (Ratnayake et al. 2017), gelatin, which has a structure similar to collagen, one of the most abundant proteins in the bone matrix (Murata 2012) and ultimately the BM-MSCs that have been able to differentiate well into osteoblasts and secrete calcium, which provides more suitable conditions for repair. In agreeing with our results Daei-farshbaf et al. examined the osteoconductivity of human adipose derived stem cells (ASCs) seeded on bioceramic-collagen scaffolds in a critical-sized rat calvarial model and their results after 6 weeks demonstrated that the combination of ASCs and Bio-

Oss-collagen scaffold synergistically enhanced bone regeneration and reconstruction compared to each one of them alone (Daei-farshbaf et al.2014).

In another study, Seyedjafari et al. demonstrated that the osteogenic differentiation potential of human cord blood derived unrestricted somatic stem cells (USSCs) was increased while cultured on nHA-coated PLLA nanofibrous scaffold in vitro. They also showed that this scaffold led to a better ectopic bone formation in mice as compared with scaffolds without nHA or USSCs (Seyedjafari et al. 2010). The importance of cells present on the scaffolds was also previously reported by Ardeshirylajimi et al., where they compared newly bone formation at the rat calvarial defect by implanting induced pluripotent stem cells (iPSCs) cultured on polyethersulfone nanofibers. Their histological and micro-computed tomography studies showed a higher bone reconstruction at the defect site when compared with animals treated with empty scaffolds (Ardeshirylajimi et al. 2013).

Conclusion

According to the results of macroscopic and microscopic studies, the PLLA/NP scaffold had a positive effect on bone regeneration of rat calvarial defect, however, in animals treated with human BM-MSC-cultured PLLA/NP scaffolds, this effect was significantly higher. It is concluded that HA and gelatin in addition to being non-toxic, when placed on the nanofiber scaffolds, provide a suitable substrate for cell growth, differentiation, and migration. It seems that the aforementioned composite scaffold after seeding with BM-MSCs plays a potent role in bone healing process and can be considered as a suitable therapeutic approach in the repair of extensive bone lesions.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in this project.

References

Amani H, Arzaghi H, Bayandori M, Dezfuli AS, Pazoki-Toroudi H, Shafiee A, Moradi L (2019) Controlling cell behavior through the design of biomaterial surfaces: a focus on surface modification techniques. Adv Mater Int 6:1900572

Andalib N, Kehtari M, Seyedjafari E, Motamed N, Matin MM (2020) Improved efficacy of bio-mineralization of human mesenchymal stem cells on modified PLLA nanofibers coated with bioactive materials via enhanced expression of integrin α2β1. PolymAdvTechnol 31:2325–2338

Ardeshirylajimi A, Dinarvand P, Seyedjafari E, Langroudi L, Adegani FJ, Soleimani M (2013) Enhanced reconstruction of rat calvarial defects achieved by plasma-treated electrospun scaffolds and induced pluripotent stem cells. Cell Tissue Res 354:849–860

Bageshlooyafshar B et al (2019) Zinc silicate mineral-coated scaffold improved in vitro osteogenic differentiation of equine adipose-derived mesenchymal stem cells. Res Vet Sci 124:444–451

Betz RR (2002) Limitations of autograft and allograft: new synthetic solutions. Orthopedics 25:S561–S570

Campbell A et al (2015) Concise review: process development considerations for cell therapy. St Cells Trans Med 4:1155–1163

Cheung H-Y, Lau K-T, Lu T-P, Hui D (2007) A critical review on polymer-based bio-engineered materials for scaffold development. Compos B Eng 38:291–300

Conway JD (2010) Autograft and nonunions: morbidity with intramedullary bone graft versus iliac crest bone graft Orthopedic. Clinics 41:75–84

Cui W, Zhou Y, Chang J (2010) Electrospunnanofibrous materials for tissue engineering and drug delivery. SciTechnolAdv Mater 11:014108

Daei-farshbaf N et al (2014) Bioceramic-collagen scaffolds loaded with human adipose-tissue derived stem cells for bone tissue engineering. MolBiol Rep 41:741–749

Dahlin RL, Kasper FK, Mikos AG (2011) Polymeric nanofibers in tissue engineering. Tissue Eng Part B Rev 17:349–364

Dolatshahi-Pirouz A, Jensen T, Foss M, Chevallier J, Besenbacher F (2009) Enhanced surface activation of fibronectin upon adsorption on hydroxyapatite. Langmuir 25:2971–2978

Fattahi FS, Khoddami A, Avinc O (2019) Poly (lactic acid)(-PLA) nanofibers for bone tissue engineering. J Text Polym 7(2):47–64

Henkel J et al (2013) Bone regeneration based on tissue engineering conceptions—a 21st century perspective. Bone Res. https://doi.org/10.4248/BR201303002

Holzwarth JM, Ma PX (2011) Biomimetic nanofibrous scaffolds for bone tissue engineering. Biomaterials 32:9622–9629

Huang B, Caetano G, Vyas C, Blaker JJ, Diver C, Bártolo P (2018) Polymer-ceramic composite scaffolds: the effect of hydroxyapatite and β-tri-calcium phosphate. Materials 11:129

Izadpanahi M, Seyedjafari E, Arefian E, Hamta A, Hosseinzadeh S, Kehtari M, Soleimani M (2018) Nanotopographical cues of electrospun PLLA efficiently modulate non-coding RNA network to osteogenic differentiation of mesenchymal stem cells during BMP signaling pathway. Mater Sci Eng C 93:686–703

Jing Y, Quan C, Liu B, Jiang Q, Zhang C (2016) A mini review on the functional biomaterials based on poly (lactic acid) stereocomplex. Polym Rev 56:262–286

Kattimani VS, Kondaka S, Lingamaneni KP (2016) Hydroxyapatite–-Past, present, and future in bone regeneration. Bone Tissue Regen Insights 7:BTRI. S36138. https://doi.org/10.4137/btri.s36138

Kechagia JZ, Ivaska J, Roca-Cusachs P (2019) Integrins as biomechanical sensors of the microenvironment. Nat Rev Mol Cell Biol. https://doi.org/10.1038/s41580-019-0134-2

Kehtari M et al (2019) Decellularized Wharton's jelly extracellular matrix as a promising scaffold for promoting hepatic differentiation of human induced pluripotent stem cells. J Cell Biochem 120:6683–6697

Mahboudi H et al (2018) Enhanced chondrogenesis of human bone marrow mesenchymal stem cell (BMSC) on nanofiber-based polyethersulfone (PES) scaffold. Gene. https://doi.org/10.1016/j.gene.2017.11.073

Mahboudi H, Sadat Hosseini F, Kehtari M, Hassannia H, Enderami SE, Nojehdehi S (2020) The effect of PLLA/PVA nanofibrous scaffold on the chondrogenesis of human induced pluripotent stem cells. Int J Polym Mater PolymBiomater 69:669–677

Monroy DAP, Bravo JMC, Mercado IES, Gómez LJV (2018) Gelatin and collagen nanofiber scaffolds for tissue engineering. Tissue Regen. https://doi.org/10.5772/intechopen.73316

Moradi SL, Golchin A, Hajishafieeha Z, Khani MM, Ardeshirylajimi A (2018) Bone tissue engineering: adult stem cells in combination with electrospunnanofibrous scaffolds. J Cell Physiol 233:6509–6522

MousaeiGhasroldasht M et al (2019) Application of mesenchymal stem cells to enhance non-union bone fracture healing. J Biomed Mater Res Part A 107:301–311

Murata M (2012) Collagen biology for bone regenerative surgery. J Korean Assoc Oral and Maxillofacial Surg 38:321–325

Pawelec KM, Planell JA (eds) (2019) Bone Repair Biomaterials, 2nd ed. Cambridge, England: Woodhead Publishing Ltd

Pesaraklou A, Mahdavi-Shahri N, Hassanzadeh H, Ghasemi M, Kazemi M, Mousavi NS, Matin MM (2019) Use of cerium oxide nanoparticles: a good candidate to improve skin tissue engineering. Biomed Mater 14:035008

Ratnayake JTB, Mucalo M, Dias GJ (2017) Substituted hydroxyapatites for bone regeneration: a review of current trends. J Biomed Mater Res Part B ApplBiomater 105:1285–1299

Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27:3413–3431

Santoro M, Shah SR, Walker JL, Mikos AG (2016) Poly (lactic acid) nanofibrous scaffolds for tissue engineering. Adv Drug Deliv Rev 107:206–212

Seshi B, Kumar S, Sellers D (2000) Human bone marrow stromal cell: coexpression of markers specific for multiple mesenchymal cell lineages. Blood Cells Mol Dis 26:234–246

Seyedjafari E, Soleimani M, Ghaemi N, Shabani I (2010) Nanohydroxyapatite-coated electrospun poly (l-lactide) nanofibers enhance osteogenic differentiation of stem cells and induce ectopic bone formation. Biomacromol 11:3118–3125

Shafiee A (2020) Design and fabrication of three-dimensional printed scaffolds for cancer precision medicine. Tissue Eng Part A 26(5–6):305–317

Shafieian R et al (2017) Effects of human adipose-derived stem cells and platelet-rich plasma on healing response of canine alveolar surgical bone defects. Arch Bone J Surg 5:406

Shibuya N, Jupiter DC (2015) Bone graft substitute: allograft and xenograft. ClinPodiatr Med Surg 32:21–34

Venkatesan J, Kim S-K (2014) Nano-hydroxyapatite composite biomaterials for bone tissue engineering—a review. J Biomed Nanotechnol 10:3124–3140

Wu S, Liu X, Yeung KWK, Liu C, Yang X (2014) Biomimetic porous scaffolds for bone tissue engineering. Mater SciEng R Rep 80:1–36

Zandi M, Mirzadeh H, Mayer C, Urch H, Eslaminejad MB, Bagheri F, Mivehchi H (2010) Biocompatibility evaluation of nano-rod hydroxyapatite/gelatin coated with nano-HAp as a novel scaffold using mesenchymal stem cells. J Biomed Mater Res Part A An Off J SocBiomaterJpnSocBiomaterAustSocBiomater Korean SocBiomater 92:1244–1255

Zhou H, Lee J (2011) Nanoscale hydroxyapatite particles for bone tissue engineering. ActaBiomater 7:2769–2781

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