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Immediate to short-term inflammatory response to biomaterial implanted in calvarium of mice

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Abstract

Scaffolds made of biodegradable materials play a very important role in repairing bone defects. Our study was conducted with the aim of investigating inflammation, vascular changes, and tissue necrosis after the placement of 3D printed scaffolds composed of beta-tricalcium phosphate (TCP-β) on the calvarial bone defect of mice. Eight samples of scalp tissue in mice were examined in four groups (one-week control, two-week control, one-week experiment, and two-week experiment). Mice with routine bone defects were selected as the control group and mice with bone defects with β-TCP scaffolds were selected as the experimental group (TCP). The groups were evaluated in terms of inflammatory cells, osteoblast and osteoclast cells, vascular changes, and the number of resorption pit and empty lacuna. The results demonstrated a decrease in inflammatory cells and an increase in osteoclast and osteoblast cells in bone defect sites placed with TCP-β scaffolds ($p<0.05$). The results of histological staining showed pit resorption and further vascularization in the place of TCP-β scaffolds, but these changes were not statistically significant ($p>0.05$). Examining the number of empty lacunae in the bone defect site showed that TCP-β could significantly reduce the number of these lacunae in the bone defect sites placed with TCP-β scaffolds ($p<0.05$). 3D printed scaffolds composed of TCP-β that were implanted in bone defect sites were effective in reducing the inflammatory responses, emptying lacunae and increasing bone regeneration.

Key Words: Inflammation, vascular changes; tissue necrosis; beta-tricalcium phosphate; bone defect; calvarium; mice.

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Injuries, tumors, infections, functional atrophies, congenital diseases, and periodontitis can cause the loss of bone tissue in the oral cavity, which affects the quality of life in addition to appearance and health.[1,2] The gold standard treatment of bone defects is usually the use of autologous bone graft, but due to limitations in bone harvesting and complications of the graft donor site, their use is limited. In recent years, with the rapid development of biological materials and the need for modern regenerative medicine, bone biomaterials have been implemented to repair bone defects, which are also biocompatible.[1] Biomaterials composed of calcium phosphate are superior due to their osteoconductivity, biocompatibility, and safety properties, but in addition to these advantages, they are also associated with some disadvantages.[3] Beta-tricalcium phosphate (TCP-β) is one of the most widely used synthetic bone grafts, which is osteoconductive and osteoinductive, and causes complete regeneration of bone defects,[4] but one of the debates regarding this biomaterial is the responses of the immune system.[5] Following the placement of biomaterial implants, inflammatory reactions are induced, and the levels of inflammatory responses of the immune system are effective in the success of implants. The activation of inflammatory processes can cause implant rejection, but it can also be effective in tissue regeneration and repair.[6,7] Macrophages are dominant cells that quickly react to biomaterial implants and play an important role in regulating inflammatory responses and tissue remodeling by secreting large amounts of bioactive mediators that

cause inflammation, cell migration, differentiation, cell remodeling, and vascularization.[6] Scaffold materials should mimic the characteristics of natural bone and provide a suitable biochemical environment and biomechanical support for adhesion, migration, proliferation, osteogenic differentiation, and angiogenesis of cells implanted on the scaffold.[8] The type and structure of the materials used can affect the efficiency of the treatment of bone defects.[6,9] In addition, the three-dimensional structure of the scaffold is effective in the response of macrophages.[6]

Better repair of bone defects and identification of the best composition and structure have always been the target of research studies, and reconstruction of facial defects is one of the constant concerns.[10,11] Until now, the use of autologous bone graft has been the gold standard treatment, but the lack of its resources, side effects of harvesting it from the donor site, and the tendency of this type of graft for erosion have limited its use.[1]ROTEXMEDICA-Germany/50mg/ml) and xylazine (Alfasan-IR 2%), and ROTEXMEDICA-Germany/50mg/ml Scaffolds made of biodegradable materials play a vital role in repairing bone defects, and it is very important to identify the materials with proper safety. Due to the existence of few studies regarding the inflammatory processes after the placement of synthetic scaffolds used for bone osteogenesis and the importance of this issue in terms of the success rate of osteogenesis, we aimed to investigate the short-term inflammatory responses after placement of the 3D printed scaffolds composed of beta-tricalcium phosphate.

Materials and Methods

Materials

Polycaprolactone (PCL) was purchased from Sigma-Aldrich Medical Instrument Institute, USA. Phosphoric acid collagen was produced by Shanghai U-sea Biotech Co., Ltd. β-TCP was a product of Beijing Modern Orient Precise Chemical Articles Co., Ltd, China.

Scaffold construction method

In order to make the paste, two combinations were used; the first paste consisted of (30wt%) TCP-β + (70wt%) polycaprolactone and the second paste consisted of 0.25 wt.% Tween 80 + (2 wt%) collagen TCP-β + PCL, so that the PCL was melted separately under direct heat inside a beaker, and then, after the reduction of temperature to room temperature, it was mixed with beta-calcium phosphate, and the mixture was placed inside the bioprinter at the same temperature. Then Tween 80 was added to the mixture since it increases the solubility of collagen, and the mixture was placed inside the device; then it was removed through a micronozzle with a thickness of 1 mm at a temperature of 90 °C and at a speed of 80 mm/min under air pressure, and the scaffolds designed by a computer with dimensions of 1 cm x 1 mm were constructed layer by layer in the orthogonal direction. TCP-β + PCL was poured in the form of lines parallel to the axis in a double layer with 65% infill to create a thickness of 1 mm. The layers of the first paste were dried at ambient temperature and finally, the second paste was printed on the previous layers at ambient temperature by a bioprinter (3DPL BIOPRINTER N-IR) N_2 and by a G22 diameter nozzle. Finally, the scaffolds were packed in two layers by a V-pack machine (Dispotech-Italy) and then the scaffolds were sterilized by gamma rays at the sterilization center of the Atomic Energy Organization.

Animals

Sprague Dawley rats aged 15 to 18 weeks were obtained from the Laboratory Animal Research Center located in Zahedan Medical Sciences Research Center. Healthy male and female mice weighing 250-300 grams were exposed to the light and dark cycle under a standard treatment regimen. Animals showing tumors, excessive weight loss, infection, and health problems were excluded from the experiment. Also, the mice were excluded from the study after inducing a bone defect, if any visible disorder occurred at the surgical site. All animal experiments were approved by the biomedical ethics committee of Zahedan University of Medical Sciences (ethics code #: IR.ZAUMS.REC.1400.1).

Grouping and surgical method

Our animal experimental protocol was approved by the biomedical ethics committee of Zahedan University of Medical Sciences (code #: IR.ZAUMS.REC.1400.1).

Eight physically healthy mice were randomly selected and were placed in two control (n=4) and experimental (n=4) groups. 8 samples of scalp tissue in mice were examined in four groups (one-week control, two-week control, one-week experiment and two-week experiment). The scalp samples of mice with bone defects were used as the control group and the mice with β-TCP scaffolds placed on the bone defect site were used as the experimental group (TCP). Mice were anesthetized by intraperitoneal injection of tribromoethanol, along with a 0.1% weight ratio of ketamine (ROTEXMEDICA-Germany/50mg/mL) and xylazine (Alfasan-IR 2%), and the hair on the head of the mice was shaved. In order to protect the eyes, sterile ophthalmic ointment Vit A (Najo/IR-250IU/G) Table 2 was placed on the eyes, and then the head area was disinfected with betadine, the body of the mouse was prepared and draped with a sterile cloth, and the head of the mouse was was placed in a downward position to prevent fluid aspiration. A full-thickness U-shaped incision was made on the scalp with the base of the flap towards the ear. After removing the periosteum, a defect site was created in the middle of the skull with dimensions of 5 x 10 mm with full bone thickness using a carbide fissure bur (China) and a micromotor handpiece (NSK/Japan). After bone removal and washing and debridement with sterile normal saline,

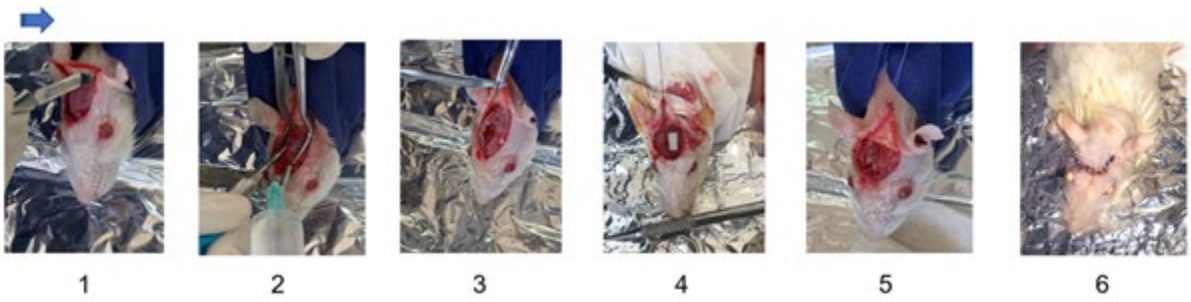

Fig 1. *The surgical steps of placement of 3D printed scaffolds; from left to right: 1) preparation of a U-shaped skin flap, 2) use of a handpiece and a carbide fissure bur to create a bone defect, 3) a bone defect above the dura tissue surface, 4) Placing the scaffold in the bone defect site, 5) Covering the bone defect site with periosteum, 6) Performing skin sutures.*

in the control groups, the fabricated scaffolds were placed in contact with the adjacent bone. Then the incision site was sutured in two layers of periosteum and skin (Figure 1). Immediately after the surgery, 2 cc of Ringer's serum (I.P.P.C/IR) and keptofen 10% (Razak) were injected into the mice as a pain reliever, and Enrosin 5% (Rooyandarou) was used as an antibiotic. During the operation, the eyes were protected from drying due to anesthesia with sterile Vit A eye ointment. After the operation and during recovery, 1cc of Ringer's lactate serum was injected into the peritoneal area to prevent dehydration, and the mice were protected inside a warm blanket to prevent body temperature drops. Enrosin was injected daily for 5 days to prevent infection. Immediately after the surgery, 2 cc of Ringer's serum (I.P.P.C/IR) and keptofen 10% (Razak) were injected into the mice as a pain reliever, and Enrosin 5% (Rooyandarou) was used as an antibiotic. During the operation, the eyes were protected from drying due to anesthesia with sterile Vit A eye ointment. After the operation and during recovery, 1cc of Ringer's lactate serum was injected into the peritoneal area to prevent dehydration, and the mice were protected inside a warm blanket to prevent body temperature drops. Enrosin was injected daily for 5 days to prevent infection. The diet of the mice was continued as usual with special rodent food approved by the Zahedan Medical Sciences Research Center. Mice were killed at intervals of 1 and 2 weeks in compliance with ethical standards and by vital perfusion method, so that the anesthetic drug was injected into the mice and surgically the desired areas containing the skin and the defect site along with a 5 mm margin of the surrounding bone and dura was removed. Scalp tissue samples were placed in 10% formalin solution (DR. MOJALLALI CO/IR) for one week. Then, the fixed samples were placed in the decalcification solution for about three weeks, the solution being changed every three days. After three weeks, radiographic imaging,[13] and confirmation of decalcification,[14] tissue processing steps were performed in a routine manner. Then the groups were examined in terms of pathology and histology.

Sampling, tissue preparation, and tissue sectioning

Based on the design in this research, the mice were killed at intervals of 1-2 weeks and following ethical standards. Scalp tissue samples were placed in formalin solution for one week. Then the fixed samples were placed in the

Table 1. *Tissue processing steps.*

Steps	Processing step	Solution used	Time (minutes)
1-2	Dehydration	80% alcohol (two containers)	60+60
3	Dehydration	95% alcohol	60
4-6	Dehydration	100% alcohol (three containers)	60+60+60
7-8	Clarification	Xylene (two containers)	60+60
9-10	Penetration-immersion	Paraffin (two containers)	60+60

Table 2. Frequency of cells in the studied groups.

Parameters (N)	Control week 1 (n=2)	Control week 2 (n=2)	TCP week1 (n=2)	TCP week 2 (n=2)	p-value
Inflammatory cells	12.5 ±1.4	10 ± 1.1	9 ± 0.7	8 ± 0.7	0.05
Osteoblast	7.5 ± 1.01	10 ± 1.2	16 ± 1.4	27 ± 2.5	0.009
Osteoclast	1 ± 0.5	1± 0.7	3 ± 1.02	4.5 ± 1.7	0.015

TCP week1: (experimental group receiving beta-tricalcium phosphate)
TCP week2: (experimental group receiving beta-tricalcium phosphate)

decalcification solution for about three weeks, and the solution was changed every three days. After three weeks, following the preparation of the radiographic images and confirming the decalcification, the tissue processing steps were performed in a routine manner.

Preparation of tissue samples

Tissue processing was performed by a device that automatically performs the process of dehydrating, clarifying, and penetrating paraffin in the tissues for about 16 hours. Preparation steps were carried out during the night and the related times were adjusted based on the values listed in Table 2.
Small tissue samples were placed in porous cassettes. The cassettes were immersed in the first solution to the transverse bar of the device. According to the timings defined for the device, the cassettes were transferred to other solutions. The tissues remained immersed until the next day when the laboratory work began, and the last step was performed, which involved being placed in molten paraffin.

Molding samples

Paraffin (Neutron/IR) with a melting point above 55°C was used for molding. Melted paraffin was poured into a hard and stable mold and the tissue sample was placed inside the paraffin using a hot clamp with a thick tip in the direction that would give us the most information. Then, paraffin was allowed to freeze at room temperature, and identification stickers, which included tissue number, were attached to the molds. During the molding process, the tissue was prepared for cutting with a microtome.

Sectioning

The molds were trimmed by a hot scalpel and then, they were attached to the base of the mold on the microtome and fixed. Then, for the immunohistochemical study, serial sections with a thickness of 5 µm were prepared from each paraffin block using a rotary microtome, and finally, the desired sections were placed on coded slides coated with albumin glue.

Hematoxylin and eosin staining:

Hematoxylin (MERCK, GERMANY) was dissolved in alcohol and aluminum alum was dissolved in distilled water in the vicinity of low heat; then, the above two solutions were mixed together and boiled quickly. At the same time, mercuric acid (MERCK, GERMANY) was added to the solution and cooled quickly by placing it in water.

Stages of staining

Paraffin removal

The paraffin around the tissues prevents the penetration of the dye into the tissue sections. Therefore, to remove the paraffin, the slides were kept in two xylene jars (for five minutes in each jar).

Hydration

At this stage, using ethyl alcohol with decreasing degrees of pure alcohol to 90, 80, and 70 percent, hydration was done. The slides were placed in each jar for five minutes and washed in running water.

Staining with hematoxylin

The slides were placed in hematoxylin dye for 15 minutes. After removing the slides from the container containing hematoxylin, they were washed in running water for 3-5 minutes.

Removing excess dye

In order to remove the excess dye and differentiation, the slides were placed in alcohol acid for 3 seconds. Then the samples were washed again in running water for 3-5 minutes.

Placing in lithium carbonate

To fix the color of the nucleus, the slides were placed in a container containing lithium carbonate (MERCK/GERMANY) for 3 minutes. Then the samples were washed in running water.

Staining with eosin

The samples were placed in a container containing eosin (MERCK/GERMANY) for 5-10 minutes and then washed in running water for 2-3 minutes.

Dehydration

At this stage, in order to dehydrate the basket containing slides, they were placed in a container containing ethyl alcohol (ALCOHOL PARS/IR) at ascending degrees (70, 80, 90%, and absolute alcohol) and in each for 3 minutes.

Table 3. Comparison of the resorption pits, diameter of blood vessels, and empty lacunae.

Parameters	Control week 1 (n=2)	Control week 2 (n=2)	TCP week1 (n=2)	TCP week 2 (n=2)	p-value
Resorption pit (μm)	2.5 ± 0.51	1.5 ± 0.78	3.5 ± 0.98	3 ± 1.4	0.31
Empty lacuna (N)	15.5 ± 2.1	14.5 ± 0.74	10 ± 1.4	9 ± 1.12	0.028
Vascular diameter (mm)	22.4 ± 1.99	19.92 ± 1.56	11.28 ± 1.7	12.3 ± 1.55	0.233

After drying, the slides were placed in two xylene containers (MERCK/GERMANY) for 5 minutes each.

Fixing slides

In the last step, after cleaning around the sample, a drop of Entellan glue was put on the stained slides and a coverslip was placed at a 45-degree angle on the slide, and then, the coverslip was pressed with tweezers until the air bubbles between the lamellae were completely removed. After drying the slides, the samples were ready for study.

Statistical analysis

Results were presented as mean and standard deviation (SD). SPSS version 25 software (IBM, USA) was used for data analysis. The correlations between the findings were analyzed with the help of a two-way analysis of variance. A level of less than 0.05 was determined as a significant level.

Results

In the four study groups, the number of inflammatory cells was almost at the same level, but the TCP groups (experimental groups receiving beta-tricalcium phosphate) showed a significant decrease in this respect ($p<0.05$) (Table 3).

Examining the number of osteoblasts in the bone defect site showed a significant increase in the number of these cells in the two-week TCP group, compared to the one- and two-week control groups ($p<0.01$) (Figure 2). The study of the osteoclast cells in the bone defect site also showed that the number of these cells in the one-week

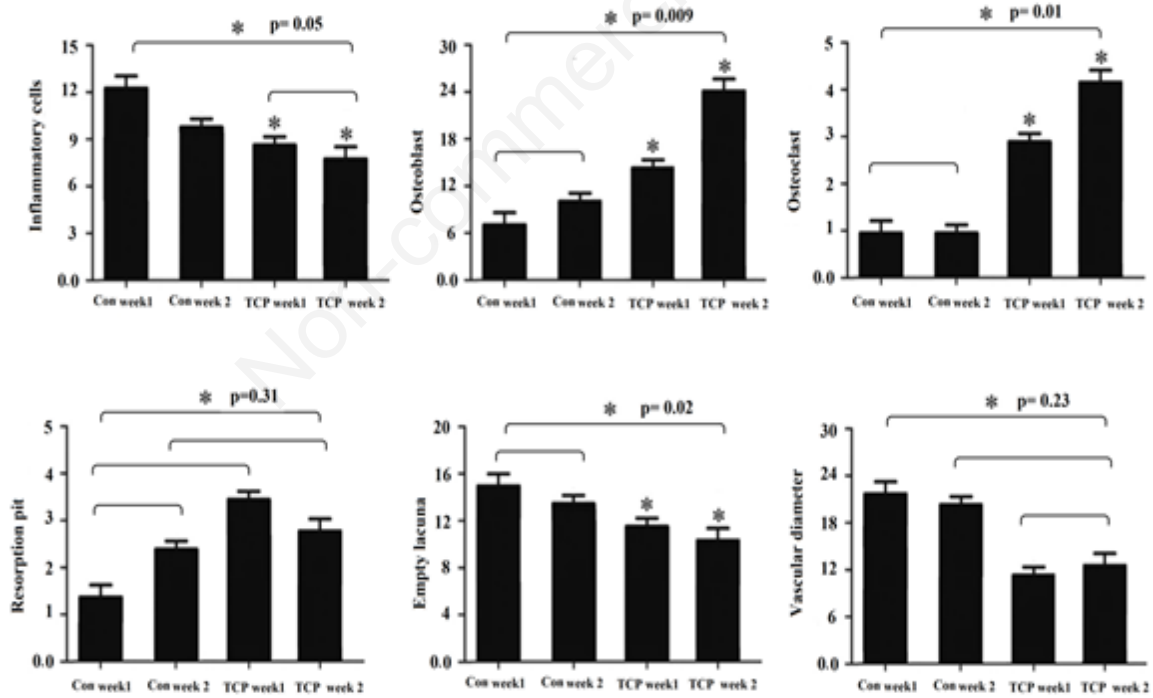

Fig 2. *Comparison of the frequency of inflammatory cells, osteoblasts, and osteoclasts in the studied groups during two weeks and also, comparison of the frequency of resorption pit cells, diameter of blood vessels, and empty lacunae in the studied groups during two weeks. In both groups, there was no significant difference between the first and second weeks in terms of the examined variables. * Significant level of p-value.*

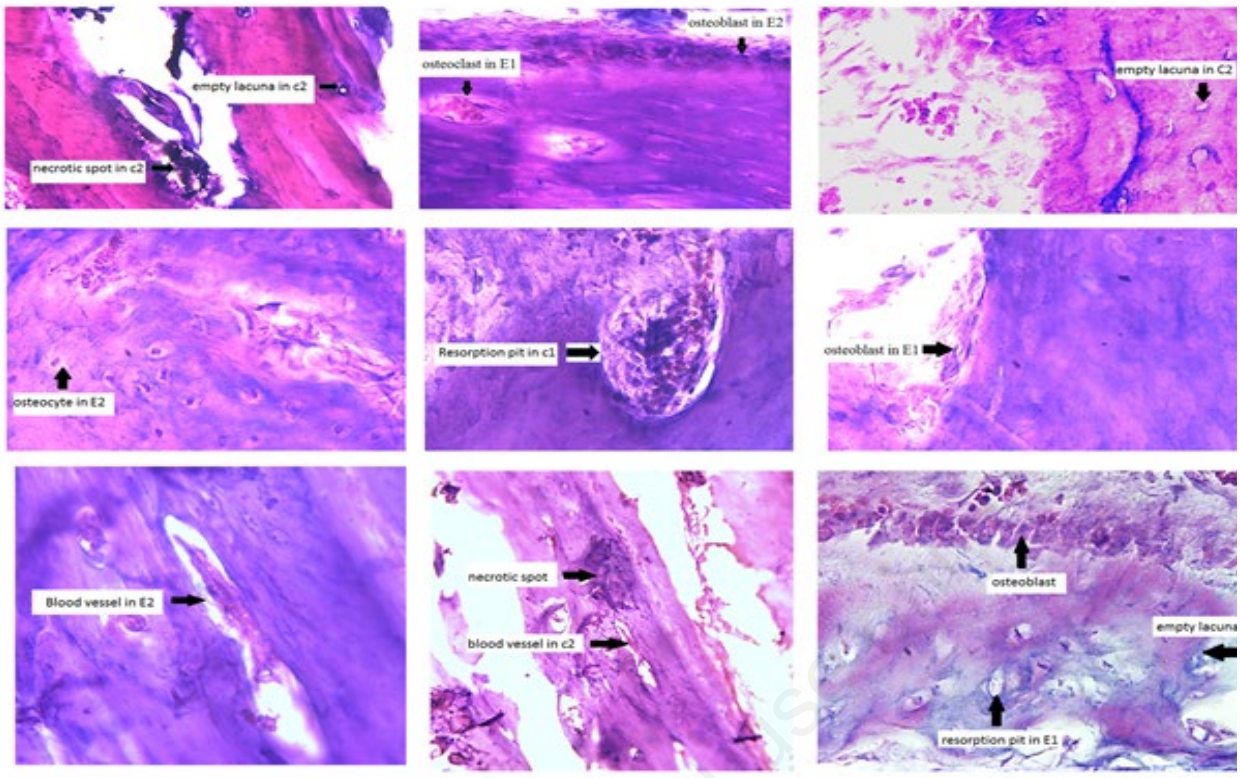

Fig 3. *Histological observations of comparison of changes frequency on necrosis, blood cells osteoblasts, and osteoclasts in the studied groups during two weeks*

C1 = control week 1, C2 = control week 2, E1 = experimental week 1, E2 = experimental week 2

and two-week TCP groups had a significant increase, compared to the one-week and two-week control groups ($p<0.05$) (Figure 3).

Discussion

This study was performed to investigate the potential effectiveness of 3D printed scaffolds on calvarial bone defect healing. Our findings showed that the number of inflammatory cells was decreased in bone defect sites implanted with TCP-β scaffold and the number of osteoblast and osteoclast cells significantly increased. Previous studies have shown similar results in terms of levels of inflammatory cells and cells effective in regeneration.[15-19] Similar studies have shown that inflammatory reactions play an important role in determining the fate of the graft towards active osteogenesis.[20] Anderson et al. (2008) and Brown and Badylak (2013) showed that tissue engineering bone grafting increases the cells associated with osteoblasts, but inflammatory reactions are the first physiological process that occurs.[7,21] During bone implantation with tissue engineering and in the initial response to inflammation, inflammatory cells secrete cytokines and chemokines, which leads to strengthening and calling dendritic cells, lymphocytes, and mononuclear macrophages to move to the defect site.[22,23] Therefore, the increase in the number of inflammatory cells in the first week in both groups indicates a physiological inflammatory reaction, which is necessary to start healing. Although the number of inflammatory cells in the second week did not show a significant decrease in both groups, this finding is consistent with previous studies.[22,23] The results of our study have shown that with the reduction of inflammatory cells in the second week, the number of osteoblast cells in the TCP group was significantly higher than in the control group. It has been reported that chemokines and cytokines produced by inflammatory reactions can increase osteogenesis, angiogenesis, and bone marrow stem cells, but modulation of the physiological inflammatory reaction is necessary to achieve osteogenesis.[24,25]

In the present study, in all groups, osteoclasts in the bone defect sites implanted with TCP-β scaffolds were significantly increased, and the significant presence of osteoclasts in the second week indicated the inducement of osteogenesis and possible bone remodeling. A similar study showed that osteoclasts, which originate from circulating monocyte cells, play an important role in the healing cascade of damaged bone;[26] therefore, the observation of analytical defects in both groups indicates the similarity of the healing processes in the intervention areas. We used 3D-printed TCP-β scaffolds for consideration of structural and chemical properties. Recent studies have concluded that the porous geometry

of PCL in 3D printed scaffolds can improve its mechanical properties.[27] Nanofiber scaffolds covered with degradable chemical compounds can also improve the repair of cranial defects.[28] Bian et al. reported that TCP can be combined with other materials such as collagen to improve its biomechanical and osteogenic properties and reduce inflammation.[29] Of course, the spread and degree of acute and chronic inflammatory responses are related to the amount of damage in the implant placement process and the type of tissue in which the implant is placed.[7] A study showed that scaffolds designed with a 3D printer can increase bone regeneration by stimulating the A2A receptor and other growth factors.[7] Also, the pore size of the scaffold is effective in improving the results.[30] Our results showed that the number of empty lacunae in the TCP group was significantly lower than in the control group. In fact, TCP-β could significantly reduce the number of these lacunae in bone defect sites implanted with TCP-β scaffold. However, the observation of an empty lacuna in histological sections does not mean the absence of osteocytes and it can appear false. At the same time, the results of recent studies have shown that tissue engineering technology allows the production of artificial bone in large quantities. These materials have the potential advantages of excellent biocompatibility, osteoinductivity, and osteoconductivity, thus, they can be a promising new method for bone repair. The construction of superior tissue engineering structures depends on three basic elements: suitable scaffolds to support tissue-cell regeneration, cytokines, and suitable implanted cells.[31] During bone regeneration, the replacement of mesenchymal stem cells and the formation of osteoblasts, as well as the secretion of extracellular matrix, lead to complete formation of bones, and it is important to pay attention to this mechanism in the construction of structures.[32]

Our study showed that there was no significant difference between the groups in terms of the diameter of blood vessels at the defect site. This means that despite the presence of 3D scaffolds in the experimental group, the presence and diameter of blood vessels did not challenge the vascular bed of the area, while the current medical practice still faces serious challenges in the treatment of large bone defects caused by trauma or disease. Arguably the most difficult aspect of treatment would be the development of bone grafts that can restore vascular function to the regenerating bone tissue. The main causes of failure of large grafts are necrosis of the internal part of the graft and lack of integration with the host tissue. The regenerative capabilities of the host tissue with a severely damaged vascular bed are limited, and the integration of a fully functional vascular bed into the bone graft is technically and biologically challenging. Therefore, non-homogeneous graft survival is a major problem and will generally lead to early graft failure.[33] However, Jiao et al. reported that the use of stem cells along with the printed scaffolds composed of beta-tricalcium phosphate could help the stability of the enzymes and biological mechanisms effective in repair and bone formation, and its use is suggested for bone repair.[34] Despite the results of this study, the identification of surgical treatments with high effectiveness in healing large bone defects remains a challenge. The 3D printed scaffolds composed of beta-tricalcium phosphate, which are associated with unique biological and molecular properties, can be effective in increasing effectiveness of surgical treatment, but larger studies are still needed.

In conclusion, 3D printed scaffolds made up of TCP-β that were implanted at bone defect sites were effective in reducing inflammatory responses and filling in gaps through increased osteogenesis, but further preclinical studies are still needed to identify safer biological compounds.

List of acronyms

PCL - polycaprolactone
SD - standard deviation
TCP - tricalcium phosphate
TCP-β - beta-tricalcium phosphate

Contributions of Authors

All authors have read and approved the final edited typescript.

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Conflict of Interest

The authors declare no conflict of interests.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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References

1. Zhang Q, Wu W, Qian C, Xiao W, Zhu H, Guo J, et al. Advanced biomaterials for repairing and reconstruction of mandibular defects. Mater Sci Eng C Mater Biol Appl. 2019;103:109858. doi: 10.1016/j.msec.2019.109858.
2. Malá E, Vejražková E, Bielmeierová J, Jindra M, Vošmik M, Novosad J, et al. [Long Term Monitoring of Nutritional, Clinical Status and Quality of Life in Head and Neck Cancer Patients]. Klin Onkol. 2015;28(3):200-14. doi: 10.14735 /amko2015200.
3. Lopez CD, Diaz-Siso JR, Witek L, Bekisz JM, Cronstein BN, Torroni A, et al. Three dimensionally printed bioactive ceramic scaffold osseoconduction across critical-sized mandibular defects. J Surg Res. 2018;223:115-22. doi: 10.1016/j.jss.2017.10.027.
4. Bohner M, Santoni BLG, Döbelin N. β-tricalcium phosphate for bone substitution: Synthesis and properties. Acta Biomater. 2020;113:23-41. doi: 10.1016/j.actbio.2020.06.022.
5. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury. 2005;36 Suppl 3:S20-7. doi:10.1016/j.injury.2005.07.029.
6. Almeida CR, Serra T, Oliveira MI, Planell JA, Barbosa MA, Navarro M. Impact of 3-D printed PLA- and chitosan-based scaffolds on human monocyte/macrophage responses: unraveling the effect of 3-D structures on inflammation. Acta Biomater. 2014;10(2):613-22. doi: 10.1016/j.act bio.2013.10.035.
7. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008;20(2):86-100. doi: 10.1016/j.smim.2007. 11.004.
8. Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, et al. Scaffolds for Bone Tissue Engineering: State of the art and new perspectives. Mater Sci Eng C Mater Biol Appl. 2017;78:1246-62. doi:10.1016/j.msec.2017.05.017.
9. Rubio-Palau J, Prieto-Gundin A, Cazalla AA, Serrano MB, Fructuoso GG, Ferrandis FP, Baró AR. Three-dimensional planning in craniomaxillo facial surgery. Ann Maxillofac Surg. 2016 Jul-Dec;6(2):281-286. doi: 10.4103/2231-0746. 200322.
10. Ishida S, Shibuya Y, Kobayashi M, Komori T. Assessing stomatognathic performance after mandibulectomy according to the method of mandibular reconstruction. Int J Oral Maxillofac Surg. 2015;44(8):948-55. doi: 10.1016/j.ijom. 2015.03.011.
11. Kumar BP, Venkatesh V, Kumar KA, Yadav BY, Mohan SR. Mandibular Reconstruction: Overview. J Maxillofac Oral Surg. 2016;15(4):425-41. doi: 10.1007/s12663-015-0766-5.
12. Sarkar SK, Lee BT. Hard tissue regeneration using bone substitutes: an update on innovations in materials. Korean J Intern Med. 2015;30(3):279-93. doi: 10.3904/kjim.2015.30.3.279.
13. Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem cells (MSCs) enhance bone regeneration in rabbit ulna critical-sized bone defect model. J Orthop Res. 2006;24(4):610-8. doi: 10.1002/ jor.20119.
14. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res. 2013;28(1):2-17. doi: 10.1002/jbmr.1805.
15. Sridharan R, Ryan EJ, Kearney CJ, Kelly DJ, O'Brien FJ. Macrophage Polarization in Response to Collagen Scaffold Stiffness Is Dependent on Cross-Linking Agent Used To Modulate the Stiffness. ACS Biomater Sci Eng. 2019;5(2):544-52. doi:10.1021/acsbiomaterials.8b00910.
16. Shi GS, Li YY, Luo YP, Jin JF, Sun YX, Zheng LZ, et al. Bioactive PLGA/tricalcium phosphate scaffolds incorporating phytomolecule icaritin developed for calvarial defect repair in rat model. J Orthop Translat. 2020;24:112-20. doi: 10.1016/ j.jot.2020.05.008.
17. Wang Z, Guo Z, Bai H, Li J, Li X, Chen G, et al. Clinical evaluation of β-TCP in the treatment of lacunar bone defects: a prospective, randomized controlled study. Mater Sci Eng C Mater Biol Appl. 2013;33(4):1894-9. doi: 10.1016/j.msec.2012. 12.041.
18. Mohd N, Razali M, Ghazali MJ, Abu Kasim NH. 3D-Printed Hydroxyapatite and Tricalcium Phosphates-Based Scaffolds for Alveolar Bone Regeneration in Animal Models: A Scoping Review. Materials (Basel). 2022;15(7). doi: 10.3390/ma15072621.
19. 19.Bi G, Mo L, Liu S, Zhong X, Yang J, Yuan Z, Chen S, Ren L. DLP printed β-tricalcium phosphate functionalized ceramic scaffolds promoted angiogenesis and osteogenesis in long bone defects. Ceramics International. 2022.24:112-20.
20. Dumas JE, Prieto EM, Zienkiewicz KJ, Guda T, Wenke JC, Bible J, et al. Balancing the rates of new bone formation and polymer degradation enhances healing of weight-bearing allograft/polyurethane composites in rabbit femoral defects. Tissue Eng Part A. 2014;20(1-2):115-29. doi: 10.1089/ ten.TEA.2012.0762.
21. Brown BN, Badylak SF. Expanded applications, shifting paradigms and an improved understanding of host-biomaterial interactions. Acta Biomater. 2013;9(2):4948-55. doi: 10.1016/j.actbio.2012. 10.025.

22. Córdova LA, Loi F, Lin TH, Gibon E, Pajarinen J, Nabeshima A, et al. CCL2, CCL5, and IGF-1 participate in the immunomodulation of osteogenesis during M1/M2 transition in vitro. J Biomed Mater Res A. 2017;105(11):3069-76. doi: 10.1002/jbm.a.36166.
23. Lu LY, Loi F, Nathan K, Lin TH, Pajarinen J, Gibon E, et al. Pro-inflammatory M1 macrophages promote Osteogenesis by mesenchymal stem cells via the COX-2-prostaglandin E2 pathway. J Orthop Res. 2017;35(11):2378-85. doi: 10.1002/jor.23553.
24. van Well GTJ, Daalderop LA, Wolfs T, Kramer BW. Human perinatal immunity in physiological conditions and during infection. Mol Cell Pediatr. 2017;4(1):4. doi:10.1186/s40348-017-0070-1.
25. Al-Maawi S, Orlowska A, Sader R, James Kirkpatrick C, Ghanaati S. In vivo cellular reactions to different biomaterials - Physiological and pathological aspects and their consequences. Semin Immunol. 2017;29:49-61. doi: 10.1016/j.smim. 2017.06.001.
26. Wright HL, McCarthy HS, Middleton J, Marshall MJ. RANK, RANKL and osteoprotegerin in bone biology and disease. Curr Rev Musculoskelet Med. 2009;2(1):56-64. doi: 10.1007/s12178-009-9046-7.
27. Rotbaum Y, Puiu C, Rittel D, Domingos M. Quasi-static and dynamic in vitro mechanical response of 3D printed scaffolds with tailored pore size and architectures. Mater Sci Eng C Mater Biol Appl. 2019;96:176-82. doi: 10.1016/j.msec.2018.11.019.
28. Nie W, Gao Y, McCoul DJ, Gillispie GJ, Zhang Y, Liang L, et al. Rapid mineralization of hierarchical poly(l-lactic acid)/ poly(ε-caprolactone) nano-fibrous scaffolds by electrodeposition for bone regeneration. Int J Nanomedicine. 2019;14:3929-41. doi: 10.2147/ ijn.s205194.
29. Diao J, OuYang J, Deng T, Liu X, Feng Y, Zhao N, Mao C, Wang Y. 3D-Plotted Beta-Tricalcium Phosphate Scaffolds with Smaller Pore Sizes Improve In Vivo Bone Regeneration and Biomechanical Properties in a Critical-Sized Calvarial Defect Rat Model. Adv Healthc Mater. 2018 Sep;7(17):e1800441. doi: 10.1002/adhm.2018 00441. Epub 2018 Jul 25.
30. Bian W, Li D, Lian Q, Li X, Zhang W, Wang K, Jin Z. Fabrication of a bio-inspired beta-Tricalcium phosphate/collagen scaffold based on ceramic stereolithography and gel casting for osteochondral tissue engineering. Rapid Prototyping Journal. 2012:18:49-62.
31. Noori A, Ashrafi SJ, Vaez-Ghaemi R, Hatamian-Zaremi A, Webster TJ. A review of fibrin and fibrin composites for bone tissue engineering. Int J Nanomedicine. 2017;12:4937-61. doi:10.2147/ijn.s 124671.
32. Wang X, Wang Y, Gou W, Lu Q, Peng J, Lu S. Role of mesenchymal stem cells in bone regeneration and fracture repair: a review. Int Orthop. 2013;37(12):2491-8. doi: 10.1007/s00264-013-2059-2.
33. Mercado-Pagán Á E, Stahl AM, Shanjani Y, Yang Y. Vascularization in bone tissue engineering constructs. Ann Biomed Eng. 2015;43(3):718-29. doi: 10.1007/s10439-015-1253-3.
34. Jiao X, Sun X, Li W, Chu W, Zhang Y, Li Y, Wang Z, Zhou X, Ma J, Xu C, Dai K, Wang J, Gan Y. 3D-Printed β-Tricalcium Phosphate Scaffolds Promote Osteogenic Differentiation of Bone Marrow-Deprived Mesenchymal Stem Cells in an N6-methyladenosine-Dependent Manner. Int J Bioprint. 2022 Mar 22;8(2):544. doi: 10.18063/ijb.v8i2.544.

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