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Calvarial bone augmentation using Bioglas Scaffold enhanced by Platelet-Rich Plasma and BMP2

Taha Chartab Mohammadi[1] , Mostafa Eslami[1] , Zahra Jafari[2] , Mohammad Reza Nourani[3*]

[1] Student Research Committee, Baqiyatalah University of Medical Sciences, Tehran, Iran
[2] Periodontology Dept., Dentistry school, Baqiyatalah University of Medical Sciences, Tehran, Iran
[3] Division of Tissue Engineering, Baqiyatalah University of Medical Sciences, Tehran, Iran
*Corresponding Author: Mohammad Reza Nourani , r.nourani@yahoo.com

Taha Chartab Mohammadi
Medical Student

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Abstract

Introduction: Calvarial bone defects are a common problem in craniofacial surgery. The use of autologous bone grafts is the gold standard for reconstruction, but it has limitations such as donor site morbidity and limited availability. Bioglass scaffolds have been used as an alternative to autologous bone grafts due to their osteoconductive properties. However, their use alone has shown limited success in calvarial bone augmentation. Platelet-rich plasma (PRP) and bone morphogenetic protein 2 (BMP2) have been used to enhance bone regeneration. This study aimed to evaluate the effectiveness of combining Bioglass scaffolds with PRP and BMP2 for calvarial bone augmentation in a rat model.

Results: The results showed that the combination of Bioglass scaffolds with PRP and BMP2 significantly increased new bone formation compared to the control group. The histological analysis also showed that the newly formed bone had a similar structure to native bone. Therefore, the combination of Bioglass scaffolds with PRP and BMP2 could be a promising approach for calvarial bone augmentation in craniofacial surgery.

Keywords: bone augmentation, Bioglas, Platelet-Rich Plasma, BMP2

1. Introduction

Bone repair is one of the most important challenges facing us in the clinic. Unfortunately, Different approaches for the preservation of bone have been investigated in recent years. Fracture healing complications, such as delayed and nonunions, are associated with 5–10 % of the over 6 million fractures occurring annually in the USA. Several conditions such as accidents/ trauma, tumor or cyst resection, inherent bone defects, and infectious require bone grafting is required to regenerate new tissue [1, 2].

Common bone graft biomaterials were proposed including autografts (a patient's bone) still considered the "gold standard" owned to their osteogenic, osteoconductive, and osteoconductive properties [3, 4]. it also reveals some limitations such as limited availability, and morbidity in the donor area, and the need for a second surgery, allografts (human cadaver bone) with osteoconductivity and osteoinductive capacities, xenografts (animal bone) and synthetic biomaterials are emerging as substitutes for bone regeneration with biocompatible, biodegradable, and bioactive properties[5-8].All of these materials, although acceptable, are not applicable in many situations, and usually require extra attention in the use of cells and signaling molecules during the decision-making process[5].

Bioactive material has been described as a material that has been designed to induce specific biological

activity [9]. that undergoes specific surface reactions. Bioactive glass (BG) is a bioactive material, when implanted into the body, leading to the formation of a hydroxyapatite-like layer on its surface, that is responsible for the formation of a strong bond with hard and soft tissues [10]. BG has been shown to improve new bone formation in vivo [88,89 own paper] and is considered to be osteoconductive as well as osteoinductive [11].

Growth factors (GFs) are expressed during different phases of tissue regeneration and are promoting tissue regeneration [12].Platelet-rich plasma (PRP) is an inexpensive way to obtain many GFs in physiological proportion and is clinically used as an autologous blood product to stimulate tissue regeneration. The growth factors produced by human platelets include platelet-derived growth factor, insulin-like growth factor, transforming growth factor b, basic fibroblast growth factor, epidermal growth factor, and vascular endothelial growth factor [13]. Osteogenesis improves by PRP administration and accelerates healing processes because of releasing the growth factors from platelets after the coagulation process in the defect site [14].

Recombinant human (rh) BMP2, has been shown to induce bone formation in a variety of indications[10, 12, 15] . A large number of animal and human studies have confirmed the successful use of rhBMP2 to induce bone regeneration for large bone defects such as mandibular resection and cleft palate defects[16-18]. Several biomaterials have been studied as carriers for rhBMP2 and rhBMP2 has been revealed to significantly enhance bone repair in vivo when delivered by an alloplastic substitute[15, 16].

This study aims to test whether PRP in combination with bioactive glass containing rhBMP2 enhances bone formation. We hypothesized that the addition of PRP to the alloplastic substitutes containing rhBMP2 could provide a highly applicable approach to clinical bone reconstruction shortly.

2. Materials and Methods

2.1. Study design

The animal experiment was approved by the Animal Care and Use Committee. All rats were housed in the animal care laboratory at our university by the standards established by the animal sciences center for the care and use of laboratory animals.

2.2. PRP Preparation

Peripheral blood was obtained immediately after anesthesia, and blood volume (10 mL) was collected from the animals of each group, using citrate dextrose as an anticoagulant . Centrifugal tubes with the blood were centrifuged at 1200 rpm for 10 min at room temperature. The blood was then separated into 3 different parts: red blood cells (at the bottom), platelet-rich plasma (in the middle), and platelet-poor plasma (at the top). The platelet-poor plasma was discarded from the tube and the remaining content was centrifuged again at 1200 rpm for 15 min. Only 1 mL PRP (platelet-rich plasma) was removed and prepared for the experiment .

2.3. Graft Preparation

The following percentage mixture was used in our research. The 45s5 BG (45% SiO2, 24% CaO, 24% na2O 6% P2O5) was utilized in this study (Mo-Sci company-USA). The particle size of 45s5 glass ranges from 100 to 300 μm. The mixture included 20 mg of bioactive glass powder with the appropriate volume of PRP and rhBMP2 according to experimental groups: 1. 20 mg BG+100 μLPPP, 2. 20 mg BG+100 μLPRP, 3. 20 mg BG+100 μLPPP+ 0.2 μg/ml rhBMP2, 4. 20 mg BG+ 100 μL PRP+0.2 μg/ml rhBMP2. The mixture was prepared in a dental glass dappen dish for procedure implantation.

2.4. Animal Experiment

20 adult male Wistar rats (average weight 200-250 g), which were classified into four groups (n=5), underwent surgery to perform subperiosteal cranial implantation. The animals were anesthetized by intraperitoneal administration of a combination of ketamine (25 mg/g) and xylazine (2.5 mg/g). Then, the skin and underlying tissues of the calvaria were raised to expose the calvarial bone. By using a periosteal elevator tool the periosteum of the frontal bone is raised and makes a little crush on the bone and putty shape scaffold is implanted on the calvarial bone. Thereafter, the skin incision was closed with silk sutures. The rats were permitted usual cage activity without immobilization until sacrifice at 4 weeks after surgery.

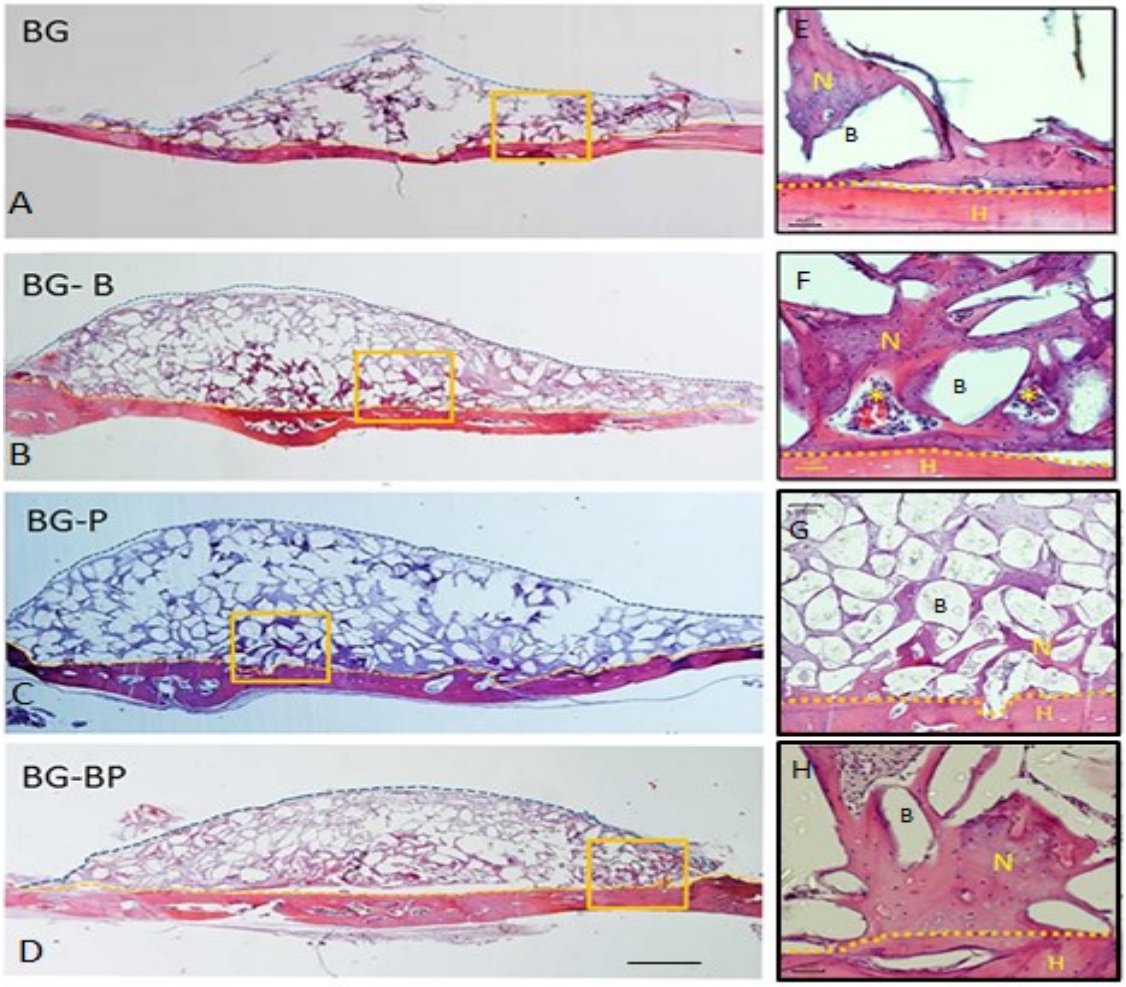

Figure1. Micrograph of histological BGs scaffold at 4 weeks after augmentation on Calvaria. (A–D). Substantial new bone formation was recognized in the vicinity of the host bone in all groups. Newly formed bone in all groups surrounded BG granules. The yellow rectangles areas in (A–D) correspond to higher magnification on the right side (E-H). Yellow dotted line indicates the boundary of the host and newly formed bone; blue dotted reveal the area of augmented bone; asterisk, Blod vessels, and bone marrows; N= New bone formation; H= Host bone; and B= Bioglass particles. scale bar in low magnification is 100 μm and in high magnification is 20μm.

2.5. Histological analysis

At 4 weeks, immediately after sacrifice, the calvarial bone samples were harvested and fixed in 4% formaldehyde for 24 h and then decalcified by ethylenediaminetetraacetic acid (EDTA) disodium salt solution according to established protocols. The samples were sectioned into layers with a thickness of 5 μm using a Microtome (SM2500, Leica, Wetzlar, Germany). Sections were taken from the area of interest and stained with the hematoxylin & eosin (H&E) staining method, and light microscopy (Olympus Co., Osaka, Japan) analysis was performed. Digital images were stored and the volume of new bone formation was processed with Image J software (NIH). The percentage of surface area occupied by new bone formation tissues was evaluated by light microscopy under X40 magnification.

2.6. Statistical analysis

For comparing the newly formed bone between groups data were presented as means ± standard deviations (SD). The SPSS for Windows v. 13.0.0 statistical software (SPSS, Inc., Chicago, IL, USA) was taken to perform statistical analysis. Statistical significance was evaluated by Student's t-test. The value of ϱ less than 0.05 was consistent to be significant.

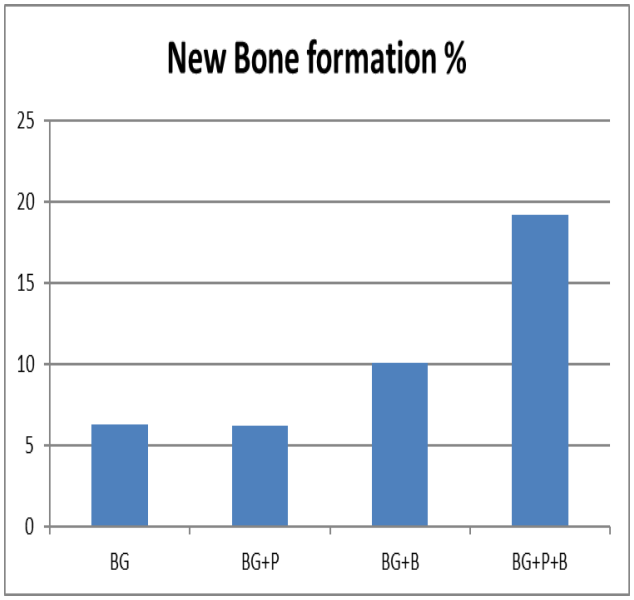

Figure. 2 Histomorphometric analysis revealed that the average percentage of new bone formation was 6.3% in the BG as the control group, 6.2% in the BG+PRP group, 10.1% in the BG+BMP2 group, and 19.2% in the BG+BMP2+PRP .

3. Result

3.1. Histological Analysis

Four weeks after implantation, a small amount of new bone was found in the vicinity of the host bone in the BG control group (Fig. 1A). However, the augmented area was mostly filled with BGs particles, which were embedded in loose connective tissue. The BG/PRP group also reveals a small amount of new bone close to the host bone (Fig. 1C). In the BG/BMP and BG/BMP/PRP groups, considerably more new bone was present in areas close to the host bone, and the new bone surrounded the b-TCP particles and connected with the host bone (Fig. 1B, D). Blood vessels could be observed throughout the specimens, showing that the blood supply was abundant (Fig 1F). Moreover, isolated new bone islands far from the host bone were observed in the sections of some samples transplanted with BG/BMP and BG/BMP/PRP in the new bone formation area (Fig. 1B, D).

3.2. Histomorphometric Analysis

Histomorphometric analysis revealed that the average percentage of new bone formation was 6.3% in the BG as the control group, 6.2% in the BG+PRP group, 10.1% in the BG+BMP2 group, and 19.2% in the BG+BMP2+PRP (Fig1). the majority of BGs were seen in many areas to be surrounded by newly formed bone. Bioglass/BMP/PRP construct considerably formed new bone compared to another group and these differences were statistically significant. However, the new bone volume produced in BG/BMP group was significantly greater than the BG control group. There was not a significant difference between BG/PRP and the control group.

4. Conclusion

The damage of bone tissue that can accompany trauma, injury, and disease can result in major socio-economic costs and highlight the requirement for novel, more reliable bone tissue regeneration strategies. Tissue engineering and regenerative medicine offer a promising new approach to de novo bone tissue formation. these approaches refer to the practice of combining innovative scaffolds, cells, and biologically active molecules that promise enhanced and more reliable bone formation strategies to improve the quality of life for many [17].

Tissue engineering scaffolds maintain the self-healing mechanism of the human body and promote the repair of injured different tissue. These implants can degrade after effective tissue regeneration reducing the immune response and the need for revision surgery.

In this study, bioactive glass was administrated as a basic component of an engineered bone substitute and control group as well. Bioactive glass is frequently used as a bone scaffold because of its special properties in bone tissue regeneration. Consequent studies have revealed that bioactive glasses and their ionic doped products promote osteogenesis in the presence of critical concentrations of Si and Ca ions by regulating osteoblast proliferation, differentiation, and gene expression [19, 20]. at the same time, osteoblasts that are not in the exact stage of the cell cycle and unable to progress toward differentiation are undergoing apoptosis by the ionic dissolution products[21].

Our data reveal that the PRP administration enhanced bone regeneration and is corresponded with a different research report that Platelet-rich plasma (PRP) is an autologous product that has an extremely concentrated number of platelets in a small volume of plasma. It has been speculated that released growth factors from platelets (insulin-like growth factor, transforming growth factor, platelet-derived growth factor …) would promote the healing of engineered tissue[22, 23].

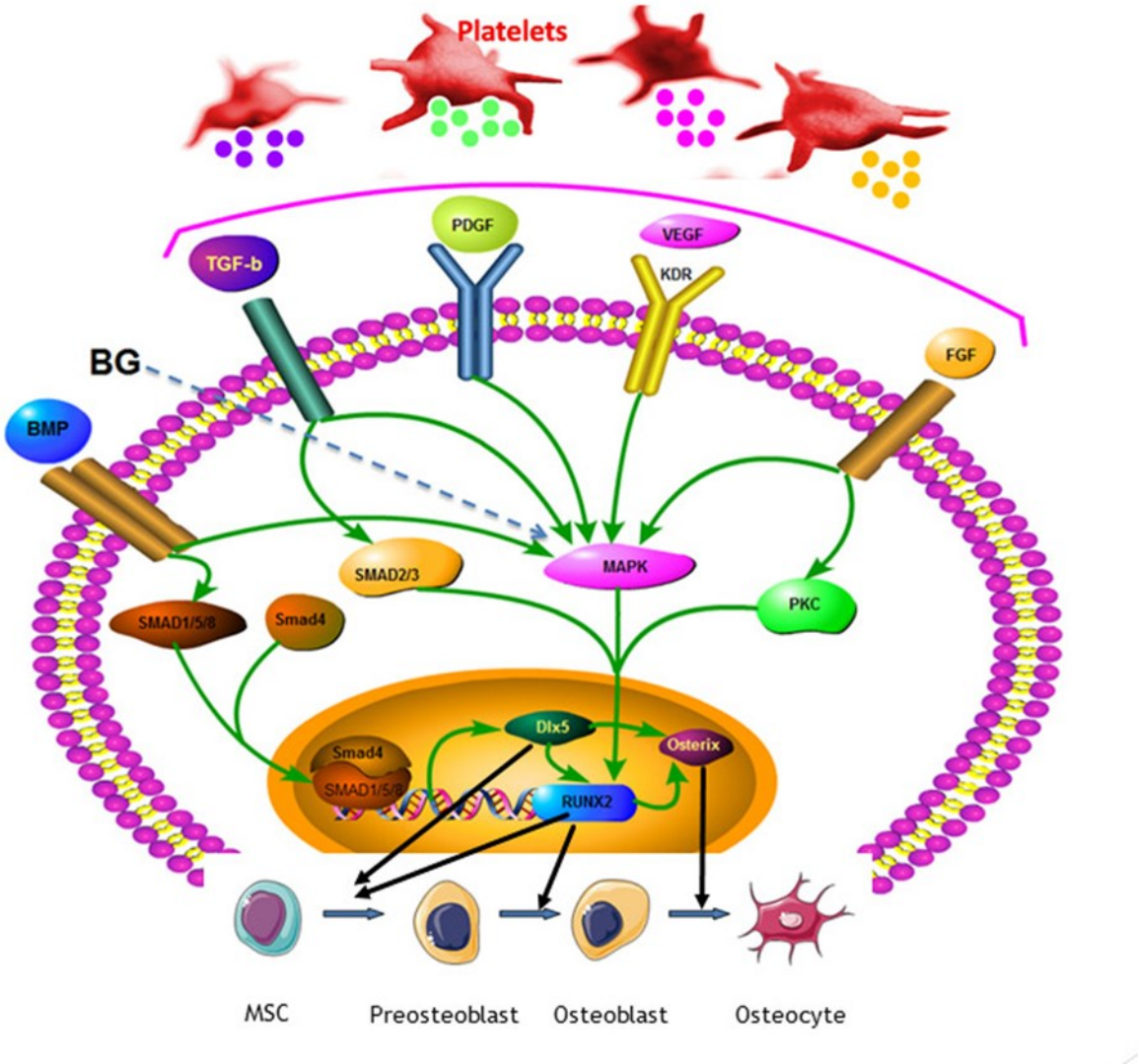

Figure 3. Basic schematic diagram showing the main stages of the mesenchymal stem cell differentiation to osteocyte induced by PRP, BMP, and bioactive glass. The main growth factors that release from platelets are TGF-b, FGF, PDGF, and VEGF. TGFβ regulates RUNX2 transcription via the Smads signaling pathway as well as by activating MAPK. Fibroblast growth factors (FGFs) signal over a group of high-affinity transmembrane receptors upregulate in RUNX2 transcription factor via the MAPK signaling pathway. PDGF receptors act on MAPK signaling pathways that can enhance osteogenesis and push MSCs to an osteoblast fate through the Runx2/ Osterix pathway. BMP signaling through the SmadS pathway binds to its receptors and then the signaling transduces to their Smads. Stimulated Smadss builds a complex with Smads4 and then translocates into the nucleus where it associates with other transcription factors to trigger target gene expression. Activated Smads regulate the expression of transcriptional factors and transcriptional coactivators important in osteoblasts (Dlx5, Runx2, and osterix). Non-Smads-dependent MAPK signaling pathway also regulates bone regeneration. BG as a scaffold involved in bone formation procedure via MAPK pathway. MSC differentiates to preosteoblast, osteoblast, and osteocyte performance under DLX5 and Runx2, Runx2, and osterix induction respectively.

This research finding reveals that the bioglass scaffold containing bone morphogenesis protein significantly enhanced bone formation in comparison with the bioglass scaffold, this data is strongly confirmed by numerous research works that BMPs regulate proliferation and/or differentiation of osteoblasts, which develop from a common population of undifferentiated mesenchymal stem cells that have pluripotency to differentiate into multiple types of cells such as adipocytes, tenocytes, and myocytes as well. Generally, osteogenic BMPs [24].

We have claimed that PRP and BMP2 associated with bioglass scaffold can strongly enhance bone formation. These findings can describe via molecular mechanisms of these elements. The main growth factors that release from platelets are TGF-b, FGF, PDGF, and VEGF [22].

TGFβ regulates RUNX2 transcription via the SMADS signaling pathway as well as by activating MAPK. Fibroblast growth factors (FGFs) signal over a group of high-affinity transmembrane receptors upregulate in RUNX2 transcription factor via the MAPK signaling pathway [25]. PDGF has been identified as an important protein for hard- and soft-tissue healing. PDGF is involved in the proliferation of stem cells at the site of a wound and angiogenesis by stimulating increased levels of VEGF[23]. PDGF receptors act on Mitogen-Activated Protein Kinase (MAPK) signaling pathways, which are all involved in cell migration and proliferation. While the effects of PDGF on bone regeneration are most likely through the mitogenic effects of these molecules, some studies have shown that PDGF-A can enhance osteogenesis. and push MSCs to an osteoblast fate through the Runx2/Osterix pathway [26].The osteoinductivity of bioglass was evaluated by microarray analysis in vitro on osteoblast. These research work data suggest that bioglass ionic dissolution products, Ca and Si, possibly mediate the bioactive effect through components of MAPK signaling pathways [26, 27](Fig 3).

4. Conclusion

Numerous studies reveal that the bioactive glass, PRP, and BMP are the major osteoinductive molecules that individually enhance bone regeneration. In this project, a bioactive glass/ BMP scaffold in associate with PRP showed dramatically increasing in bone regeneration. These parameters induce the expression of Runx2 via Smads and MAPK signaling pathways. Runx2 plays an important role in modulating the osteogenesis action of differentiation of mesenchymal stem cells toward pro -osteoblast under physiological or pathological conditions.

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