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Clinical and Radiological Evaluation of Transplanted Fresh Ear Cartilage Impregnated With the Mesenchymal Cells & PRP in Treatment of Growth Plate Injury in Lamb

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Abstract:

BACKGROUND: Growth plate is responsible for bone elongation and its injury could result in severe orthopedic problems.

OBJECTIVES: This study was aimed to investigate the radiographic changes after transplanting the fresh autogenous ear cartilage impregnated with PRP and MSc in treatment of growth plate injury in lamb.

METHODS: Cranial half (½) in length in full depth of the right hind limb growth plate was resected with bone oscillator and was transplanted with autogenous fresh harvested ear cartilage at the time of surgery in all of the 15 male lambs. Subsequently they were divided into 3 subgroups of 5 lambs in each group, control, PRP and MSCs. Radiographs were obtained from operated limbs at surgery time and 60 post-operative.

RESULTS: Clinically, all lambs showed same lameness degree and mostly apparent after cast removal but gradually improved in the treated group which showed normal weight bearing after one month. Early physeal closure was seen in growth plate injured site only in control group without limb shortening or angular deformity. Lambs with MSCs showed mild bone bridge formation (1.4b ± 0.4b) as compared with PRP group (1.8 ± 0.37ab) and control group (2.8 ± 0.2 a) (P<0.05).On 60 days postoperatively control group showed significant mineralization (by 2.8±0.2a) while less mineralization was detected in MSCs and PRP groups (by 1.4± 0.4b and 2.4± o.4 ab).

CONCLUSIONS: This study showed that MSCs & PRP can quite effective in preventing bone bridge formation and mineralization besides convergence of the transplanted tissue into growth plate cartilage.

Keywords:

Cartilage, Growth plate, Mesenchymal cell, PRP, Radiography

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Introduction

Growth plates exist in the metaphysis of growing animal and children. They consist of a hyaline cartilage layer that is involved in the longitudinal growth of long bones. As it is prone to various type injuries (Saheri et al., 2015). It is common to have a bony bridge formation at the injury site when the growth plate is injured by trauma even by fractures, tumours, infection or side effects after radiation therapy. Injured growth plate cartilage and the bony bridge can result in leg length discrepancy, angular deformities and rotational deformities (Dabash et al., 2018, Apte and Kenwright 1994, Salamon, 2013, Chung 2014). Apart from trauma, other causes of physeal injury include infection, neoplasm, radiation, ischemia metabolic abnormalities, thermal injury, sensory neuropathy, and iatrogenic injury (Jawetz et al., 2015). To overcome these problems, we focused on ear cartilage. This has been used as a satisfactory graft for surgery in lamb model of animal. No major side effects have been reported over long observation periods. In this study, ear cartilage was transplanted for treatment of a partial growth plate injury in a lamb model to evaluate its healing ability. It has been highly cited that cartilage is a highly differentiated tissue and therefore has a limited capacity for self-repair. Recently, several studies (Aston and Bentley1986, Cunniffe 2017, Brittberg et al., 1994) have been reported of the successful repair of osteochondral defects by the transplantation of cultured chondrocytes, but the method requires that a sufficient number of cells are obtained from the donor site in the articular cartilage (Brittberg et al., 1996, Erickson, 2017). Osteochondral progenitor cells or mesenchymal stem cells are undifferentiated cells found in small numbers in the preiosteum or in the bone marrow which are capable of differentiation to chondroblasts or osteoblasts (Yoshida, 2012). These cells are able to re-establish chondrogenesis during the developmental process when implanted in osteochondral defects (Wakitani et al., 1994). The successful repair of a large, full-thickness defect by the implantation of cultured mesenchymal osteochondral progenitor cells isolated from the periosteum and bone marrow in the rabbit was reported (Wakitani, et al., 1994; Awang et al., 2013, Lie Xie et al., 2014). So we have planned to investigate radiographic changes while using a biological material such as ear cartilage impregnated with mesenchymal stem cells from bone marrow and PRP from peripheral blood samples for repairing of growth plate, these materials definitely potentiate the differentiation of grafted cartilage into variable cells including fibroblasts and chondroblasts.

Material and Methods

This protocol was approved by the Ethics Committee for the Analysis of Research Projects by the Experts Research Committee in the Faculty of Veterinary Medicine University of Tehran, Protocol 2345/95/2016. All lambs received human care in compliance with the experimental protocols of the Ethical Principles in Animal Experiments adopted by the Faculty of Veterinary Medicine Research Committee (FVMRC). For in vivo study, fifteen 4 month-old male lambs weighing 15 to 20 Kg/bw were housed five per box and were given food and purified tap water. Before experiment all lambs were acclimatized to their new environment for 2

weeks. They were subsequently subdivided into 3 subgroups of five lambs in each group control, PRP and MSc group. On operation day lambs were taken off feed for 12 h and water was withheld for 6 h. The lambs were given intramuscularly KetariarR (ketamine HCL, 11mg/kg)[a] and Rompun (xylazine HCL, 0.22 mg/kg)[b]. Lambs were placed in a sterile field on a heated surgery table and after proper positioning endotracheal intubation was done and they were connected to anesthetic machine with AErrance (isoflurane 1-2 % for maintenance)[c]. The latero-medial portion of proximal right tibia including stifle joint was shaved and subjected to disinfection with betadine 7.5 % and last touch with betadine 10% and sprayed with septicidine and then covered with a sterile surgical drape.

The skin incision was given longitudinally 5 cm in length, on the latero-medial surface of the tibia region and after careful separation of subcutaneous tissue the metaphyseal area was well exposed. Before surgery the exact growth plate line was radiographyically demarked and with the help of bone oscillator exactly half of the full length (½) of growth plate with full thickness 5 mm in width was resected in all lambs. The collected sample of ear cartilage 2 cm in length and 1 cm in width was transplantation in the bony gap in control group (I) (Figure 1), whereas the sample was impregnated with 1ml PRP and infiltrated after transplantation in group II whereas in group (III) the cartilage samples were impregnated with 2 ml MSCs broth and infiltrated after transplantation in excavated space. The area was sutured as routine. Coaptation splint was used for 15 days for better stability of hind limb.

PRP Preparation: In order to prepare autologous PRP, five lambs were selected and anesthetized.10 ml venous whole blood was collected from each lamb and immediately transferred into tubes containing anticoagulant (ACD). Blood samples were centrifuged (SmartPReP2, Centrifuge, Harvest Plymouth .MA) for 5 min at 2000 RPM so that three separate layers were formed based on density of blood components. The buffy coat layer was closely collected by a sampler and transferred into a separate tube which was centrifuged for a second time for 5 min at 2000 RMP so that PRP portion was obtained from the surface. 1.5 to 2.5 ml of PRP product was obtained. A volume of 1 ml of PRP containing $882\pm199\times10^3$ platelet/µl was used for each lamb (Salma et al., 2013).

Bone-Marrow–Derived MSCs: In order to collect bone–marrow derived mesenchymal nonhemopoietic cells (BM-MSCs), one month before the actual experiment, five lambs were selected and anesthetized and under strict asepsis using Jamshidi bone marrow needle 4ml bone marrow was collected from iliac crest region and immediately transferred into a 5 ml syringe with 2 ml Dulbecco,s Phosphate Saline (PBS), 2% Fetal Bovine Serum (FBS, stem cell Technologies) and 5 IU heparin/ml connected with a hypodermic needle (20G/40mm) then bone marrow blood (4 ml) was deposited over 3 ml of Ficol Paque ™ PLUS (Stem cells Technologies, Vancouver Canada) in a 50 ml conical tube and centrifuged for 30 min at 400 ×g. The mononuclear cells were washed once with PSB containing 2% FBS before being plated in tissue culture flasks at a density of 4000 cells per cm2. The isolated MSCs were cultured in MesenCult@ MSC Basal Medium supplemented with MesenCult@ Mesenchymal Stem Cell Stim-

ulatory Supplements (STEMCELL Technologies) at 370 °C in a humidified incubator with 5% CO2.The adherent MSCs were passaged at 60-80% confluence. At passage 2, 1×10^6 MSCs were resuspended in 50 µl culture medium and then mixed with 50 µl 2% alginate. Of the alginate mixture, 40 µl (containing 4×10^5MSCs) was added onto the PGA scaffold and alginate polymerization was subsequently initiated with CACl2 (100mM). Finally, MSCs were embedded within alginate gel (Lew et al., 2016, Pauline & Yee 2015).

Post–Operative care: After surgery, the lambs were kept warm until they recovered consciousness. Antibiotic intramedicine[d] 1 ml for each 10 kg, Vitacoid[e] 2ml I.M and Vitaforte[f] 2 ml I.M were administered every 24 h for five days. During the time they were kept housed, and skin sutures were removed on 12 post-operative days.

Radiographic analysis: Each lamb was subjected to radiological examination on the day of transplantation and 60 days post-operatively. The operated right tibias were radiographed using an MX-20 Cabinet X-ray System under specific conditions (35 kV, 300 µA and 240 s). For the radiographic evaluation, the extent of bone bridge formation and mineralization was considered (Figure 2). The medial proximal tibia angle (MPTA), defined as the angle between the mechanical axis of the tibia and proximal tibia articular surface, was measured at eight weeks post-operatively. The proximal tibia articular surface was defined as the line from the medial corner of the articular surface to the lateral corner of the articular surface. The mechanical axis was defined as the line from the center of the articular of the knee to the center of the articular of the foot.

Table 1. Bone bridge formation study showing means and stander errors in control, PRP and MSC groups after 60 days of grafting. ab Different letters within column indicates significant differences ($P< 0.05$).

group	Before surgery	After surgery	After 60 days
control	0.00	0.00	2.8 ± 0.2^{a}
PRP	0.00	0.00	1.8 ± 0.37^{ab}
MSCs	0.00	0.00	1.4 ± 0.4^{b}

Table 2. Stages of mineralization study showing means and stander errors in control, PRP and MSC groups after 60 days of grafting. abDifferent letters within column indicates significant differences ($P< 0.05$).

group	Before surgery	After surgery	After 60 days
control	0.00	0.00	2.8 ± 0.2^{a}
PRP	0.00	0.00	2.4 ± 0.4^{ab}
MSCs	0.00	0.00	1.4 ± 0.4^{b}

Statistical analysis: Data were analyzed statistically, and values are expressed by median. Normality of the variables was assessed. In order to assess whether there was any difference the Repeated Measure Analysis of Variance was used, level of significance was accepted at $P<0.05$. Data were analyzed using the statistical package SPSS17.0 (Chicago, IL).

Results

No reactions or complications were observed before and after operation in the animals of the three groups (MSCs, PRP, and control groups). Two months after inducing physeal defect in the proximal tibia and autogenous cartilage transplantation, early physeal closure was seen in the growth plate injured site of control group without limb shortening or angular deformity. On the contrary, the physeal closure was not observed in MSCs or PRP groups where the transplanted autogenous cartilage covered with MSCs or PRP (Figure 2). Results measurement of bone bridge development table (1) and (Figure 3) show that the damaged growth plate of proximal tibia transplanted with autoge-

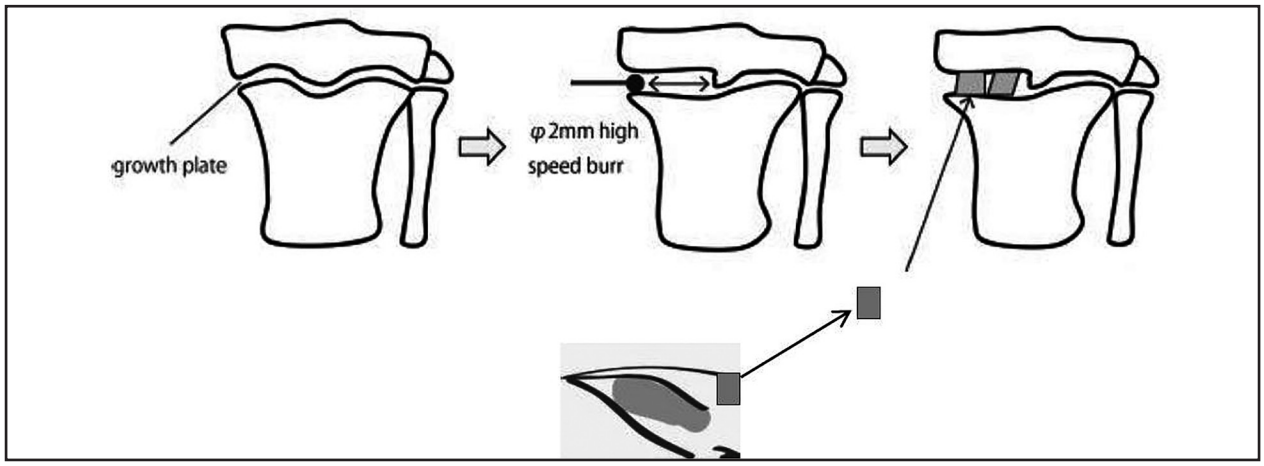

Figure 1. Schematic showing the surgical procedure. The medial half of the growth plate of the right proximal tibia was drilled from the inside using a high-speed steel bur, and injured in full (1/2) length and 5 mm in width. Fresh autogenous ear cartilage was transplanted to the excavated space.

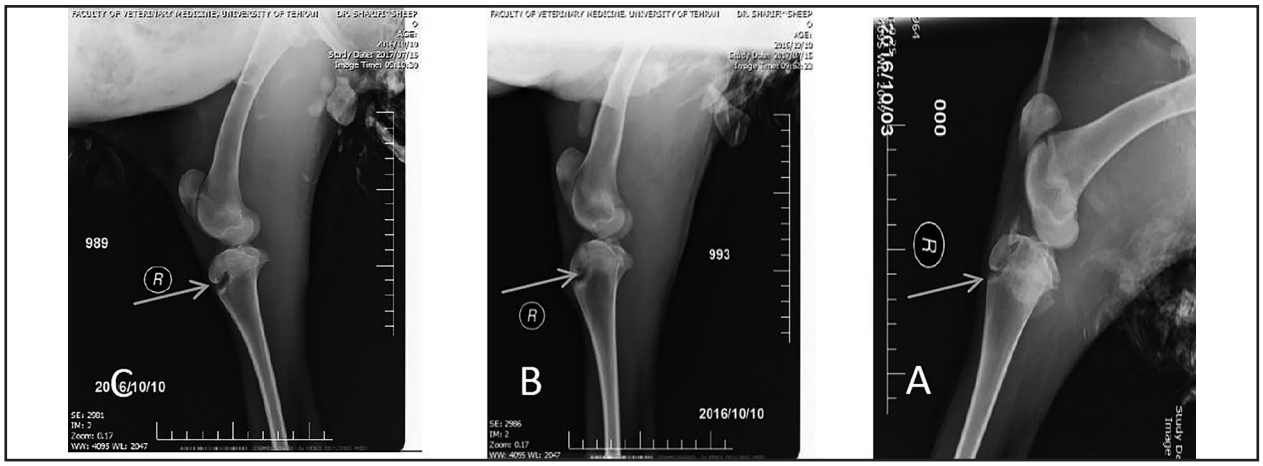

Figure 2. Radiographic images showed bone bridge formation and mineralization in 60 days postoperatively in control (A), PRP(B), and MSCs (C) groups.

nous cartilage covered with MSCs have a mild bone bridge formation (1.4b ± 0.4b) as compared with both PRP groups in which the physeal defect is filled with autogenous cartilage covered with PRP (1.8 ± 0.37ab) and control group with autogenous cartilage transplantation only (2.8 ± 0.2 a) (P<0.05). Concerning the influence of PRP, a trend to more bone bridge prevention in PRP-group compared to control group was detected 60 days postoperatively. The measurement of mineralization of the injured growth plate site in the control group where autogenous cartilage was transplanted without MSCs or PRP revealed significant mineralization (by 2.8±0.2[a]), while in the MSCs and PRP groups mineralization was less than control group (by 1.4± 0.4b and 2.4± o.4 ab) Table (2) and Figure 4.

Discussion

Physeal injuries are common and are crucial to recognize, as morbidity and the sequelae of missed physeal injury are not easily remedied. Trauma, both acute and chronic, is by far the most common cause of physeal injury. While acute traumatic events may result in physeal damage, chronic low-grade trauma such as in overuse injuries can also injure the vulnerable growth plate.

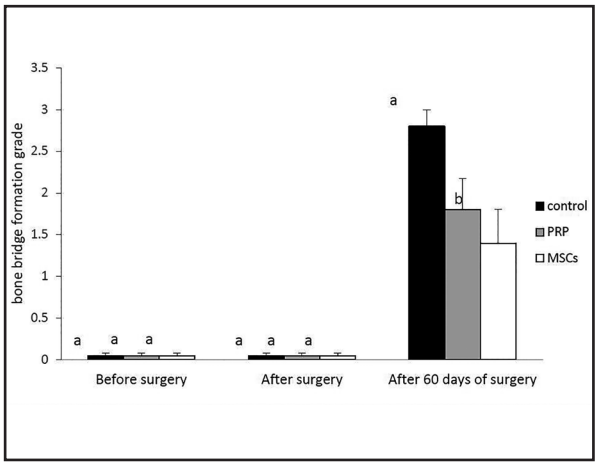

Figure 3. bone bridge formation in injured growth plate in control, PRP and MSCs groups after 60 days postoperatively. ab Different letters among groups indicates significant differences (P<0.05).

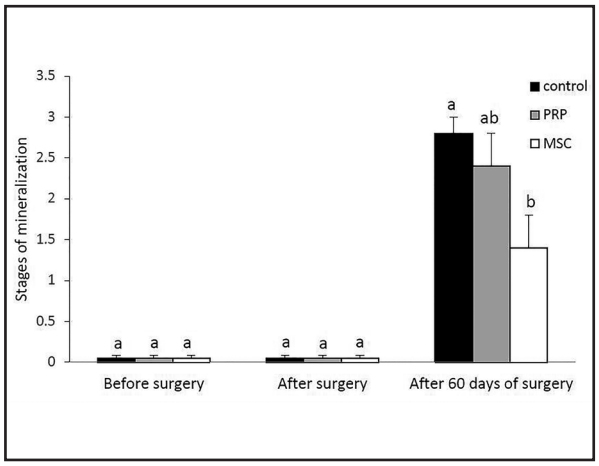

Figure 4. Stages of mineralization in injured growth plate in control, PRP and MSCs groups after 60 days postoperatively. ab Different letters among groups indicates significant differences (P<0.05).

With increasing youth involvement in organized sports, (Paterno et al., 2013) the phenomenon of overuse injuries is a frequent reality among children. The intense training schedules, intense focus on a single sport, and lack of off-season rest all contribute to the overuse injuries seen in children (Davis 2010). In the current age of competitive sports, 53% of children between the ages of 5 and 17 years sought medical care for an overuse injury in the cohort evaluated (Stracciolini et al., 2014). But debate persists as to whether the incidence of pediatric sports-related injuries is actually increasing. Whether the incidence of injury is trending upward, this article aims to highlight the utility of imaging in the medical workup of this patient population.

Our hypothesis was that the healing of a growth plate defect would be promoted by providing an excessive amount of mesenchymal stem cells capable of differentiating into cartilage or bone in accordance with the surrounding milieu (Dirsko and Charles 2009, Goldberg 1999). Because the artificially-induced defect reached subchondral bone, mesenchymal cells would also be recruited, although in small amounts, from underlying bone marrow in the control group. The newly-formed cartilage of the experimental group would be more resistant to degenerative changes than that of the control group. The junction between the regenerated cartilage and the surrounding normal cartilage could be identified without difficulty and could become a starting point for cartilage degeneration after our experimental period of 60 days. One theoretical advantage of implanting mesenchymal stem cells is that they are undifferentiated cells capable of more mitotic divisions. Mammalian cells have an almost fixed number of possible cell divisions after which they undergo programmed cell death (Cotran 1999). Chondrocytes are highly differentiated, and cells expanded in culture would have a more limited capacity for proliferation after implantation in the osteochondral defect. Our results agreed with those of recorded research (Takahashi et al., 1995), who used an autogenous callo-osseous graft as a source of mesenchymal stem cells for the repair of osteochondral graft in rabbits (Takahashi et al., 1995). Obtained imaging

from all the lambs offers clinically relevant information about areas of physeal injury. The radiographic results showed that, the mild bone bridge formation and amount of mineralization was in MSCs and PRP then Control group. We therefore assumed that the chondrocytes had potential to be alive and active. Moreover, in the operated control, the growth plate was discontinuous, whereas the growth plate in the MSCs and PRP groups was continuous. In summary, there were two differences between the transplantation of ear cartilage. First, there were no varus deformities in the PRP and MSCs group than in the control group. Second, the cartilage was continuous in the MSCs & PRP groups, whereas it was discontinuous in the group control with no varus deformities. Despite no difference in the bony bridge rate between the PRP and MSCs groups. The cartilage that was impregnated with MSCs broth was at the same level as the normal growth plate for eight weeks after operation. In contrast the PRP image shown moved to the distal side as the tibia grew longitudinally. This might have been because ear cartilage is biological material. Therefore, autogenous ear cartilage may be more suitable as an interpositional material, supporting our assumption that the growth plate cartilage was partially repaired by ear cartilage impregnated with MSCs transplantation. However, a long-term functional analysis using larger animal models is required to prove the repair of growth plate defect functionally.

In conclusion: Noninvasive imaging is extremely important in the management of pediatric patients with physeal injuries, both in the acute traumatic and chronic overuse settings. Radiographs have roles in evaluating these injuries. Knowledge of the location and extent of physeal damage and injury to the extraphyseal structures allows for a more comprehensive assessment of the risk of future complications. This study showed a decrease in angular deformities and bony bridge formation and suggests the growth plate has potential to be partially repaired by the transplantation of ear cartilage graft impregnated with MSCs. The ear cartilage graft is cartilage extracellular-matrix-rich and safe, and is a good interpositional biological material. Ear cartilage may therefore be a suitable graft for the treatment of growth plate injury. Further study is required using Autograft & Allograft and evaluating the long-term function of repaired growth plate in larger animal models.

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Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۴، ۲۹۲-۲۸۳

ارزیابی بالینی و رادیوگرافی بافت پیوند شده غضروف تازه گوش پوشش داده شده با سلول‌های مزانشیمی و پلاسمای غنی از پلاکت در درمان آسیب صفحه رشد در بره

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چکیده

زمینه مطالعه: صفحه رشد مسئول افزایش طول در استخوان می‌باشد و آسیب به آن می‌تواند سبب بروز مشکلات شدید ارتوپدی شود.

هدف: هدف از مطالعه حاضر، ارزیابی تغییرات رادیوگرافی بعد از پیوند شدن بافت غضروفی تازه گوش پوشش داده شده با پلاسمای غنی از پلاکت و سلول‌های بنیادی مزانشیمی در درمان آسیب صفحه رشد در بره است.

روش کار: نیمه قدامی در طول و به صورت تمام ضخامت از صفحه رشد اندام خلفی سمت راست به وسیله اره نوسانگر مخصوص استخوان برداشته شد و غضروف گوش که در همان زمان جراحی استخراج شده بود در محل آن به صورت اتولوگ در پانزده بره پیوند شد. متعاقباً حیوانات در سه گروه پنج‌تایی قرار داده شدند: گروه کنترل، گروه پلاسمای غنی از پلاکت و گروه سلول‌های بنیادی مزانشیمی. در زمان جراحی و شصت روز پس از آن، رادیوگراف از اندام‌های جراحی شده گرفته شد.

نتایج: از لحاظ بالینی همه بره‌ها به یک میزان علائم لنگش را نشان می‌دادند و بیشترین میزان مربوط به بعد از برداشت کست بود اما به مرور در گروه درمان بهبودی مشاهده شد که وزنگیری نرمال بعد از یک ماه نشان می‌داد. بسته شدن صفحه رشد در محل آسیب صفحه رشد تنها در گروه کنترل دیده شد که همراه با کوتاه شدن اندام و بدشکلی نبود. گروه بره های سلول‌های بنیادی مزانشیمی میزان خفیفی (۱/۴b ± ۰/۴b) تشکیل پل استخوانی را در مقایسه با گروه پلاسمای غنی از پلاکت (۱/۸ ± ۰/۳۷ab) و گروه کنترل (۲/۸ ± ۰/۲ a) نشان می‌دادند ($P<0/05$). شصت روز بعد از جراحی گروه کنترل بطور معنی‌داری مینرالیزاسیون (۲/۸ ± ۰/۲ a) را نشان می‌داد درحالی که مینرالیزاسیون کمتری در گروه‌های سلول‌های بنیادی مزانشیمی و پلاسمای غنی از پلاکت (۱/۴ ± ۰/۴ b and ۲/۴ ± ۰/۴ ab) یافته شد.

نتیجه‌گیری نهایی: نتایج این مطالعه نشان می‌دهد که سلول‌های بنیادی مزانشیمی و پلاسمای غنی از پلاکت می‌توانند کاملاً در جلوگیری از تشکیل پل استخوانی و مینرالیزاسیون و همچنین همگرایی بافت پیوند شده در صفحه رشد مؤثر باشند.

واژه‌های کلیدی:
غضروف، صفحه رشد، سلول‌های مزانشیمی، پلاسمای غنی از پلاکت، رادیوگرافی

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