Am J Stem Cells 2023;12(4):83-91
www.AJSC.us /ISSN:2160-4150/AJSC0152242

Original Article

Evaluation of osteoconductive effect of polycaprolactone (PCL) scaffold treated with *Aloe vera* on adipose-derived mesenchymal stem cells (ADSCs)

Maryam Teymori[1], Ehsan Karimi[1], Ehsan Saburi[2,3]

[1]Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran; [2]Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Received July 16, 2023; Accepted October 17, 2023; Epub October 20, 2023; Published October 30, 2023

Abstract: Background: Adipose-derived mesenchymal stem cells (ADSCs) hold promise for bone tissue engineering because of their ability to differentiate into a variety of cell lineages. In tissue engineering, composite scaffolds made of natural and synthetic polymers have also attracted interest. Modification of scaffolds with various substances, including Aloe Vera, is expected to play a useful role in the repair of damaged tissues, including bone. Method: ADSCs were isolated and seeded in three groups on an Aloe Vera-modified PCL scaffold: 1. Polycaprolactone (PCL) scaffold group, 2. PCL/Aloe Vera scaffold group, and 3. TCPS (Tissue Culture Polystyrene) group. Subsequently, staining with Oil red and Alizarin Red was performed to assess the ability of ADSCs to differentiate into fat and bone cells. Cell viability was determined by the resazurin assay on days 1, 3, and 5. Calcium content and alkaline phosphatase activity (ALP) were determined with kits on days 7, 14, and 21. RNA was extracted, and cDNA was synthesized. Finally, the expression of marker genes for bone differentiation like osteogenic markers such as Osteonectin (ON), Osteocalcin (OC), RUNX Family Transcription Factor 2 (RUNX2), Collagen type I alpha 1 (COL1) was evaluated by real-time PCR. Results: Aloe vera-treated PCL scaffolds showed improved biocompatibility compared with untreated scaffolds ($P<0.05$). In addition, treated scaffolds promoted osteogenic differentiation of ADSCs, as evidenced by increased expression of osteogenic markers such *ON*, *OC*, *RUNX2*, *COL1* compared with PCL scaffold and TCPS ($P<0.05$). Furthermore, ALP and calcium content assay confirmed improved mineral deposition on PCL scaffolds treated with Aloe vera, indicating enhanced osteoconductivity ($P<0.05$). Conclusion: Our data suggest that a PCL scaffold mixed with Aloe Vera gel has promising osteoconductive potential, which can be used as a natural polymer for tissue engineering of bone and promote bone regeneration.

Keywords: Mesenchymal stem cell (MSCs), bone tissue engineering, PCL, scaffold, Aloe vera

Introduction

Tissue engineering (TE) has emerged as a promising solution to replace injured tissue-related organs by developing in vitro tissues to in vivo repair damage [1]. The process of TE requires three factors: I. suitable cells, II. suitable scaffolds, and III. growth factors and differentiation stimuli to achieve the appropriate cell lineage [2]. With the advent of functionally appropriate materials and biocompatible and biodegradable materials, there is an increasing demand for technologies that can correct bone defects naturally, which has led to a great interest in bone tissue engineering research [3]. The extracellular matrix (ECM) is produced by cells located in tissues and organs [4]. In developing scaffolds, it is better to imitate some special properties of the ECM, such as large-volume surface properties, biocompatibility, and biodegradability. Besides, it is better to produce scaffolds with high mechanical strength and low-cost [5]. Extracellular matrix scaffolds can create a desirable regenerative microenvironment, promote TE, and serve as an induction model for repair and regeneration [4]. Scaffolds have been developed to enhance adhesion, growth, cell division, and migration functions of cultured cells. Therefore, they facilitate the adhesion of cells and help them grow.

Poly-capro-lactone (PCL), like poly-glycolic-acid (PGA) and poly-lactic-acid (PLA), are linear aliphatic polyesters and synthetic biopolymers [6, 7]. Due to their controllable biodegradability, they are mainly used to construct temporary tissue engineering scaffolds. The degradation rate of PCL is lower than that of PGA and PLA. The porosity size and three-dimensional structure of PCL mimic the standard ECM and can enhance adhesion, growth, proliferation, and cell differentiation [2, 8]. PCL is a biodegradable, biocompatible, flexible, and non-toxic polymer with hydrophobicity, easy processability, low antigenicity, and low melting point (about 60°C) [9, 10]. When the PCL polymer enters the body, the ester bond is broken down into water and carbon dioxide and has no adverse effects on cell metabolism [11, 12].

Using macromolecules and natural materials in combination or the form of a coating can increase the biocompatibility and hydrophilicity of scaffolds and provide better adhesion of cells to the scaffold [13]. Aloe vera is a natural polymeric gel from the lily family (Liliaceae) with antibacterial, antioxidant, hydrophilic, and anti-inflammatory properties. The gel in the leaves of the aloe vera plant is a clear liquid, which consists of 99% water and also contains 20 amino acids, including the essential amino acids. It has vitamins such as A, C, thiamine, niacin, and B12 and contains choline and folic acid [13].

Another important component of tissue engineering is appropriate cells, such as MSCs. They are multipotent cells with high proliferative capacity [14]. The best sources for obtaining MSCs are adipose tissue and bone marrow [15]. MSCs derived from adipose tissue (AD-MSCs) can differentiate into bone, adipose tissue, joint, muscle, and nerve. These cells have attracted more attention in bone tissue engineering because they are readily available in large quantities, easy to obtain, and relatively non-invasive [16, 17]. Adipose tissue is considered an unlimited and reliable source of stem cells. Unlike bone marrow, biopsy of adipose tissue is painless, and there are no moral restrictions for obtaining adipose tissue. High proliferation rate and low invasiveness after surgery are among the advantages of ADSCs for cell therapy [16].

This study presents a novel approach of incorporating Aloe vera extract into PCL scaffold for tissue engineering applications. Aloe vera, widely known for its regenerative capabilities, is used to improve scaffold performance and stimulate tissue regeneration [13]. The main objective of this study is to evaluate the osteoconductive properties of Aloe vera-infused scaffold, which plays a crucial role in supporting bone formation and promoting important cellular processes such as attachment, proliferation and differentiation. Through the synergistic combination of Aloe vera and PCL, this research contributes to the expanding field of using natural products in conjunction with biomaterials, advancing the field of tissue engineering strategies. Therefore, the current study was conducted to evaluate the osteoconductive effect of PCL scaffold modified with Aloe Vera on ADSCs. The results showed that the coating of PCL by the Aloe Vera gel could be considered a potential candidate for bone tissue engineering.

Materials and methods

Cell culture

ADSCs, as described previously, were isolated from adipose tissue (Imam Hospital, Tehran, Iran) [18]. ADSCs were isolated and cultured (12×10^3 cells per well) in a T-75 culture flask (75 cm^2) in DMEM (Dulbecco's Modified Eagle Medium) high glucose medium with 15% fetal bovine serum (FBS). Then, the fourth passage of cells was planted in 48 well plates (12×10^3 cells per well). When 80% of the surface of a culture vessel was covered with cells, the cells were cultured to test their differentiation ability and then seeded onto prepared scaffolds (**Figure 1**).

The differentiation ability of ADSCs

Oil red staining: At the end of the 21-day period of bone differentiation, cells were washed three times with PBS, then fixed with formalin (10%), and incubated for 10 minutes. Formaldehyde (10%) was added and incubated for 80 minutes. Cells were washed with isopropanol (60%) for 5 minutes and allowed to dry. Oil red was added, washed with DEPC-treated water, and finally, the cells were observed by an inverted microscope.

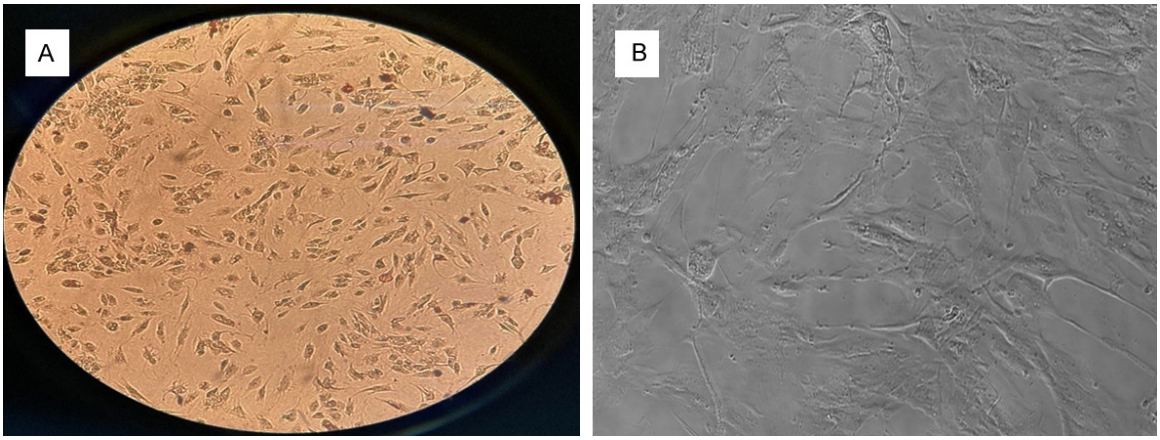

Figure 1. ADSCs under the inverted microscope. (A) 40×, (B) 400×.

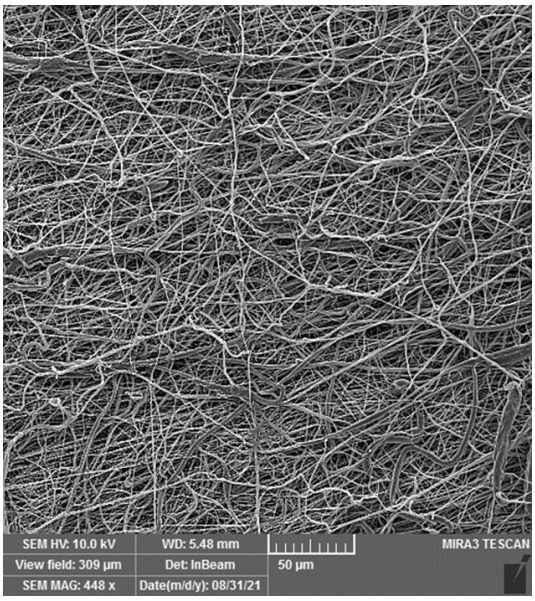

Figure 2. SEM microscopy of PCL scaffold (Magnification is 448×).

Alizarin red mineral staining: At the end of the 21-day period of bone differentiation, cells were washed three times with PBS and fixed with paraformaldehyde (4%) and incubated for 15 minutes. Cells were washed with DEPC-treated water, stained with alizarin red, and incubated for 15 minutes. Then they were washed with DEPC-treated water and observed with an inverted microscope.

Scaffold production

This scaffold was prepared by electrospinning. 680,000 g/mol PCL powder was dissolved in the solvent (2,2,2 trifluoroacetyl; Merck, Germany) [19]. The PCL solution (2 wt%) was thrown from the syringe head (the distance between the needle tip and the collector was 15 cm) into the collector (target side) with a voltage potential of 20 kV and a flow rate of 0.6 ml/h. This work was performed 8-10 h for the scaffold preparation. The prepared scaffold was sterilized with plasma, ethanol, and UV. Then the prepared Aloe Vera gel from the plant leaf, was placed on the scaffold and incubated overnight in the refrigerator [13] (**Figure 2**).

Hydrophilicity of scaffolds

The fabricated nanofiber scaffolds were tested using a contact angle conveyor (Krüss, Hamburg, Germany) to check the hydrophilicity of these scaffolds at room temperature.

Mechanical properties of the scaffold

The tensile test was performed using the Universal Materials Testing Machine (SANTAM Company, Model: STM 20, Tehran, Iran) to evaluate the mechanical properties of the PCL and PCL/Aloe Vera nanofiber scaffolds. In this test, a cell load of 200 N was applied at a rate of 1 mm/min. Subsequently, the nanofiber scaffolds were cut into 30×5×1 mm stretch strips.

Cell viability assay

The resazurin assay (7-hydroxy-10-oxidophenoxazine-10-yum-3-van) was used to assess the cell viability or toxicity of the prepared scaffolds. Cells were plated into 96-well plates (12×10^3 cells per well). After three periods (24

Table 1. Primer sequences used in this study

Gene	Primer Sequences (5'>3')	Product Size (bp)
B2m-F	TGGAAAGAAGATACCAAATATCGA	201
B2m-R	GATGATTCAGAGCTCCATAGAGCT	
Collagen I-F	TGGAGCAAGAGGCGAGAG	121
Collagen I-R	CACCAGCATCACCCTTAGC	
Runx2-F	GCCTTCAAGGTGGTAGCCC	66
Runx2-R	CGTTACCCGCCATGACAGTA	
Osteonectin-F	AGGTATCTGTGGGAGCTAATC	224
Osteonectin-R	CGTTACCCGCCATGACAGTA	
Osteocalcin-F	GCAAAGGTGCAGCCTTTGTG	80
Osteocalcin-R	GGCTCCCAGCCATTGATACAG	

hours, 3, and 5 days), the dye resazurin was added to the cells, and they were incubated in a CO_2 incubator for 2 hours. Then, the results were read with the Epoch Plate Reader (BioTek, United States) at 630 nm. ADSCs cultured on TCPS plates were considered controls compared with the PCL and PCL/Aloe Vera groups.

Alkaline phosphatase activity

ALP was measured after cell seeding according to the instructions of the Pars Azmoun kit for quantitative detection of alkaline phosphatase (Pars Azmoun, Tehran, Iran) on days 7, 14, and 21. Cells were lysed with the radioimmunoprecipitation (RIPA) buffer and then centrifuged at 15,000 rpm for 15 minutes at 4°C. Reagents R1 and R2 were added and optical density was read at 405 nm at intervals of 0, 1, 2, 3 minutes.

Calcium content assays

The calcium content of the three groups was extracted with HCL (0.6 N). The steps were performed according to the instructions of the kit (Pars Azmoun, Tehran, Iran), and the optical density was read at 650 nm using a spectrophotometer.

Osteogenic genes evaluation

The expression of the bone marker genes, including *ON*, *OC*, *RUNX2*, *COL1*, was evaluated in comparison with the b-actin reference gene. First, RNA extraction was performed using an RNA extraction kit (Parstous, Tehran, Iran), and then complementary DNA was synthesized using a cDNA synthesis kit (Parstous, Tehran, Iran). Real-time PCR was performed according to the kit instructions (AMPIQON, Denmark) using the primers for bone marker genes listed in **Table 1**.

Statistical analysis

Quantitative data from ALP, calcium content, and level of gene expression were expressed as mean ± standard error of the mean (SEM). Depending on the type of variable, independent t-tests or Mann Whitney U tests (IBM SPSS Statistics 20) were performed to evaluate the statistical significance of all tested values. Real-time data RT-PCR were analyzed using Rest-2009 software. Means obtained from the tests were compared using Graphpad-Prism Software Inc. (La JOLLA, California), using one-way analysis of variance (ANOVA). Statistical significance was considered at $P \leq 0.05$, indicating a significant difference between groups.

Results

AD-MSC differentiation potential

Oil-red staining: Oil red staining was used to evaluate the differentiation of MSCs into adipocytes, in which fat droplets are stained red. After 21 days, the cells exposed to the adipose differentiation medium were stained with oil red. The results were viewed with an inverted microscope. The cells differentiated into fat were observed as red fat droplets (**Figure 3**).

Alizarin-red staining: Alizarin red is used to detect calcium in tissue sections and cells cultured in vitro. The reaction of calcium with this dye leads to the formation of the alizarin red S-calcium complex. Cells were cultured in 48 well plates (12×10^3 cells per well) in bone differentiation medium for 21 days. The specialization of the cells and the formation of a mineral matrix around them were observed by Alizarin Red staining in the form of red masses (**Figure 3**).

Cell viability

Resazurin is an oxidative blue dye that is released through the cell membrane to enter the cell, where it is reduced to a fluorescent pink resorufin compound. Dead cells are unable

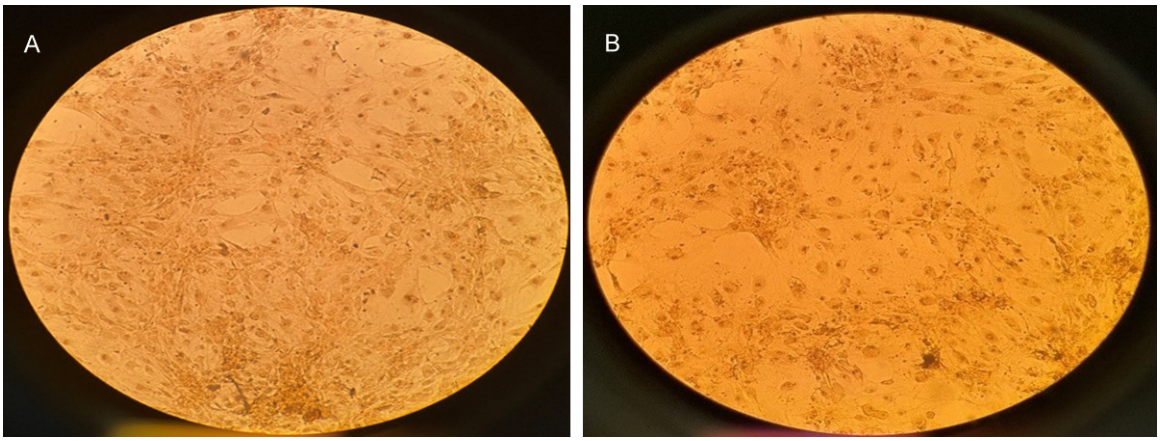

Figure 3. Differentiation of ADSCs into adipocyte and osteocyte cells. A. Alizarin-red staining. B. Oil-red staining (40×).

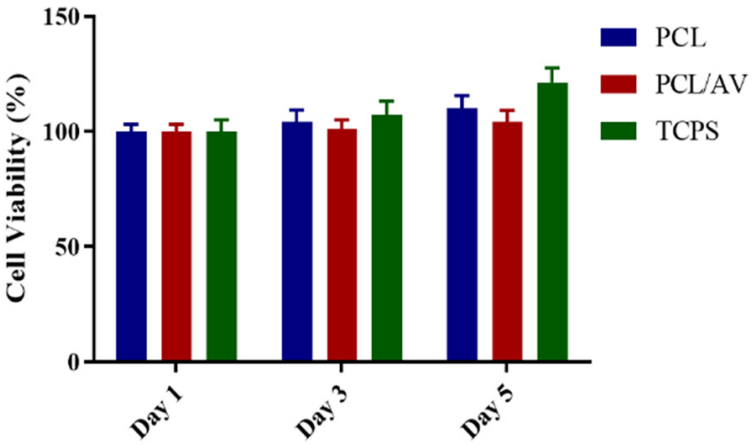

Figure 4. Cell Viability. Cell viability of the PCL and PCL-gel compared with TCPS (as control). The PCL and the PCL-gel have no toxic and lethal effect.

to reduce resazurin. The result showed that the PCL scaffold had no toxic or lethal effect. Aloe vera, which was added to PCL to modify the surface of the PCL scaffold and improve its hydrophilic properties, also had no toxic or deadly effect (**Figure 4**).

Alkaline phosphatase activity

ATP activity was assessed after differentiation induction on days 7, 14, and 21. ATP activity demonstrated the efficacy of the PCL scaffold compared with TCPS in differentiating ADSCs into bone cells ($P<0.05$). Alkaline phosphatase activity also increased in cells grown on PCL/Aloe Vera scaffold compared with cells grown on the PCL scaffold ($P<0.05$). Therefore, we conclude that the Aloe Vera-modified PCL scaffold plays a more effective role in differentiating adipose-derived stem cells into bone cells (**Figure 5**).

Calcium content

Calcium content is an important parameter in osteogenic differentiation. The results showed that the cells grown on the PCL/Aloe Vera scaffold had higher calcium content than the PCL scaffold and TCPS ($P<0.05$). Therefore, the PCL/Aloe Vera scaffold showed a more effective role in differentiating AD-MSCs into bone cells (**Figure 6**).

Gene expression

The differentiation of AD-MSCs into bone cells was assessed by the expression level of bone marker genes (*OC*, *ON*, *COL1*, *RUNX2*) using real-time RT PCR between three groups (PCL scaffold, PCL/Aloe Vera scaffold, and TCPS) on days 7, 14 and 21. The results showed a significant increase in RUNX2, COL1, OC, and ON genes in the PCL/Aloe Vera scaffold compared with the PCL scaffolds and TCPS ($P<0.05$). The expression of these genes was also significantly higher in the PCL scaffold than in the TCPS ($P<0.05$) (**Figure 7**).

Discussion

Bone marrow is the first and best-known source of MSCs, but the method of surgically col-

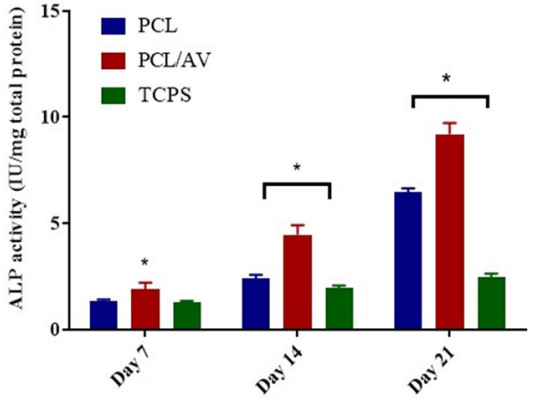

Figure 5. Alkaline phosphatase activity (*P<0.05).

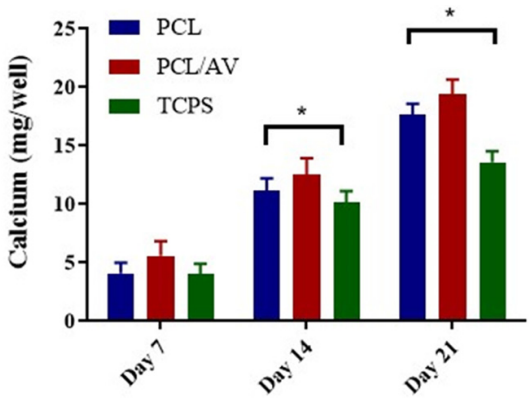

Figure 6. Calcium content (*P<0.05).

lecting bone marrow samples is very invasive. It can cause many complications, and in addition, only a small number of cells are obtained from each sample. Therefore, it is very important to use alternative sources to get MSC instead of bone marrow for laboratory and clinical studies. Adipose tissue contains many MSCs, and it is easy to obtain large quantities of them. Studies have shown that MSCs isolated from adipose tissue are similar to the same cells isolated from bone marrow in terms of morphology and surface markers, such as CD105 and CD90. The MSCs isolated from adipose tissue resemble the stem cells isolated from bone marrow in their cellular behavior in the culture medium and in their ability to differentiate into bone [20], cartilage, and fat cells. Therefore, adipose tissue was used as a valuable source of MSCs in this study.

Adipose tissue can be obtained by various methods, such as liposuction or tissue cutting. Similarly, Nejati Koushki et al. used ADSCs in stem cell therapy, which are promising candidates for effective therapy [21]. Also, Hany et al. selected adipose-derived stem cells for their study because they are an available source with high flexibility. This study was performed to evaluate the differentiation of ADSCs to bone by PCL-nano-hydroxyapatite alginate (PCL-nHA) scaffold [22]. Lee et al. who studied the two types of stem cells from bone marrow and adipose tissue, confirmed our findings and demonstrated diffuse bone tissue formation with both stem cell types using micro-computed tomography (micro-CT). They also indicated that both cell types have the same osteogenesis [23].

On the other hand, scaffolds with properties that induce bone differentiation may be more effective in osteogenesis. Due to the biocompatibility and nontoxicity of PCL polymer, it can promote cell adhesion, growth, proliferation, and differentiation by mimicking the natural extracellular matrix [24]. According to the results of the current study, the PCL scaffold was more effective than TCPS control in inducing ADSCs into bone cells. Shuang Zhao et al. used a PCL scaffold with magnesium, an important component of bone, to culture mesenchymal stem cells derived from rat bone marrow. They showed that this mixture is well-suited for bone induction and has good biological activity. They prepared PCL in different amounts in four groups: pure PCL, PCL with 5% magnesium, PCL with 10% magnesium, and PCL with 15% magnesium. Increasing the magnesium content improved the biocompatibility and bioactivity of PCL, and PCL with 10% magnesium showed the best results in repairing bone defects [12]. Therefore, the surface of PCL scaffolds can be modified by combining them with synthetic and natural polymers or other materials that improve the properties of the scaffold. These composite scaffolds are very effective for TE.

Gahar War et al. designed a hybrid PCL scaffold with nano-clay to differentiate human-MSCs into osteocytes cells. This composite scaffold showed better bioactivity in MSCs [25]. Thus, combining PCL with other polymers could help to make PCL more biocompatible and can be effective on the differentiation of MSCs cultured on it into other tissues. In another study, the deposition of hydroxyapatite on a scaffold

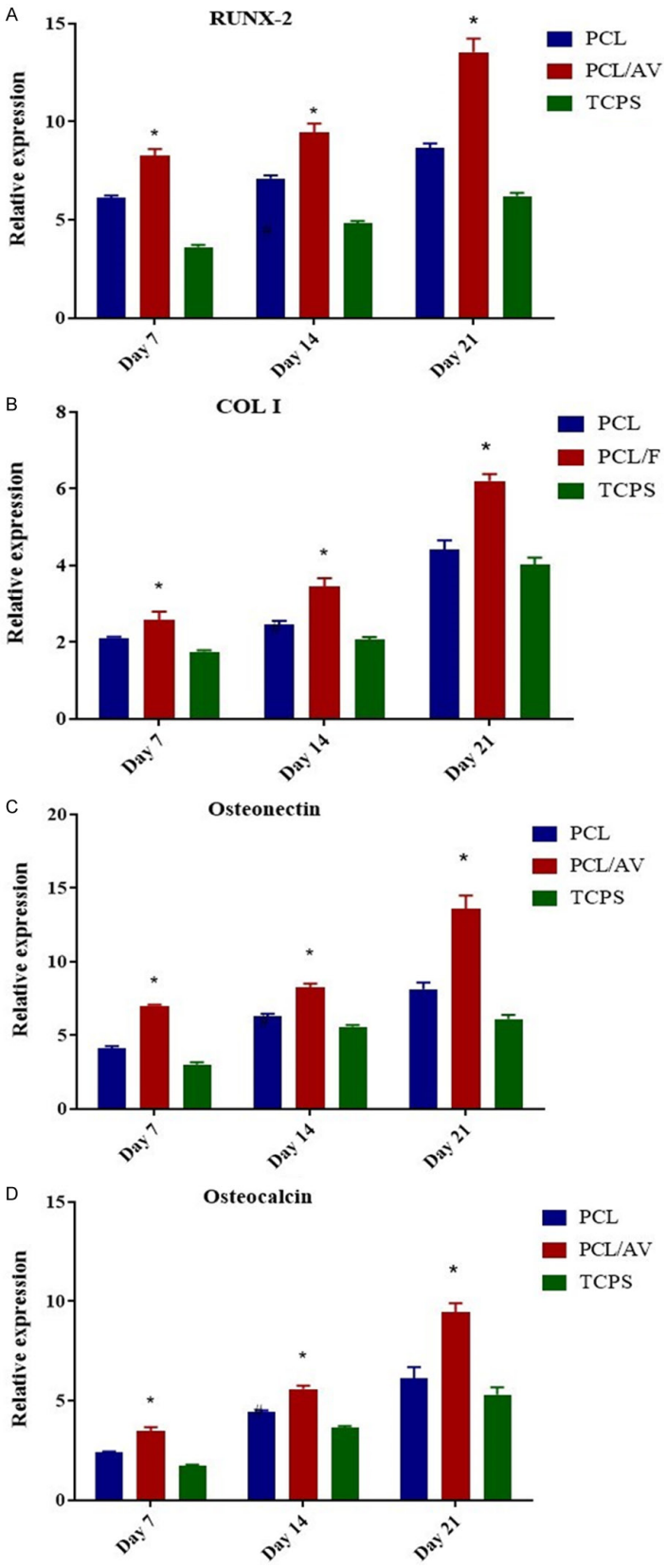

Figure 7. Relative expression of bone marker genes including Runx-2 (A), Collagen-I (B), Osteonectin (C), and Osteocalcin (D) in PCL/Aloe Vera group compared with the control groups (PCL and TCPS) (*P<0.05).

of PCL/Aloe Vera/silk fibroin increased alkaline phosphatase and expression of the osteocalcin gene, suggesting enhanced osteogenesis [26]. Several studies have also shown that the combination of PCL with materials such as Aloe Vera [27], magnesium ferrite [28], and tetracycline hydrochloride [29] can effectively improve the properties of PCL. Similarly, the results of a study showed that Aloe Vera could be effective in cell differentiation as a natural polymer for scaffolds with hydrophilic properties. Oryan et al. revealed that Aloe Vera has a healing effect on wounds. Their results also demonstrated that the Collagen-Aloe Vera scaffold maintains and improves cell viability [30]. Also, the results of Tahmasebi et al. confirmed our findings. They studied PHPV and PHPV scaffolds with Aloe Vera. They showed that PHPV scaffolds with Aloe Vera have better biocompatibility and also increase the growth and proliferation rate of stem cells and increase the rate of cell attachment to the scaffold in the early stages [13]. This study also proved the efficacy of Aloe Vera on other scaffolds. Similarly, using a natural polymer, Aloe Vera, in a mixture with PCL, Carter et al. showed that this scaffold exhibited physical, chemical, and biological properties such as hydrophilicity, mechanical strength, chemical structure, and cellular compatibility [31]. These results suggest that Aloe Vera, as a natural polymer, can be a good candidate for promoting tissue regeneration and growth.

Conclusion

According to the results of this study, we concluded that the PCL scaffold coated with Aloe Vera caused more significant differentiation of AD-MSCs into bone cells compared with the PCL scaffold. Consequently, this study showed that Aloe Vera is effective in modifying PCL scaffolds and could be effective as a natural polymer for

treating bone diseases in bone tissue engineering.

Limitation

However, it is important to note that this study focused on in vitro evaluations and experiments. Although it provided valuable insights into the osteo-conductive effect of the aloe vera-treated scaffold on adipose-derived mesenchymal stem cells, further studies in animal models and humans are needed to validate these findings.

Acknowledgements

The results described in this paper were part of student thesis.

Disclosure of conflict of interest

None.

Address correspondence to: Ehsan Saburi, Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-9151048897; E-mail: ehsansaburi@yahoo.com; Sabourie@mums.ac.ir

References

[1] Sharma P, Kumar P, Sharma R, Bhatt VD and Dhot PS. Tissue engineering; current status & futuristic scope. J Med Life 2019; 12: 225-229.

[2] Basiri A, Amooabediny G, Vasei M and Solimani M. Preparing pcl/plga hybrid nanofiber scaffold capable of controlled releasing of insulin for cartilage tissue engineering application. Journal of Shahid Sadoughi University of Medical Sciences and Health Services 2014; 22: 1175-1186.

[3] Wubneh A, Tsekoura EK, Ayranci C and Uludag H. Current state of fabrication technologies and materials for bone tissue engineering. Acta Biomater 2018; 80: 1-30.

[4] Yi S, Ding F, Gong L and Gu X. Extracellular matrix scaffolds for tissue engineering and regenerative medicine. Curr Stem Cell Res Ther 2017; 12: 233-246.

[5] Samadian H, Farzamfar S, Vaez A, Ehterami A, Bit A, Alam M, Goodarzi A, Darya G and Salehi M. A tailored polylactic acid/polycaprolactone biodegradable and bioactive 3D porous scaffold containing gelatin nanofibers and Taurine for bone regeneration. Sci Rep 2020; 10: 13366.

[6] Abazari MF, Hosseini Z, Zare Karizi S, Norouzi S, Amini Faskhoudi M, Saburi E, Enderami SE, Ardeshirylajimi A and Mohajerani H. Different osteogenic differentiation potential of mesenchymal stem cells on three different polymeric substrates. Gene 2020; 740: 144534.

[7] Mirzaei A, Saburi E, Islami M, Ardeshirylajimi A, Omrani MD, Taheri M, Moghadam AS and Ghafouri-Fard S. Bladder smooth muscle cell differentiation of the human induced pluripotent stem cells on electrospun Poly(lactide-co-glycolide) nanofibrous structure. Gene 2019; 694: 26-32.

[8] Hosseini M, Dadashi-Noshahr K, Islami M, Saburi E, Nikpoor AR, Mellati A, Mossahebi-Mohammadi M, Soleimanifar F and Enderami SE. A novel silk/PES hybrid nanofibrous scaffold promotes the in vitro proliferation and differentiation of adipose-derived mesenchymal stem cells into insulin producing cells. Polym Adv Technol 2020; 31: 1857-1864.

[9] Soleimanifar F, Hosseini FS, Atabati H, Behdari A, Kabiri L, Enderami SE, Khani MM, Ardeshirylajimi A and Saburi E. Adipose-derived stem cells-conditioned medium improved osteogenic differentiation of induced pluripotent stem cells when grown on polycaprolactone nanofibers. J Cell Physiol 2019; 234: 10315-10323.

[10] Saburi E, Atabati H, Kabiri L, Behdari A, Azizi M, Ardeshirylajimi A, Enderami SE, Ghaderian SM, Nafar M, Parvin M and Omrani MD. Bone morphogenetic protein-7 incorporated polycaprolactone scaffold has a great potential to improve survival and proliferation rate of the human embryonic kidney cells. J Cell Biochem 2019; 120: 9859-9868.

[11] Huang SH, Hsu TT, Huang TH, Lin CY and Shie MY. Fabrication and characterization of polycaprolactone and tricalcium phosphate composites for tissue engineering applications. J Dent Sci 2017; 12: 33-43.

[12] Zhao S, Xie K, Guo Y, Tan J, Wu J, Yang Y, Fu P, Wang L, Jiang W and Hao Y. Fabrication and biological activity of 3D-printed polycaprolactone/magnesium porous scaffolds for critical size bone defect repair. ACS Biomater Sci Eng 2020; 6: 5120-5131.

[13] Tahmasebi A, Shapouri Moghadam A, Enderami SE, Islami M, Kaabi M, Saburi E, Daei Farshchi A, Soleimanifar F and Mansouri V. Aloe vera-derived gel-blended PHBV nanofibrous scaffold for bone tissue engineering. ASAIO J 2020; 66: 966-973.

[14] Al-Huthaifi R, Dehghanifard A, Kaviani S, Noruzinia M, Rezaei S, Azad M, Mashhadikhan M and Solali S. Evaluation of APC gene expression and methylation pattern following bone marrow-derived mesenchymal stem cell differentiation into osteoblasts category. Journal of Payavard Salamat 2016; 9: 541-555.

[15] Hoang DH, Nguyen TD, Nguyen HP, Nguyen XH, Do PTX, Dang VD, Dam PTM, Bui HTH, Trinh MQ, Vu DM, Hoang NTM, Thanh LN and Than UTT. Differential wound healing capacity of mesenchymal stem cell-derived exosomes originated from bone marrow, adipose tissue and umbilical cord under serum- and Xeno-free condition. Front Mol Biosci 2020; 7: 119.

[16] Foroutan T, Javid MA, Manoochehri M and Naji T. Comparison of expression of reprogramming genes of induced pluripotent stem cells and mesenchymal stem cells derived bone marrow and adipose tissues. Pajoohande 2014; 19: 37-44.

[17] He Y, Liu W, Guan L, Chen J, Duan L, Jia Z, Huang J, Li W, Liu J, Xiong J, Liu L and Wang D. A 3D-printed PLCL scaffold coated with collagen type I and its biocompatibility. Biomed Res Int 2018; 2018: 5147156.

[18] Enderami SE, Soleimani M, Mortazavi Y, Nadri S and Salimi A. Generation of insulin-producing cells from human adipose-derived mesenchymal stem cells on PVA scaffold by optimized differentiation protocol. J Cell Physiol 2018; 233: 4327-4337.

[19] Daliri Shadmehri F, Karimi E and Saburi E. Electrospun PCL/fibrin scaffold as a bone implant improved the differentiation of human adipose-derived mesenchymal stem cells into osteo-like cells. International Journal of Polymeric Materials and Polymeric Biomaterials 2022; 1-8.

[20] Arab F, Aghaee Bakhtiari SH, Pasdar A and Saburi E. Evaluation of osteogenic induction potency of miR-27a-3p in adipose tissue-derived human mesenchymal stem cells (AD-hMSCs). Mol Biol Rep 2023; 50: 1281-1291.

[21] Nejati-Koshki K, Pilehvar-Soltanahmadi Y, Alizadeh E, Ebrahimi-Kalan A, Mortazavi Y and Zarghami N. Development of Emu oil-loaded PCL/collagen bioactive nanofibers for proliferation and stemness preservation of human adipose-derived stem cells: possible application in regenerative medicine. Drug Dev Ind Pharm 2017; 43: 1978-1988.

[22] Hany E, Yahia S, Elsherbeny MF, Salama NM, Ateia IM, Abou El-Khier NT, El-Sherbiny I and Abou Elkhier MT. Evaluation of the osteogenic potential of rat adipose-derived stem cells with different polycaprolactone/alginate-based nanofibrous scaffolds: an in vitro study. Stem Cell Investig 2020; 7: 14.

[23] Lee JW, Chu SG, Kim HT, Choi KY, Oh EJ, Shim JH, Yun WS, Huh JB, Moon SH, Kang SS and Chung HY. Osteogenesis of adipose-derived and bone marrow stem cells with polycaprolactone/tricalcium phosphate and three-dimensional printing technology in a dog model of maxillary bone defects. Polymers (Basel) 2017; 9: 450.

[24] Ghassemi T, Shahroodi A, Ebrahimzadeh MH, Mousavian A, Movaffagh J and Moradi A. Current concepts in scaffolding for bone tissue engineering. Arch Bone Jt Surg 2018; 6: 90-99.

[25] Gaharwar AK, Mukundan S, Karaca E, Dolatshahi-Pirouz A, Patel A, Rangarajan K, Mihaila SM, Iviglia G, Zhang H and Khademhosseini A. Nanoclay-enriched poly(varepsilon-caprolactone) electrospun scaffolds for osteogenic differentiation of human mesenchymal stem cells. Tissue Eng Part A 2014; 20: 2088-2101.

[26] Shanmugavel S, Reddy VJ, Ramakrishna S, Lakshmi BS and Dev VG. Precipitation of hydroxyapatite on electrospun polycaprolactone/aloe vera/silk fibroin nanofibrous scaffolds for bone tissue engineering. J Biomater Appl 2014; 29: 46-58.

[27] Ghorbani M, Nezhad-Mokhtari P and Ramazani S. Aloe vera-loaded nanofibrous scaffold based on Zein/Polycaprolactone/Collagen for wound healing. Int J Biol Macromol 2020; 153: 921-930.

[28] Thompson Z, Rahman S, Yarmolenko S, Sankar J, Kumar D and Bhattarai N. Fabrication and characterization of magnesium ferrite-based PCL/Aloe vera nanofibers. Materials (Basel) 2017; 10: 937.

[29] Ezhilarasu H, Ramalingam R, Dhand C, Lakshminarayanan R, Sadiq A, Gandhimathi C, Ramakrishna S, Bay BH, Venugopal JR and Srinivasan DK. Biocompatible Aloe vera and tetracycline hydrochloride loaded hybrid nanofibrous scaffolds for skin tissue engineering. Int J Mol Sci 2019; 20: 5174.

[30] Oryan A, Alemzadeh E, Mohammadi AA and Moshiri A. Healing potential of injectable Aloe vera hydrogel loaded by adipose-derived stem cell in skin tissue-engineering in a rat burn wound model. Cell Tissue Res 2019; 377: 215-227.

[31] Carter P, Rahman SM and Bhattarai N. Facile fabrication of aloe vera containing PCL nanofibers for barrier membrane application. J Biomater Sci Polym Ed 2016; 27: 692-708.