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Original Article

Investigation of the effect of the aqueous-alcoholic extract of *Colchicum kotschyi* on the polycaprolactone (PCL) scaffold to adipose-derived mesenchymal stem cells (AD-MSCs) in the process of osteogenesis

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Abstract: Objectives: Bone tissue engineering (BTE) is a promising approach for improving bone regeneration. Mesenchymal stem cells (MSCs) and scaffolds are key components in BTE, and various materials are being investigated to improve bone tissue regeneration. Colchicum kotschyi extract has been investigated for its potential to promote the proliferation and osteogenic differentiation of MSCs. Its bioactive compounds and reported anti-inflammatory properties may make it a promising candidate for bone regeneration. The aim of this study was to investigate the effect of Colchicum kotschyi extract on the osteogenic differentiation of adipose-derived (AD)-MSCs, particularly in conjunction with polycaprolactone (PCL) scaffolds. Methods: PCL scaffolds were produced using the electrospinning technique. AD-MSCs were cultured on the PCL and PCL/Colchicum scaffolds. Osteogenic differentiation of AD-MSCs was analyzed using: Alamar blue assay (cell viability), alkaline phosphatase (ALP) activity, alizarin red staining (mineralization), calcium content assay, and quantitative real-time reverse-transcription PCR (qRT-PCR) assay for osteocalcin (OC), collagen type I alpha 1 (COL1), osteonectin (ON) and runt-related transcription factor 2 (RUNX2) gene expression. Results: The synergistic effect of Colchicum extract and the PCL scaffold resulted in increased ALP activity, increased mineralization (measured by Alizarin Red staining and calcium content), and increased expression of osteogenic marker genes (OC, COL1A1, ON, and RUNX2) compared with the controls ($P<0.05$). Conclusions: The findings demonstrate that Colchicum extract may modulate the fate of MSCs toward the osteogenic lineage. The combination of Colchicum extract and PCL scaffolds enhances the osteogenic differentiation of AD-MSCs, suggesting a potential role in promoting bone tissue regeneration.

Keywords: Polycaprolactone (PCL), adipose-derived mesenchymal stem cells (AD-MSCs), bone tissue engineering, scaffold, Colchicum

Introduction

Infections, tumors, and trauma are among the growing causes of large bone defects [1]. Since these large defects cannot be regenerated by themselves, therapeutic options to repair them remain a major challenge at the clinic [2]. Today, one of the main treatments for bone lesions is bone grafting, which is associated with problems such as the risk of infection, bleeding, nerve damage, and nonunion. Therefore, scientists have been looking for alternative ways [3]. Alternative options include tissue engineering, which was proposed in the early 1990s as the design and construction of new tissue to restore the function of lost tissue [4].

Bone tissue engineering (BTE), which is proposed for the strengthening and regeneration of bone tissue. By overcoming the disadvantages of conventional methods, it has given rise to much hope [5]. Advances in regenerative medicine and 3D printing have also made this method a viable alternative to bone grafting [6]. BTE

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comprises three essential components, namely, bone progenitor cells, biochemical agents, and scaffolds. Among the various cell sources, mesenchymal stem cells (MSCs) have gained immense attention from researchers for their stemness [7], and ability to differentiate into multiple cell lineages, including bone cells (osteoblasts), a process known as osteogenesis [8, 9]. This differentiation is triggered by specific signals, including growth factors and signaling pathways that activate a cascade of molecular events in AD-MSCs [10]. These events lead to the expression of genes and proteins specific to osteoblasts, which ultimately results in the formation of mature bone cells [11].

The osteogenic differentiation of AD-MSCs involves a complex interplay of molecular and cellular processes. Initially, AD-MSCs adopt a more osteoblast-like morphology characterized by an increased expression of osteoblast-specific transcription factors such as runt-related transcription factor 2 (Runx2) and Osterix [12]. At the same time, the expression of osteoblast-related proteins, including COL 1 and ALP, is upregulated [13]. These proteins are essential for the formation and mineralization of the extracellular matrix, the basis of bone tissue [14]. As differentiation progresses, AD-MSCs synthesize a mineralized matrix and deposit it, leading to the formation of new bone [15]. This osteogenic potential of AD-MSCs represents a promising avenue for the development of innovative therapies aimed at combating bone loss and promoting bone regeneration.

Among other main concepts of BTE is to understand cell functions and create an environment that mimics the extracellular matrix. This requires ample knowledge of scaffold design to generate a suitable environment to support cells [16]. Scaffolds are structures that are used to promote the growth of cells and tissues in the human body. They require essential properties such as biocompatibility, biodegradability, good mechanical strength, and suitable porosity to ensure their effectiveness [17]. To construct these scaffolds, such natural and synthetic polymers are used. polycaprolactone (PCL) is a commonly used polymer due to its properties such as proper solubility, compatibility with most drugs, and low cost [18, 19]. PCL, a bioresorbable aliphatic polymer, has been approved by the Food and Drug Administration (FDA) for potential applications in cartilage and bone repair [20, 21]. In 2003, Yoshimoto et al. proposed that electrospun PCL could be a great option for BTE applications [22].

However, the pure PCL scaffold alone cannot promote enough bone growth, so various materials are added to enhance cell connection and differentiation [23]. Herbal medicines are one such material that has been used for centuries in many countries to treat bone fractures and other bone diseases. Flavonoids are the main biologically active substances in these herbal medicines [24], which are synthesized in plants as secondary metabolites and have various medicinal properties [25]. According to reports, natural flavonoids and their glycosides are utilized to treat complications and disorders associated with osteoporosis. By promoting the differentiation of MSCs, flavonoids strengthen osteogenesis and display an anti-osteoporosis effect [26-28]. In Europe, nutritional studies have confirmed that daily flavonoid consumption contributes to bone health [29] because flavonoids possess anti-inflammatory, antioxidant, antimicrobial, and antitumor properties beneficial for bone regeneration [30, 31]. There are several medicinal plants that are used in various communities to treat many diseases such as arthritis, gout, cancer, inflammation, and jaundice [32]. One of these plants is the Colchicum kotschyi species, also known as “Sorenjân” [33]. Colchicum belongs to the Colchicaceae family and contains tropolone alkaloids and flavonoids, among other compounds [34].

Colchicine is one of the effective substances extracted from Colchicum, which is an anti-inflammatory drug [35] used to treat gout, familial mediterranean fever (FMF), and skin diseases [36, 37]. In addition, colchicine has been found to be effective in treating heart diseases such as pericarditis and coronary artery disease [38]. It has also been used to treat acute ulcers in calcium pyrophosphate deposition disease [39]. While the anti-inflammatory effect of colchicine is well established, its impact on bone metabolism is not fully understood. A studyby Holtrop et al. revealed that colchicine may reduce the number of osteoclasts and interfere with bone resorption in bones treated with parathyroid hormone [40]. According to a study conducted on rats, the use of colchicine over an extended period resulted

in a decrease in bone strength, inhibited fracture healing, and harmed fracture healing [41]. Additionally, other studies have shown that colchicine acts as an inhibitor of proliferation in osteosarcoma cells and also exhibits non-selective mitosis inhibition as well as selective mineralization inhibition [42].

However, the available evidence regarding the effects of Colchicum-derived compounds on bone metabolism remains conflicting. Therefore, the present study aimed to investigate the effect of a Colchicum extract incorporated into a PCL scaffold on osteogenesis.

Materials and methods

Preparation of PCL scaffold by electrospinning method

The PCL scaffold was created using electrospinning technology, following a previously documented process [43]. Briefly, PCL powder (Sigma-Aldrich, St. Louis, MO, USA; MW = 680,000 g/mol) was combined with a 10 ml solution of 7.5 mL chloroform and 2.5 mL N, N-dimethylformamide (DMF; Merck, Berlin, Germany). This polymer was placed in a 5 ml nozzle at a distance of 15 to 18 cm from the collector of the device and was targeted on the target plate in an electric field with a voltage of 20 kV. As a result of the movement of the fluid at a voltage of 20 kV, the solvent in the polymer solution was evaporated in the path of the electric field and threads with a diameter of less than one micrometer was produced on the collector. They appeared in one sheet. The sheet was irradiated with gamma rays to create and strengthen covalent bonds between the fibers. This plate was used as a PCL scaffold. The prepared scaffold underwent sterilization using 70% ethanol and UV exposure.

Scaffold hydrophilicity and scanning electron microscope (SEM) analysis

The scaffold's hydrophilic characteristics were evaluated at room temperature utilizing a contact angle conveyor (Krüss, Hamburg, Germany). Furthermore, the scaffold's morphology was examined using scanning electron microscopy (SEM S-4500; Hitachi, Tokyo, Japan).

Preparation of the aqueous-alcoholic extract of Colchicum kotschyi

The plant material, locally known as Sorenjan, was collected from the mountainous area near Mashhad, Razavi Khorasan Province, northeastern Iran. The plant was botanically identified as Colchicum kotschyi Boiss. through consultation with a herbarium. The species name and collection locality have been specified to ensure accurate botanical identification of the plant material used in this study. After collecting the Colchicum kotschyi plant, its bulb was first ground completely. Then 100 g of the powder was dissolved separately in three beakers, including water, 70% ethanol and 96% ethanol. (The final volume of each beaker was 500 ml). Then, the Rotary evaporator device (Heidolph, US), which is used in evaporation and extracting the sample solvent, was used. And in the next step, the Freeze Dryer (CHRIST, Germany) device was used. Then, 1 mg of the prepared powder was weighed and dissolved in 45 ml of H_2O and 5 ml of dimethyl sulfoxide (DMSO) (Merck, Germany). And then the solution was filtered. PCL scaffolds were immersed in 100 µL of the aqueous-alcoholic extract of Colchicum kotschyi Boiss. overnight. Following incubation, the scaffolds were washed with phosphate-buffered saline (PBS) to remove residual extract and were immediately used for cell seeding while still wet. The extract treatment was performed before cell seeding; the extract was not directly added to the culture medium as part of this scaffold-treatment procedure. Finally, we used Colchicum kotschyi extract (named Colchicum extract) with 90% ethanol after assessing cell viability with the resazurin assay (7-hydroxy-10-oxidophenoxazine-10-yum-3-van).

Cell culture and seeding

Human adipose-derived mesenchymal stem cells (AD-MSCs) were obtained from the ACECR Stem Cell Bank, Mashhad, Iran, and were isolated from adipose tissue according to the previously described procedure [44]. Cells from passages 3-5 were used for the experiments. The cells were cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM-Gibco, US) supplemented with 15% (v/v) fetal bovine serum (FBS-Gibco, US) and

1% (v/v) penicillin/streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Upon reaching approximately 80% confluence, the cells were harvested and seeded onto the PCL-based scaffolds at a density of 25 × 10^3 cells/cm^2 [44].

Differentiation ability of AD-MSCs

The cell suspension with a concentration of 25 × 10^3 (cells/cm^2) was placed in 24-well plates with osteogenic cell differentiation medium for 21 days and stained with Alizarin Red S (Sigma, USA) according to the following method: First, cells were washed three times with phosphate-buffered saline (PBS) and fixed with paraformaldehyde (4%) and incubated for 15 minutes. Then they were washed with diethylpyrocarbonate (DEPC) treated water, stained with alizarin red and incubated for 15 minutes. Finally, they were washed with DEPC-treated water and observed with an inverted microscope.

In addition, the differentiation ability of cells was assessed using oil red staining as follows: 21 days after cells were cultured with adipogenic cell differentiation medium, the cells were washed three times with PBS and then fixed with formaldehyde (10%) for 10 minutes. Then the cells were washed with isopropanol (60%) and stained with Oil Red O (Sigma, USA). Finally, they were rinsed with DEPC-treated water and observed under an inverted microscope.

Alamar blue assay

In this study, we used the resazurin assay, also known as the Alamar Blue assay, because it provides a simple, rapid, and sensitive measurement of cell viability [45]. Cell viability was measured on days 1, 2, and 3 after cell seeding in 3 groups: 1. TCPS (Tissue Culture Polystyrene) as control group, 2. PCL scaffold group, and 3. PCL/Colchicum group. The PCL/Colchicum group consisted of cells cultured on PCL scaffolds that had been pretreated overnight with the aqueous-alcoholic extract of Colchicum kotschyi Boiss, and subsequently washed with PBS. No additional extract was added directly to the culture medium after cell seeding. Then, to the PCL/Colchicum group, 500 µl of osteogenic differentiation medium and 100 µl of the aqueous-alcoholic extract of Colchicum species were added. Also, in the PCL scaffold group, the cells were cultured in 600 µl of osteogenic differentiation medium. And in the TCPS group, the same cells number were seeded in 24 well plates with 600 µl of DMEM medium. After three periods (24 hours, 2, and 3 days), The dye resazurin (Alamar Blue assay reagent; Sigma, USA) was added to the cells and 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.

ALP activity and calcium content assays

The ALP enzyme is a naturally occurring in all body tissues, with elevated levels typically observed in the liver, kidneys, and bones (osteoblasts). The enzyme's activity serves as a characteristic indicator of osteoblast activity during the process of osteogenesis [46]. ALP assay was performed according to the instructions of the Pars Azmoun kit (Pars Azmoun, Tehran, Iran) on days 7, 14, and 21. More than 99% of calcium is found in bones and teeth, and its deficiency causes osteoporosis. This calcium is crystallized as hydroxyapatite calcium phosphate [47]. The calcium content was measured according to the instructions of the Pars Azmoun kit (Pars Azmoun, Tehran, Iran) on days 7, 14, and 21.

Real-time quantitative (qRT-PCR)

For the evaluation of osteogenic marker gene expression, including osteocalcin (OC), collagen type I alpha 1 (COL1A1), osteonectin (ON), and RUNX2, total RNA was extracted from AD-MSCs using an RNA extraction kit (Parstous, Iran), followed by complementary DNA (cDNA) synthesis using a cDNA synthesis kit (Parstous, Iran), according to the manufacturers' instructions. Quantitative real-time PCR was performed using a SYBR Green-based master mix (AMPIQON, Denmark). Each 20-µL reaction contained 10 µL of master mix, 2 µL of cDNA template, 10 pmol of each forward and reverse primer, and nuclease-free water to the final volume. β-Actin was used as the reference gene for normalization. The amplification protocol consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 20 s. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for the

Table 1. Primer sequences used for quantitative real-time polymerase chain reaction (qRT-PCR) analysis of osteogenic marker gene expression

Target gene	Primer sequence
β-Actin	F: 5'-TCATGAAGTGTGACGTGGACATC-3'
	R: 5'-CAGGAGGAGCAATGATCTTGATCT-3'
RUNX2	F: 5'-GCC TTC AAG GTG GTA GCC C-3'
	R: 5'-CGT TAC CCG CCA TGA CAG TA-3'
OC	F: 5'-GCA AAG GTG CAG CCT TTG TG-3'
	R: 5'-GGC TCC CAG CCA TTG ATA CAG-3'
ON	F: 5'-AGG TAT CTG TGG GAG CTAATC-3'
	R: 5'-ATT GCT GCA CAC CTT CTC-3'
COL1A1	F: 5'-TGG AGC AAG AGG CGA GAG-3'
	R: 5'-CAC CAG CAT CAC CCT TAG C-3'

target and reference genes are presented in **Table 1**.

Statistical analysis

All experiments were performed using three independent biological replicates, with three technical replicates for each biological replicate. Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, USA). Because independent biological samples were used at each time point and the same biological sample was not repeatedly measured over time, repeated-measures ANOVA was not applicable. For each time point, differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons test for post hoc pairwise comparisons. Technical replicates were averaged before statistical analysis so that the biological replicate was considered the experimental unit. A P value ≤0.05 was considered statistically significant.

Results

Scaffolds morphology

Figure 1 shows the SEM image of the electrospun PCL scaffold. The SEM analysis demonstrated the formation of a continuous, interconnected fibrous architecture with relatively uniform nanofibers. The electrospinning process resulted in submicron fibers with diameters below 1 μm, confirming the successful formation of the intended nanofibrous scaffold structure. The interconnected fibrous morphology provides an appropriate three-dimensional surface architecture for cell attachment and interaction with the scaffold.

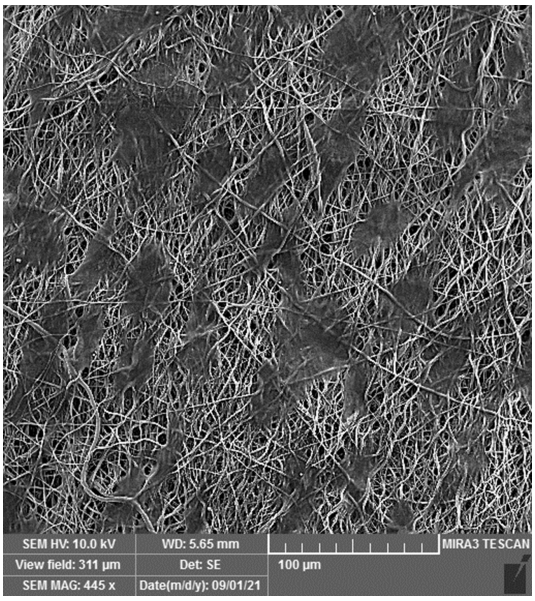


Figure 1. SEM images of 3D PCL at 445× magnification.

Osteogenic and adipogenic differentiation of AD-MSCs

The ability for adipogenic or osteogenic differentiation is a crucial criterion in the identification of MSCs [48]. On the 21st day after differentiation, the cells were stained with Alizarin Red and Oil Red. The results showed that AD-MSCs were successfully differentiated into adipogenic and osteogenic lineages. In cells stained with oil red, the fat mass in the cytoplasm of fat cells was stained red (**Figure 2A**). Also, mineralized nodule formation and calcium deposition in the cytoplasm of cells differentiated into osteoblast cells was observed in the form of red masses (**Figure 2B**).

Cell survival

Cell viability and proliferation were evaluated using the resazurin assay on days 1, 2, and 3. Cell viability increased progressively over the observation period in all experimental groups. No statistically significant difference was observed between the PCL/Colchicum scaffold and the control groups at the evaluated time

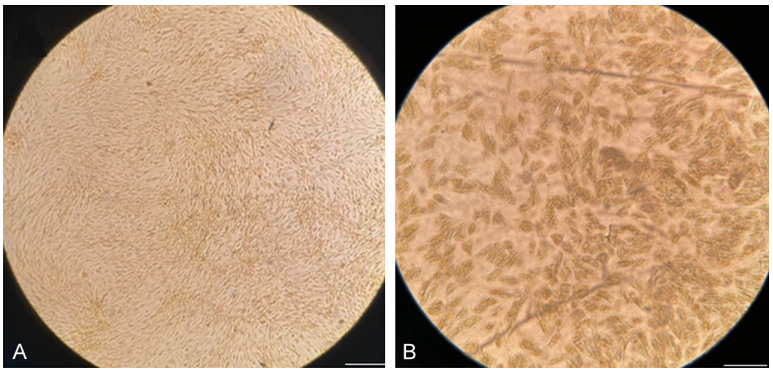


Figure 2. AD-MSCs staining, 21 days after differentiation induction by an inverted microscope. A. Oil red staining. B. Alizarin red staining (magnification: 100×; scale bar = 100 μm).

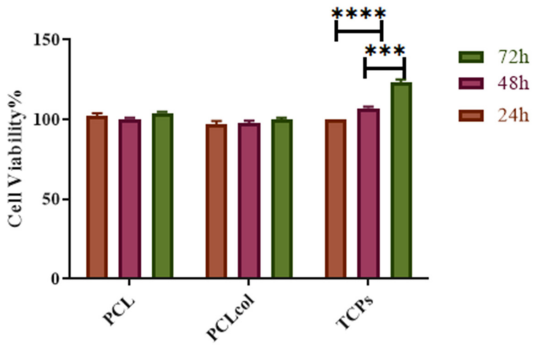


Figure 3. Cell viability of AD-MSCs seeded on scaffolds in osteogenic induction medium after 1, 2, and 3 days (***P<0.001, ****P<0.0001).

points (**Figure 3**), indicating that incorporation of the Colchicum extract into the PCL scaffold did not adversely affect cell viability under the experimental conditions.

ALP activity and calcium content assays

ALP activity was evaluated on days 7, 14, and 21 of osteogenic differentiation. ALP activity increased progressively during the differentiation period in the experimental groups. Cells cultured on the PCL/Colchicum scaffold exhibited higher ALP activity than the control groups, with statistically significant differences observed on days 14 and 21 ($P<0.05$; **Figure 4**). The data are presented as mean ± SD. Intracellular calcium content was measured on days 7, 14, and 21 of osteogenic differentiation. Calcium deposition increased progressively during the differentiation period. Cells cultured on the PCL/Colchicum scaffold showed a greater increase in calcium content compared with the control groups, particularly at the later stages of differentiation (**Figure 5**). The data are presented as mean ± SD, and statistically significant differences were determined using ANOVA followed by Tukey's multiple-comparisons test.

Expression of bone marker genes

The expression levels of osteogenic marker genes, including RUNX2, COL1, OC, and ON, were evaluated on days 7, 14 and 21 using qRT-PCR. Compared with the PCL scaffold, cells cultured on the PCL/Colchicum scaffold showed increased expression of all evaluated osteogenic markers (**Figure 6**). The results are presented as relative expression levels normalized to β-actin and expressed as mean ± SD. Statistical significance was determined using ANOVA followed by Tukey's multiple-comparisons test.

Discussion

This study is the first study to investigate the effect of a Colchicum kotschyi extract in combination with scaffolds on the differentiation of AD-MSCs. Scaffolds are crucial in tissue engineering as they mimic the extracellular matrix and provide mechanical support for cell growth and differentiation. When scaffolds are combined with bioactive molecules, they can increase the differentiation efficiency of stem cells by creating a suitable microenvironment for bone formation. The findings of the present study demonstrated that the combination of the Colchicum extract with the scaffold could enhance the osteogenic differentiation of MSCs.

In this study, we developed a PCL nanofibrous scaffold using the electrospinning technique, which provided a structural framework for cell attachment, growth, and differentiation. The results showed that the PCL scaffold by mimicking the extracellular matrix and providing mechanical support for the growth and differentiation of cells had an effective role in inducing osteogenic differentiation of AD-MSCs compared with the TCPS control group. Similarly,

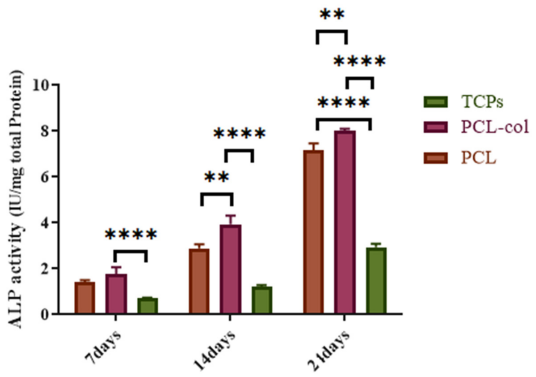


Figure 4. Alkaline phosphatase activity of AD-MSCs cultured under the indicated experimental conditions at days 7, 14, and 21. Data are presented as mean ± SD from three independent biological replicates, with three technical replicates performed for each biological replicate. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (**P<0.01, ***P<0.001, ****P<0.0001).

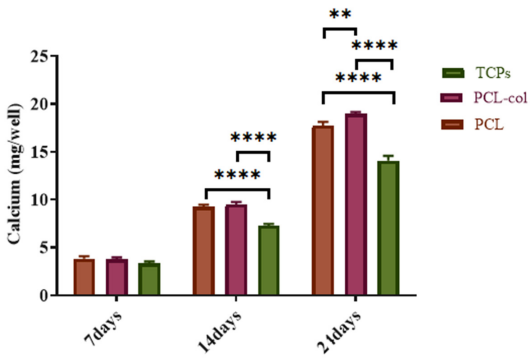


Figure 5. Calcium content of AD-MSCs cultured under the indicated experimental conditions at days 7, 14, and 21. Data are presented as mean ± SD from three independent biological replicates, with three technical replicates performed for each biological replicate. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (**P<0.01, ***P<0.001, ****P<0.0001).

the results of our previous studies also proved that PCL, as a biodegradable and biocompatible polymer, has a suitable mechanical property for use in BTE [43, 49]. In addition, Marino et al., in a study that investigated the growth potential and endothelial differentiation of ADSC on PCL, showed that PCL could be a suitable material for scaffolding to facilitate the regeneration of new endothelial tissue [7]. Considering that the pure PCL scaffold alone cannot achieve sufficient bone growth, it is used in combination with synthetic and natural polymers or other materials that improve the properties of the scaffold [23]. For example, the bioactive compounds of plants, shown in various studies, play a main role in increasing the proliferation and differentiation of MSCs [27]. For example, using quercetin plant extract mixed with MSCs ceramic scaffold, Huang et al. showed that quercetin promotes cell proliferation on the scaffold by stimulating cell division and leads to osteogenesis differentiation [50].

In the present study, we used a Colchicum extract incorporated into the PCL scaffold. Colchicum extract contains various bioactive compounds, including flavonoids, which may enhance MSC proliferation and osteogenic differentiation, as well as modulate the microenvironment for bone regeneration [51]. Colchicine is currently prescribed for its anti-inflammatory effects in the treatment of several conditions, including gout [52], FMF [53], and arthritis [54, 55]. By incorporating the Colchicum extract into PCL scaffolds, we found that the resulting scaffold enhanced cell survival, osteogenic differentiation, and osteogenesis. In the present study, an increase in mineral deposition was observed by measuring the cellular calcium content in AD-MSCs seeded on scaffolds. Calcium deposition 21 days after osteogenesis induction showed that mineralization was significantly higher in the PCL/Colchicum scaffold group than in the PCL scaffold group. It has also been reported that ALP is an early marker before matrix maturation, suddenly increasing in the early phase of osteogenesis and gradually decreasing in the later stages [56]. Similarly, we have shown that the cells seeded on PCL/Colchicum scaffold increase ALP activity more than the PCL scaffold. Examination of the gene expression of important bone markers such as Col1, which is known as a primary marker for osteoprogenitor cells [16], also showed a significant increase in the PCL/Colchicum scaffold group. Type I collagen increases the rate of cell attachment and bone differentiation of MSCs by activating key signaling pathways in bone formation [57].

However, several studies investigating the relationship between bone metabolism and colchicine have published contradictory results. Salai et al. showed that colchicine can prevent heterotopic ossification (HO) in patients with FMF who have undergone total hip replace-

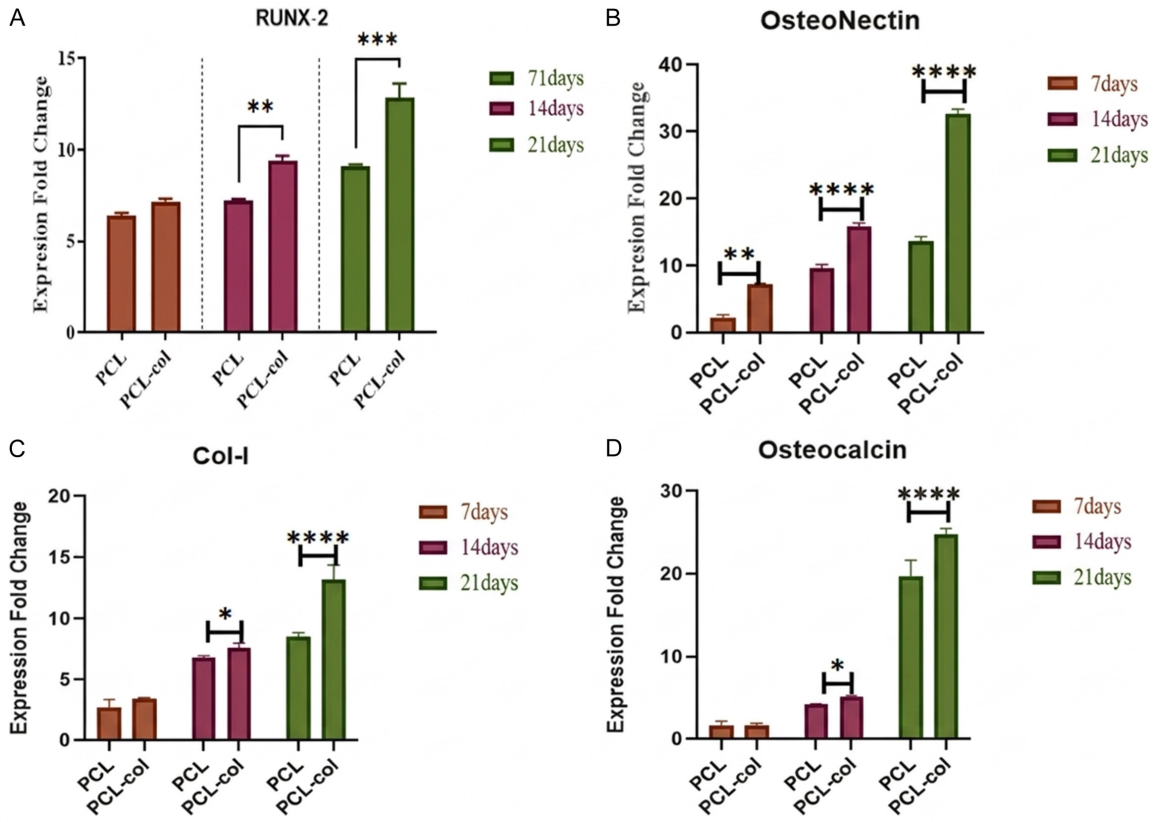


Figure 6. Relative expression of osteogenic marker genes in adipose-derived mesenchymal stem cells (AD-MSCs) cultured on polycaprolactone (PCL) and *Colchicum*-extract-treated PCL scaffolds. A. Runt-related transcription factor 2 (RUNX2) expression. B. Collagen type I alpha 1 chain (COL1A1) expression. C. Osteocalcin (OC) expression. D. Osteonectin (ON) expression. Gene expression levels were normalized to β-actin as the reference gene. Data are presented as mean ± SD from three independent biological replicates (n = 3), with three technical replicates performed for each biological replicate. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparisons test (**P<0.01, ***P<0.001, ****P<0.0001).

ment [58]. Reports also indicate that colchicine has led to a significant decrease in tissue mineralization by inhibiting the proliferation of osteoblasts in vitro [42]. In addition, many of patients who have taken colchicine for various complications, deleterious effects on bone have been noted at both the cellular tissue and whole bone levels [42]. Potential effects of colchicine have also been reported in higher animals or humans, in long-term clinical studies in FMF patients [59-61], and in studies on rabbits [62].

In contrast, the study by Arai et al. reported that after the administration of 1 mg/kg colchicine, the expression of collagen type 1 was increased in osteoblasts, leading to the formation of inappropriate calcified tissue [63]. Jisun Oh et al. also showed that colchicine inhibits the cell cycle of osteosarcoma by inducing apoptosis, confirming the results of our study [64]. As shown in our study, combining the Colchicum extract with the scaffold created a dynamic environment that led to increased bone differentiation of MSCs. In other words, the results of our study showed that colchicine can positively influence various cellular processes, including gene expression and cytoskeletal dynamics, which are crucial for determining the fate of MSCs to bone lineages. However, further research is needed to elucidate the exact mechanisms by which colchicine regulates MSC differentiation and to explore its therapeutic potential in bone tissue regeneration.

In other words, our study revealed a synergistic effect between the Colchicum extract and the PCL scaffold, which promoted an environment that significantly enhanced osteogenic differ-

entiation of AD-MSCs [21]. This enhancement was reflected in the increased expression of key osteogenic markers such as Runx2 and OC, as well as the upregulation of genes involved in collagen synthesis and mineral deposition. These findings suggest that the Colchicum extract in combination with the scaffold is effective in promoting the binding of AD-MSCs to the bone lineage. Further analysis revealed that the Colchicum extract probably exerts its influence via a multi-layered mechanism. Our results suggest that the Colchicum extract plays a crucial role in regulating cytoskeletal dynamics and promoting cell adhesion to the scaffold. In addition, the Colchicum extract appears to modulate the expression of genes involved in cell signaling pathways, ultimately steering AD-MSCs toward osteogenic differentiation. This complex interplay of the Colchicum extract's effects on cellular processes, including cytoskeletal dynamics, gene expression, and cell signaling, contributes to the enhancement of osteogenesis observed in our study. This study faces limitations in the field of complementary tests confirming osteogenic differentiation, such as Western blot or immunocytochemistry, which can be considered as future research.

Conclusion

Flavonoids are natural compounds found in plants like Colchicum, known for their medicinal benefits. They have a wide range of health benefits and are readily available. Flavonoids play a crucial role in bone repair by promoting the development of bone cells and possessing anti-inflammatory and antioxidant properties. MSCs, combined with Colchicum extract, containing naturally occurring flavonoids and other bioactive compounds, compounds showed great potential for regenerating bone tissue. Colchicine can help increase the proliferation and differentiation of MSCs, while also modulating the microenvironment during osteogenesis. This makes it a promising option for bone regeneration treatments. Although the concentration of the extract and its long-term effects on the cells should be verified to ensure its safety and efficacy, the above can be considered as one of the limitations of this study. In addition, this study focused on in vitro evaluations and experiments. Although it provided valuable insights into the osteo-conductive effect of the Colchicum-treated scaffold on AD-MSCs, further studies in animal models and humans are needed to validate these findings.

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Disclosure of conflict of interest

None.

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