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Use of transforming growth factor-β loaded onto β-tricalcium phosphate scaffold in a bone regeneration rat calvaria model

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Abstract

Background: Transforming growth factor-β (TGF-β_1) enhances mesenchymal stem cell (MSC) differentiation into osteoblasts.

Purpose: The aim of the study was to assess whether TGF-β_1 loaded onto β-tricalcium phosphate (β-TCP) synthetic scaffold enhances bone regeneration in a rat calvaria model. The release kinetics of TGF-β_1 from β-TCP scaffold was evaluated in vitro.

Materials and Methods: TGF-β_1 in various concentrations (1-40 ng/mL) was loaded onto the β-TCP scaffold, and release kinetics was monitored by ELISA. The effect of TGF-β_1 on the proliferation of MSCs was assessed using AlamarBlue, and MSC differentiation was evaluated by Alizarin Red quantification assay.Bone augmentation following transplantation of TGF-β_1 loaded onto β-TCP in a rat calvaria model was evaluated in vivo.

Results: Greater TGF-β_1 release from the 40 ng/mL concentration was found. A suppressive effect of TGF-β on the MSCs proliferation was observed with maximum inhibition obtained with 40 ng/mL compared to the control group (P = .028). A positive effect on MSCs osteogenic differentiation was found.Bone height and bone area fraction in vivo were similar with or without TGF-β_1; however, blood vessel density and degradation of the scaffold were significantly higher in the TGF-β_1 group.

Conclusion: TGF-β_1 adsorbed to β-TCP stimulated angiogenesis and scaffold degradation that may enhance bone formation.

KEYWORDS

animal model, biomaterials, bone regeneration, bone tissue engineering, calvaria

1 | INTRODUCTION

Tissue engineering is a potent tool in managing bone loss. It involves the use of scaffolds with growth factors (GFs) and/or stem cells.[1] Transforming growth factor-β (TGF-β) is a secreted protein participating in proliferation, differentiation, migration, and apoptosis, and has an important role in embryogenesis, tissue repair, and immunomodulation.[2] TGF-β is abundant in bone and plays a central role in bone remodeling.[3] Mesenchymal stem cells (MSCs), osteoclasts, osteoblasts, and osteocytes are involved in bone resorption and formation.[4] TGF-β can provide competence in the early stages of osteoblastic differentiation, but inhibition at the late stages of osteoblastic differentiation.[5]

Rina Elimelech and Nizar Khoury contributed equally to this study.

Important mediators of bone regeneration are GFs; however, they exhibit a limited half-life in vivo.[6] Active TGF-β_1 half-life is about 2 to 3 minutes, whereas latent TGF-β_1 binding protein half-life is >100 minutes.[7] Therefore, controlled-release scaffolds have been proposed as a feasible means of overcoming this obstacle. Owing to advances in biomaterial research, the use of synthetic bone ceramic materials, such as tricalcium phosphate (TCP), $Ca_3(PO_4)_2$, is feasible. TCP is a biologically active biodegradable ceramic. There are two types of TCP: alpha (α), which is stable between 1125°C and 1430°C, and beta (β), which is stable below 1125°C. Owing to the greater stability and biodegradation rate of β-TCP, it has been widely used in bone regeneration.[8] Furthermore, the stimulation of the differentiation of mesenchymal cells toward osteoblasts and induction of new bone formation has shown that β-TCP shows potentially osseoinductive properties. In later stages of bone regeneration, hydrolysis and active phagocytosis gradually disintegrates the material and it is replaced by newly formed bone tissue.[9] The bioactivity of calcium phosphate cements is affected by their porosity. Pore size influences both the ingrowth of bone and angiogenesis, mechanical strength, and shape and increased porosity improves contact with body fluids on the surface area.[10]

Low doses of TGF-β loaded onto biological scaffolds have been shown to enhance bone growth.[11,12] Blumenfeld et al. showed enhanced bone defect wound healing with a single dose of TGF-β (10 mg) at 4 weeks in a rat model.[13] Similarly, Lind et al. reported enhancement of bone growth by adsorbing 300 mg of rhTGF-β_1 to TCP-ceramic coated implants.[11] The primary aim of the study was to load TGF-β_1 onto β-tricalcium phosphate (β-TCP) synthetic scaffold; to follow the release kinetics and to investigate the biological effect of TGF-β_1 on the function of MSCs. The secondary aim was to evaluate the effect of TGF-β_1 loaded onto the β-TCP synthetic scaffold on bone regeneration in a rat calvaria model.

2 | MATERIALS AND METHODS

2.1 | Loading and release kinetics of TGF-β_1 from the β-TCP scaffold

Based on previous results of comparative studies on grafting materials performed in our lab, synthetic β-TCP scaffold was chosen for this study.[14] Human recombinant TGF-β_1 (R&D system, Minneapolis, Minnesota) was loaded onto β-TCP scaffold (LASAK Ltd, Prague, Czech Republic) and the release kinetics was evaluated by ELISA. Then, 0.2 g of β-TCP granules were placed as a dense monolayer in a 24-well plate, and 100 μL of TGF-β_1 in different concentrations (10, 20, and 40 ng/mL) was added to each well to create three testing groups, and one control group containing β-TCP and phosphate buffered saline (PBS) without TGF-β_1. After a 24 hours adsorption time of TGF-β_1 onto β-TCP granules, 500 μL of PBS was added to each well; samples of 100 μL were taken from the supernatant for each measurement. Measurements after 0, 1, 3, 6, and 9 hours were performed. The samples were placed in polypropylene tubes. The release kinetics was measured with specific ELISA assay for TGF-β_1 (ab119558; Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions. The experiment was performed twice in duplicates.

2.2 | Investigating the biological effect of TGF-β_1 on MSCs function in vitro

2.2.1 | Isolation, culture, and characterization of rat bone marrow MSCs

This animal study was approved by the ethical committee of the Technion Institute of Technology (approval no. IL 0450314). Four- to six-week-old male Sprague-Dawley rats (300 g) were sacrificed via CO_2 asphyxiation, and the femora and tibiae bones were collected under sterile conditions, and bones cut at both ends. Bone marrow was collected by flushing the bones with Dulbecco modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 100 U/mL penicillin, 200 mM L-glutamine (all chemicals were purchased from Biological Industries Ltd, Beit Haemek, Israel). Three days post-culturing of the cells, non-adhesive cells were gently washed with PBS and removed, while the remaining attached cells were constantly cultured with DMEM medium. MSCs (at passage 4) were characterized using fluorescence activated cell sorting (FACS). Cells were labeled with specific antibodies CD90, CD14, CD31, CD34, and IgG (BD Biosciences, San Jose, California). A total of 5×10^5 cells were suspended in PBS and incubated for 30 minutes with antibodies according to the manufacturer's recommendations. Following three washing cycles with PBS, the cells were resuspended in PBS and analyzed via FACS scan and the CellQuest software (Becton Dickinson, Franklin Lakes, New Jersey).

2.2.2 | The effect of TGF-β_1 on the proliferation of MSCs

A total of 3×10^4 MSCs were seeded into a 12-well plate with 1 mL of DMEM. The medium was loaded with three different concentrations of TGF-β_1 (40, 10, and 1 ng/mL) and one group of MSCs without TGF-β_1 served as the control group. Cell metabolic activity was measured with AlamarBlue (Invitrogen, Paisley, United Kingdom) after 24 and 48 hours and the fluorescence signal was measured using a spectrophotometer (excitation at 545 nm, emission at 590 nm; fluorometer, FLUOstar, BMG Labtech, Ortenberg, Germany). The data were expressed as fluorescence intensity, which is directly proportional to the number of proliferating and living cells.[15]

2.2.3 | The effect of TGF-β_1 on osteogenic differentiation of MSCs

A total of 2×10^4 MSCs in passage 3 to 4 were seeded in 12-well plate and cultured in DMEM to attain 70%-80% confluence. The medium was then replaced by differentiating medium composed of DMEM supplemented with 10% FCS, 50 μg/mL ascorbic acid, 10 nM dexamethasone, and 10 mM β-glycerol phosphate (Sigma Chemical Co, St. Louis, Missouri). Based on previous studies[16–18] that presented an enhancement of MSCs differentiation by loading a single low dose (0.1-1 ng/mL) of TGF-β, we chose to investigate the effect of TGF-β_1 on MSCs differentiation. MSCs were cultured in DMEM with 1 ng/mL TGF-β (group A) or without TGF-β (group B), and in osteogenic

medium with 1 ng/mL TGF-β_1 (group C) or without TGF-β (group D). Cells were kept in differentiating medium for 21 days. Osteogenic differentiation was evaluated by Alizarin Red staining (ARS) and Alizarin Red Quantification Assay (Sciencell, Carlsbad, California) according to the manufacturer's instructions. Calcium deposits can be visualized by their red color under a light microscope.

2.2.4 | Alizarin Red S Staining Quantification Assay

Cells were washed with PBS and fixed with 4% paraformaldehyde (Sigma) for 15 minutes at room temperature. Then, cells were washed thrice with diH_2O and stained with 1 mL of 40 mM ARS (Sciencell) per well and incubated for 30 minutes at room temperature with gentle shaking. Alizarin was aspirated and the wells were washed at least five times with diH_2O. Then, 2 mL of 10% (v/v) acetic acid was added to each well, after 30 min, the monolayer was scraped off the well with a cell scraper and transferred to centrifuge tube. After vortexing for 30 seconds, the tubes were sealed with paraffin film and heated to 85°C for 10 minutes, then centrifuged at 20,000*g* for 15 minutes and 500 μL of the supernatant was removed to a new 1.5 mL tube. Then, 200 μL of 10% (v/v) ammonium hydroxide was added to neutralize the acid, and 150 μL of the supernatant was read in triplicate at 405 nm.

2.2.5 | The influence of TGF-β_1 on the attachment of MSCs to β-TCP

β-tricalcium phosphate granules were added into 48-well plate and loaded with 100 μL PBS either with 40 ng/mL TGF-β_1 or without TGF-β_1 as the control. It was incubated for 24 hours to allow adsorption of TGF-β_1 to β-TCP. A total of 5×10^4 MSCs were dripped over the β-TCP layer and incubated for 1 hour. The medium containing the nonadhering cells was removed and collected, rinsed once again, and incubated for another 30 minutes. Incubation was repeated twice. The granules were gently removed to allow trypsinization of the cells adhered to the well. The cells in the supernatant and those at the bottom of the initial seeding wells were collected and counted, and the seeding efficiency was calculated: (initial cell number − remaining cell number)/initial cell number × 100%.

2.2.6 | Evaluation of TGF-β_1 on bone regeneration in a rat calvaria model

Fourteen male Sprague-Dawley rats (350 g) received an intramuscular injection of 100 mg/kg bw ketamine (ketaset, Fort Dodge, Iowa) and 5 mg/kg bw Xylazine (Eurovet, Cuijk, Holland) for anesthesia. Exposure of the calvaria was performed via a U-shaped incision (full thickness skin flap raised). The periosteum, containing osteoprogenitor cells, was reflected and four cortical perforations of 1 mm diameter were performed under water irrigation in the calvaria via electric hand drill and diamond burr. A rigid gold dome (7 mm in radius and 5 mm in height) served as guided bone regeneration model (GBR) and preserved the space between the calvaria and the dome. The GBR technique was used to create a barrier that prevents migration of fibroblasts and epithelial cells from the surrounding soft tissue. The following groups were transplanted under the gold dome: 0.2 g β-TCP alone served as the control group (n = 7), and the test group (n = 7) where the TGF-β_1 (40 ng/mL) was loaded on 0.2 g β-TCP 24 hours before the surgical procedure (as described earlier), without adding MSCs to any group. Fixation screws secured the domes to the calvaria, surgical flaps were repositioned, and horizontal mattress resorbable sutures with minimal tension were performed. Post-op, each rat was kept in a separate cage and all rats were fed chow and water ad libitum. Then, 0.1 mg/kg bw cephalexin (Norbrook Laboratories, Monaghan, Ireland) and 0.2 mg/kg bw Buprenorphine (Vetmarket, Shoham, Israel) were injected by a subcutaneous method preoperation and 3 days postoperation. Rats were sacrificed following 3 months via Isofloran and CO_2 asphyxiation. Following euthanasia, the domes were removed and the regenerated tissue together with the underlying original calvaria was removed, and the specimens were fixed immediately in 4% paraformaldehyde for 2 days.

2.2.7 | Histological preparation

The specimens were fixed with 4% paraformaldehyde and decalcification with 10% EDTA (Sigma-Aldrich, Natick, Massachusetts) for 4 weeks. Specimens were then sliced into half in the midline, embedded in paraffin, and sectioned (5 μm). Determination of bone morphology was acquired via two stains: (1) hematoxylin and eosin (H&E) and (2) Masson's trichrome.

2.2.8 | Histomorphometric analysis

The Masson's trichrome stained sections were scanned by a panoramic digital slide scanner (3DHISTECH panoramic MIDI, Budapest, Hungary). The calibration scale and morphometric analyses were determined by the Image-Pro software (Rockville, Maryland). The histomorphometric analysis was accomplished by isolating the rat's original calvaria from the newly augmented tissue to analyze only the regenerated bone tissue. The following parameters were calculated for each specimen using the Image-Pro software: *Gained vertical bone height*: maximal gained bone height (mm) which was measured from the superior border of the calvaria to the crest of the newly formed bone. *Bone area fraction (new bone)*: percentage of bone area from the whole newly regenerated tissue under the dome (excluding the original calvaria). *Blood vessel density (BVD)*: Luminal structures were identified using anti-rat CD31. Blood vessels (BV) were quantified in 10 random areas within each specimen (magnification: ×40), and an average was calculated for individual specimens (BV/mm^2). BVD was defined as mean number of BV in a microscopic field (260 × 444 μm). *Residual scaffold (RS) and connective tissue (CT)*: percentage of RS and CT area from the whole newly regenerated tissue under the dome.

2.2.9 | Statistical analysis

A SSPS program was used. Descriptive statistics included means and medians, ranges, and standard error. Comparisons between the

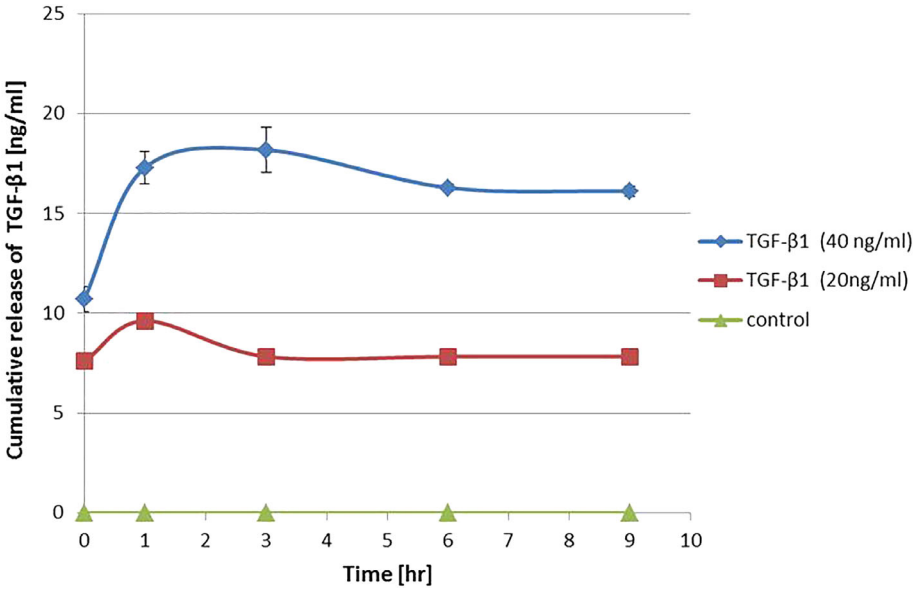

FIGURE 1 Release kinetics of TGF-β_1 from β-TCP. A larger amount of TGF-β_1 was released from the scaffold loaded with 40 ng/mL compared to 20 ng/mL. In scaffold loaded with 40 ng/mL, 26% of initial TGF-β_1 concentration was released in the first hour and remained relatively constant for 9 hours. β-TCP, β-tricalcium phosphate; TGF-β_1, transforming growth factor-β

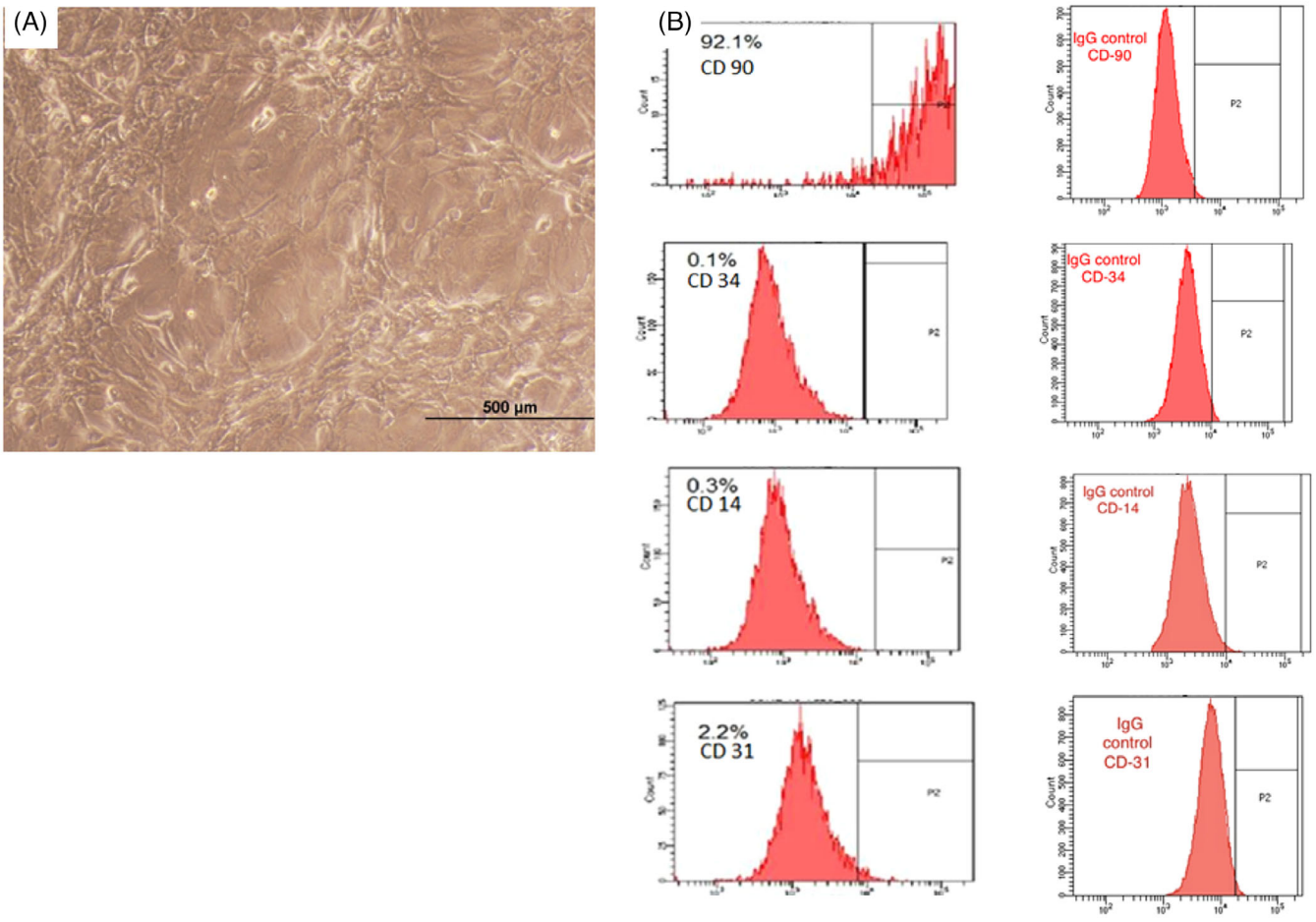

FIGURE 2 MSCs characterization via FACS analysis. A, Cultured MSCs were positive to CD90 and negative to: CD34, CD14, and CD31 compared with IgG control. B, FACS, fluorescence activated cell sorting; MSCs, mesenchymal stem cells

groups in the proliferation assay were performed using Wilcoxon and Kruskal Wallis test. Comparisons between the groups in the differentiation assay were performed using Mann-Whitney nonparametric test. Comparisons between the groups in histomorphometric analysis were performed using a Student *t* test for unpaired observation (two-tailed). A threshold of $P \leq .05$ was set to determine true significance.

3 | RESULTS

3.1 | TGF-β_1 release kinetics from β-TCP

Higher concentrations of TGF-β_1 were released from the 40 ng/mL compared with 20 ng/mL loaded scaffolds. In the scaffold loaded with 40 ng/mL TGF-β_1, 26% of the initial TGF-β_1 concentration was released in the first hour and remained relatively constant for 9 hours (Figure 1).

Similarly, in the 20 ng/mL TGF-β_1 loaded scaffold, an initial burst release occurred within the first hour, followed by a steady release. The release kinetics of the 10 ng/mL TGF-β_1 loaded scaffold was below the detection level.

3.2 | MSCs culture and characterization

Cells rapidly replicated and formed a monolayer of homogenous spindle shape morphology. FACS analysis revealed that more than 92% of the cells were CD90 positive (MSCs surface marker). Importantly cells were negative for CD14, CD31, and CD34 (markers of hematopoietic and endothelial cells, respectively; Figure 2).

3.3 | TGF-β_1 suppress MSCs proliferation

This study demonstrated the effect of (1, 10, and 40 ng/mL) TGF-β_1 on the proliferation of MSCs. After the first 24 hours, we found a suppressive effect of TGF-β_1 on the MSCs proliferation in a dose-dependent manner (Figure 3), maximum inhibition was observed with 40 ng/mL compared to the control group ($P = .028$). There were no significant differences between 24 and 48 hours.

3.4 | TGF-β_1 enhances MSCs osteogenic differentiation

Twenty-one days after culturing cells in osteogenic differentiation medium, AR demonstrated positive red staining in the groups cultured in osteogenic medium, especially in the group treated with TGF-β_1. Weak stain was observed in the DMEM (standard medium) groups ($P = .021$; Figure 4).

3.5 | TGF-β_1 did not influence MSCs adhesion to β-TCP

According to the results, adsorption of TGF-β_1 to β-TCP did not influence MSCs attachment to the scaffold. In the TGF-β_1 group, 86% of the seeded MSCs were attached to the scaffold compared to 85% in the control group.

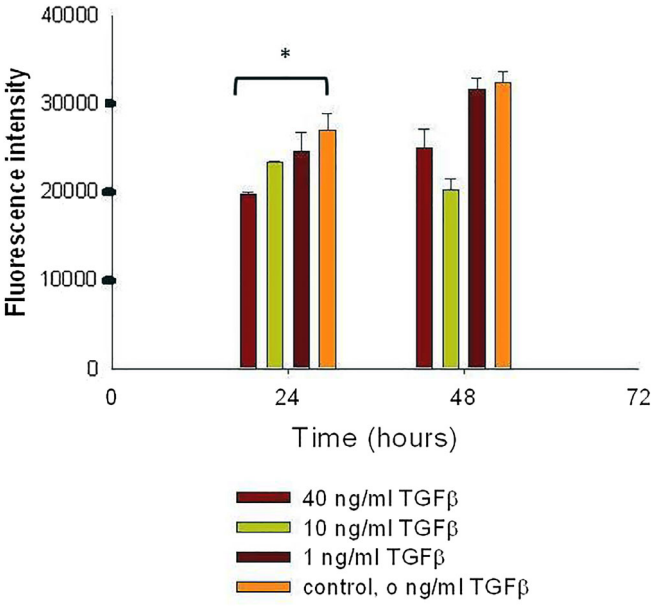

FIGURE 3 MSCs proliferation in response to TGF-β_1. TGF-β_1 suppressed MSCs proliferation in a dose-dependent manner. After 24 hours, a statistically significant difference was observed between the groups 40 ng/mL and the control group *P*, * = .028. MSCs, mesenchymal stem cells; TGF-β_1, transforming growth factor-β

3.6 | TGF-β_1 stimulates scaffold degradation and angiogenesis, in vivo

Thirteen rats survived the experiment. Rats had no locomotive problems and gained weight over time. The surgical sites healed completely and the domes remained in place for the entire experimental duration. Macroscopically, following the removal of the gold domes, the new augmented hard tissue was tightly attached to the rat's original calvaria and filled the entire space under the dome in all rats.

Histologically, sections showed that the composition of the space under the dome consisted of bone, RS and CT in different proportions. The newly formed compact bone was continuous with the original calvaria in the lower part of the specimens. The upper or distal part of the augmented tissue consisted of RS surrounded by vascularized, dense CT (Figure 5).

The newly formed BV invaded the β-TCP scaffold in both groups, with a higher BVD in the TGF-β_1 group ($P = .0009$). The percentage of the new bone and CT were similar among the test and control groups, however degradation of the RS was significantly enhanced in the TGF-β_1 group ($P = .036$; Table 1).

4 | DISCUSSION

Growth factors play an important role in bone regeneration. However, due to their high cost, safety, and limited half-life only a few recombinant GFs have been approved for clinical use (eg, BMP-2 [rhBMP-2], recombinant BMP-7 [rhBMP-7], recombinant human platelet-derived GF-BB and β-TCP [rhPDGF-BB/β-TCP]).[19] Furthermore, the roles of vascular

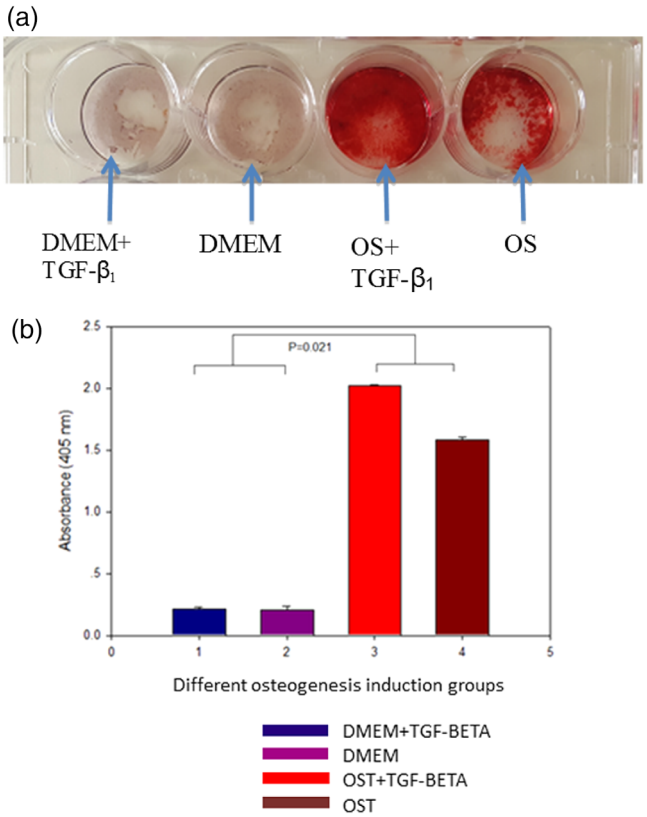

FIGURE 4 MSC differentiation in response to TGF-β_1. A, Alizarin Red staining of MSCs cultured in four different groups for 21 days demonstrating differences between the two major groups DMEM (standard MSC culture medium) and OS (osteogenic medium). B, Quantification assay demonstrating significant difference between the two major groups DMEM and OS, *P* = .021. DMEM, Dulbecco modified Eagle's medium; MSCs, mesenchymal stem cells; TGF-β_1, transforming growth factor-β

endothelial growth factor (VEGF), fibroblast growth factor, Insulin-like growth factor, and TGF-β in bone regeneration are currently under investigation.

Controlled-release scaffolds have been proposed as a feasible means of overcoming the short half-life and rapid degradation of GFs. In the current study, synthetic β-TCP was chosen as the scaffold based on the superior results achieved with the use of β-TCP in a vertical bone augmentation rat calvaria model performed previously in our lab.[14] TGF-β_1 was adsorbed to a synthetic scaffold and the effect of released TGF-β_1 on cultured MSCs and bone formation was followed. In vitro, we found a burst release during the first hour in which more than 26% of the 40 ng/mL TGF-β_1 was released. This burst release was followed by a slow release of TGF-β_1 for up to 9 h. This initial burst release may be due to the desorption of proteins on or near the surface of the scaffold, or insufficiently interacting with the scaffold.[20] The slow release can be attributed to electrostatic forces between molecules as well as environmental conditions (eg, temperature). Furthermore, the continued release of low doses may be attributed to the solubility of the scaffold.

The effect of TGF-β_1 on osteogenesis and angiogenesis is dependent on the dose. Due to the carcinogenic potential of TGF-β_1, the minimal concentration that achieves desired results should be used.[21] We examined the influence of TGF-β_1 on cell function in various concentrations (1, 10, 20, and 40 ng/mL). The seeding efficacy of MSCs to the scaffold (with or without TGF-β_1) was very high ~86% after 1 hour of incubation. The proliferation assay results showed that TGF-β_1 inhibits cell proliferation in a dose- and time-dependent manner. The maximum inhibition was obtained with 40 ng/mL concentration after 24 hours. Studies have suggested a mechanism for inhibition of cell proliferation is that TGF-β binds to the TGF-β receptor and activates the receptor's intracellular protein kinase domain. This leads to the phosphorylation of Smad proteins that enter the nucleus and bind to promoters of genes to control transcription, for example, the p21 gene that controls the arrest of the cell cycle.[21–24] In this study, the addition of TGF-β_1 to osteogenic culture medium enhanced differentiation of MSCs and increased calcium deposits. TGF-β cooperates with Wnt signaling, and promotes osteoblast differentiation of MSCs.

In the in vivo study, we used an extra-cortical GBR model that is well established.[25] Extra-cortical bone formation is limited to only a few millimeters due to limited blood supply, nutrients, and cells.[26] Therefore, the addition of TGF-β to the scaffold may improve angiogenesis and osteogenesis. In this study, histological analysis of the regenerated tissue under the dome revealed the presence of lamellar and woven bone together with residual grafting material and CT. The percentage of new bone was almost identical in both test and control

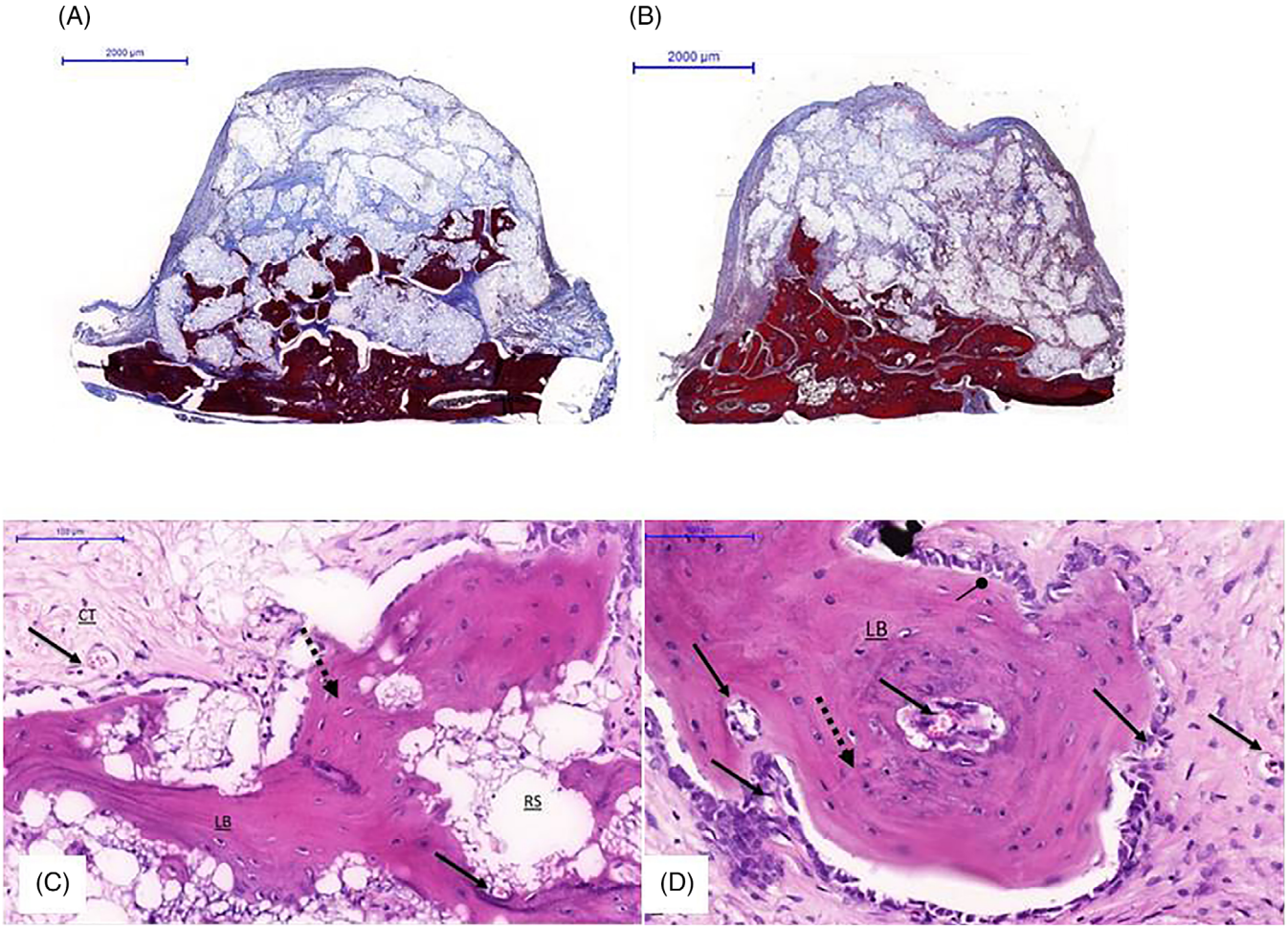

FIGURE 5 Histological view of the newly regenerated tissue. A and B, Samples were stained with Masson's trichrome. Augmented tissue consisted: bone (dark red), residual scaffold, and connective tissue. Control group (β-TCP alone) (A) compared to the test group (β-TCP + TGF-β) (B). C and D, Samples were stained with Hematoxylin and Eosin. Augmented tissue consisted: connective tissue (CT), lamellar bone (LB), and residual scaffold (RS). The black arrows pointing on blood vessel, the dotted arrows pointing on osteocytes, and the circular arrow pointing on osteoblasts. Control group (β-TCP alone) (C), test group (β-TCP + TGF-β) (D). β-TCP, β-tricalcium phosphate; TGF-β, transforming growth factor-β

groups (~11.5%). Similar bone regeneration among the groups can be attributed to several factors. In the current in vivo study, low concentration of TGF-β_1 was used (40 ng/mL). Better results might have been achieved using higher concentrations.[11,27,28] In this rat extracortical calvaria model, the microenvironment in the transplantation site is cell depleted (especially MSCs) due to the thin and cortical calvaria bone. Therefore, addition of MSCs to the transplantation site might improve results. Finally, a longer observation period may be necessary in this type of model. A previous study performed in our lab on the same rat calvaria model demonstrated an increase in vertical bone height gain and bone fraction from 3 to 5 months that was significant.[14]

TABLE 1 Histomorphometric analysis of bone regeneration

	β-TCP + TGF-β	β-TCP	*P*-value
New bone (%)	11.56 ± 2.8	11.86 ± 2.2	.943
Connective tissue (%)	16.8 ± 2.4	10.9 ± 0.4	.1223
Residual scaffold (%)	18.7 ± 2.1	27.39 ± 1.9	.0362
BVD (BV/mm^2)	11.62 ± 1.1	2.56 ± 0.2	.0009
gVBH (mm)	1.55 ± 0.3	2 ± 0.7	.4042

Abbreviations: β-TCP, β-tricalcium phosphate; BVD, blood vessel density; gVBH, gained vertical bone height; TGF-β, transforming growth factor-β.

Scaffold degradation was enhanced in the test group (TGF-β_1 + β-TCP), this phenomena may be related to the fact that activation of RhoA signaling by low concentration of active TGF-β_1 can induce macrophage migration.[29] As macrophages are precursors for osteoclasts, it is possible that TGF-β_1 increased scaffold degradation by these cells. Therefore, TGF-β_1 stimulates both the differentiation and survival of osteoclastic cells.[30]

An additional known effect of TGF-β_1 is the stimulation of extracellular matrix formation as TGF-β_1 acts to maintain a net accumulation of extracellular matrix.[31] Indeed, CT percentage was higher in the test group compared to the control group (P = .1223). The regulation of TGF-β_1 in collagen synthesis gave similar results.

Angiogenesis in regeneration is critical.[32] In vivo, angiogenesis is induced by TGF-β_1 through an indirect mechanism, by inducing

expression of VEGF in epithelial cells or osteoblasts.[33] In our study, newly formed BV invading the β-TCP scaffold in both groups, with a higher BVD in the TGF-β group (P = .0009) was found.

In conclusion, this study shows that TGF-β_1 can be successfully adsorbed to synthetic β-TCP scaffold and demonstrated an initial burst release profile that was followed by a slow release profile for up to 9 hours. In vitro, TGF-β_1 increased MSC differentiation toward osteoblasts and decreased MSC proliferation. In vivo, the percentage of the new bone was similar among test and control groups, however, cotransplantation of TGF-β_1 adsorbed to β-TCP stimulated angiogenesis and scaffold degradation that may, in the future, be followed by enhanced bone formation. Future studies may determine the ideal concentration of TGF-β_1 for bone regeneration using a synthetic scaffold.

CONFLICT OF INTERESTS

The authors (R.E, N.K, T.T, I.B, Z.G, H.Z) declare that they did not receive any benefits for personal or professional use and there are no conflicts of interest in this study. The study was funded by the Research Institute for Bone Repair, Rambam Health Care Campus, Haifa, Israel.

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