Comparative Evaluation of BOSS, A-OSS, and Osteon II Collagen in the Repair of Experimental Mandibular Bone Defects in Rabbits

Ikramov Sh.Sh.

Tashkent state medical university, Tashkent, Uzbekistan

Abstract. Background. Reconstruction of mandibular bone defects remains a major challenge in craniofacial surgery. Various bone graft materials are used to promote regeneration, but their biological effectiveness may differ significantly. This study aimed to compare the osteogenic, angiogenic, and anti-inflammatory properties of three bone graft materials - BOSS, A-OSS, and Osteon II Collagen -using an experimental model in rabbits. Methods. Forty-eight adult Chinchilla rabbits were randomly assigned to four groups (n=12): control (unfilled defect), BOSS, A-OSS, and Osteon II Collagen. A standardized 5×5 mm bone defect was created in the body of the mandible and filled according to group assignment. Histological evaluation was performed at 7, 14, 30, 60, and 90 days post-operation. Tissues were analyzed for graft resorption, osteogenesis, angiogenesis, inflammatory response, and defect closure using standardized scoring systems. Results. All tested materials enhanced bone regeneration compared to the control. BOSS showed the most pronounced osteogenic and angiogenic activity, along with minimal inflammation, leading to complete structural restoration by day 90. A-OSS demonstrated effective osteoconduction but slower bone remodeling. Osteon II Collagen exhibited good angiogenesis but lagged in bone tissue density and defect closure. Statistical analysis confirmed the superiority of BOSS across multiple regenerative parameters ($p<0.05$). Conclusion. BOSS exhibited the highest biological performance among the materials tested, making it a promising candidate for clinical applications involving large mandibular bone defects. Its rapid integration, biodegradability, and stimulation of mature bone formation position it as a material of choice in reconstructive maxillofacial surgery.

Keywords: Bone regeneration, Mandibular defect, Bone graft materials, Osteogenesis.

Introduction. The reconstruction of bone defects in the maxillofacial region remains one of the pressing challenges in modern reconstructive surgery. The causes of such defects vary and may include traumatic injuries, tumor resections, congenital anomalies, and inflammatory conditions. Despite the wide range of available bone grafting techniques, the search for biocompatible, osteointegrative, and predictably resorbable materials continues, as the ideal graft should simultaneously provide structural support, stimulate osteogenesis and angiogenesis, and minimize inflammatory response [1,2,6,9].

Among modern bone graft substitutes, particular attention is given to materials based on xenogenic, allogenic, and synthetic components. BOSS (Bio-Organic Structural Substitute), A-OSS (Anorganic Osteoconductive Xenograft), and Osteon II Collagen (a composite material based on β-tricalcium phosphate and collagen) represent different approaches to the development of biomaterials with osteoconductive and osteoinductive properties. However, direct comparative studies of their biological effects under a unified experimental protocol remain limited, particularly with regard to assessing their comprehensive regenerative potential in mandibular bone defects [3,4,5,7,8].

The objective of the study was to experimentally evaluate the osteogenic, angiogenic, and anti-inflammatory properties of various bone graft materials (BOSS, A-OSS, and Osteon II Collagen) in the repair of standardized mandibular bone defects in laboratory animals.

Materials and Methods. The experimental study was conducted at a certified animal facility in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123), and was approved by the local institutional ethics committee (protocol No. __, approval date to be specified). A total of 48 clinically healthy adult Chinchilla rabbits (average weight: 2.8 ± 0.3 kg; age: 5–6 months) were included and randomly allocated into four groups (n = 12 per group) using a block randomization method.

All animals underwent the creation of a standardized critical-size bone defect (5×5 mm in size and extending to the compact bone layer) on the body of the mandible, ensuring uniform regenerative conditions. The animals were divided as follows:

— Control group: the defect remained unfilled;

— BOSS group: the defect was filled with BOSS (a biomechanically stabilized bone allograft);

— A-OSS group: the defect was filled with A-OSS (an anorganic matrix of xenogenic origin);

— Osteon II Collagen group: the defect was filled with a composite material based on β-tricalcium phosphate and collagen.

Prior to surgery, all animals received general anesthesia consisting of xylazine (5 mg/kg, intramuscularly) and ketamine (35 mg/kg, intravenously), ensuring adequate analgesia and muscle relaxation. Once a sufficient depth of anesthesia was achieved, the surgical site was aseptically prepared with povidone-iodine solution. A 2 cm skin incision was made over the mandibular body, followed by a layered dissection to expose the bone. Using a dental surgical drill under continuous irrigation with isotonic saline, a bone defect of standardized size was created.

Depending on group allocation, the defect was filled with the respective bone graft material according to the manufacturer's instructions. The soft tissues were then closed in layers using absorbable sutures (Vicryl 4-0). Postoperative care included daily monitoring, analgesia with meloxicam (0.2 mg/kg, subcutaneously, for 3 days), antibiotic prophylaxis with enrofloxacin (5 mg/kg, subcutaneously, for 5 days), and maintenance of hygienic housing conditions.

Animals were euthanized at predetermined time points — on postoperative days 7, 14, 30, 60, and 90 (2–3 animals per group at each time point) — via barbiturate overdose. The mandibular segments containing the defect and surrounding soft tissues were harvested for histological examination. Samples were fixed in 10% neutral buffered formalin for 48 hours, processed using standard protocols, embedded in paraffin, and sectioned at a thickness of 5–7 μm.

For morphological evaluation, sections were stained with hematoxylin and eosin (H&E), as well as Van Gieson's picrofuchsin. The main histological parameters assessed included:

— Degree of graft resorption (scored 0–4);
— Osteogenic activity, including osteoid and mature bone formation (scored 0–4);
— Angiogenesis, based on vascular density and maturity (scored 0–4);
— Defect closure (scored 0–4);
— Inflammatory response, scored 0–4 according to inflammatory infiltration and signs of tissue destruction.

Histological evaluation was performed in a blinded manner by two independent pathologists using a light microscope (Olympus CX43, Japan) with digital image capture and subsequent morphometric analysis. In cases of score discrepancies ≥1 point, a consensus review was conducted.

Statistical analysis was performed using SPSS Statistics v.26.0 (IBM, USA) and Microsoft Excel 2019. All quantitative data were expressed as mean ± standard deviation (M ± SD). The normality of distribution was tested using the Shapiro–Wilk test. For intergroup comparisons, Student's *t*-test was used when normality was confirmed, and the Mann–Whitney U test was applied otherwise. Differences were considered statistically significant at $p<0.05$.

Results. The experimental phase of the study in laboratory animals involved a series of sequential surgical procedures. Initially, rabbits received intravenous general anesthesia, ensuring complete immobilization and absence of pain perception. Upon achieving an adequate anesthetic depth, the surgical field was aseptically prepared, followed by a skin incision in the mandibular region. After careful dissection of the soft tissues and exposure of the bone, the surgical site was delicately prepared under visual control. A standardized bone defect of predetermined size and shape was then created using a surgical drill. The resulting defect cavity was filled with the

corresponding bone graft material depending on the animal's group allocation. Following material placement, the soft tissues were sutured in layers to ensure airtight wound closure. The final stage included monitoring the surgical field and postoperative observation of the animal.

During the study, a comprehensive evaluation of reparative processes in the area of bone grafting was conducted in rabbits treated with three different materials: BOSS, A-OSS, and Osteon II Collagen (Tables 1–5). According to the data presented in Tables 1–5, each material demonstrated specific biological properties influencing the dynamics of osteogenesis, angiogenesis, inflammation, and graft biodegradation. As early as day 30, the BOSS group showed significantly greater signs of material resorption and replacement with newly formed bone, accompanied by intensive development of mature bone structure, a dense vascular network, and minimal inflammatory response. By day 90, the remodeling process was nearly complete in this group.

Table 1

Dynamics of graft biodegradation (resorption) in rabbits

Score	Description
0	Graft fully preserved; no signs of resorption
1	Surface changes; ≤25% resorption
2	Moderate resorption; 25–50% of material lost
3	Pronounced resorption; ≤25% of graft remains
4	Complete resorption; replaced by bone or connective tissue

Time point	BOSS	A-OSS	Osteon II	Control
15 days	1,0 ± 0,3*#	1,0 ± 0,4*#	1,0 ± 0,4*	0,2 ± 0,1
30 days	3,1 ± 0,4*#^	2,1 ± 0,3*#^	2,0 ± 0,4*^	1,0 ± 0,2
60 days	4,0 ± 0,0*#^	3,2 ± 0,3*#^	3,1 ± 0,2*^	2,1 ± 0,4
90 days	4,0 ± 0,0*^	4,0 ± 0,0*^	3,0 ± 0,3*	2,0 ± 0,3

* — statistically significant difference compared to control group; # — statistically significant difference compared to Osteon II group; ^ — statistically significant difference compared to previous time point (within-group dynamics);
No symbol — differences not statistically significant; Statistical analysis was performed using Student's t-test, significance level $p < 0.05$

Comparative analysis of osteogenic activity demonstrated that the BOSS material supported the fastest and most complete bone regeneration, reaching the maximum score of 4 by day 60, indicating the presence of mature lamellar bone. A-OSS also showed high effectiveness, though the regeneration process was slower, likely due to more stable preservation of the graft volume. Osteon II, despite exhibiting good angiogenic activity, lagged behind the other materials in terms of osteogenesis and bone tissue density. Angiogenesis indicators were highest in the BOSS and Osteon II groups, with a mature capillary network forming by days 60–90.

Table 2

Dynamics of Osteogenic Activity in Rabbits.

Score	Description
0	No new bone tissue or osteoid structures detected
1	Foci of osteoid formation observed at the defect margins
2	Formation of individual bone septa occupying ≤50% of the defect volume
3	Predominance of trabecular bone tissue; more than 50% of the defect filled
4	Mature lamellar bone formed; complete structural restoration achieved

Time point	BOSS	A-OSS	Osteon II	Control
15 days	1,1 ± 0,2*#	1,0 ± 0,3*#	1,0 ± 0,3*	0,1 ± 0,1
30 days	2,0 ± 0,4*#^	1,2 ± 0,3*#^	2,0 ± 0,3*^	0,3 ± 0,1
60 days	4,0 ± 0,0*#^	3,1 ± 0,3*^	3,0 ± 0,3*^	1,2 ± 0,3
90 days	4,0 ± 0,0*^	4,0 ± 0,0*^	3,0 ± 0,3*	1,0 ± 0,2

* — statistically significant difference compared to control group; # — statistically significant difference compared to Osteon II group; ^ — statistically significant difference compared to previous time point (within-group dynamics);
No symbol — differences not statistically significant; Statistical analysis was performed using Student's t-test, significance level $p < 0.05$

Table 3

Dynamics of Angiogenesis in Rabbits.

Score	Description
0	No blood vessels detected
1	Single capillaries observed at the periphery of the defect
2	Moderate vascularization; vessels penetrate the graft area
3	Well-developed capillary network throughout the defect
4	Dense, mature vascular network with pronounced vascularization

Time point	BOSS	A-OSS	Osteon II	Control
15 days	2,0 ± 0,3*^	1,0 ± 0,3*#	2,0 ± 0,2*^	1,0 ± 0,2
30 days	3,0 ± 0,3*#^	2,0 ± 0,3*^	3,0 ± 0,3*^	1,0 ± 0,3
60 days	4,0 ± 0,0*#^	3,1 ± 0,3*^	3,0 ± 0,2*^	2,0 ± 0,3
90 days	4,0 ± 0,0*^	4,0 ± 0,0*^	3,0 ± 0,2*	2,0 ± 0,3

* — statistically significant difference compared to control group; # — statistically significant difference compared to Osteon II group; ^ — statistically significant difference compared to previous time point (within-group dynamics);
No symbol — differences not statistically significant; Statistical analysis was performed using Student's t-test, significance level $p < 0.05$

Analysis of the inflammatory response revealed that all three materials contributed to a reduction in inflammation by day 90. The most favorable results were observed in the BOSS group, where no signs of inflammation were detected as early as day 60. This contrasted with the control group, which exhibited persistent moderate inflammatory infiltration and incomplete defect closure. In terms of defect closure dynamics, BOSS again demonstrated superior performance: by day 30, the geometry of the mandibular body was restored, and by days 60–90, full structural

regeneration was achieved. These findings support the superiority of BOSS across all morphological parameters studied and suggest that it may be considered the material of choice for reconstruction of large mandibular bone defects.

Table 4

Dynamics of Defect Closure in Rabbits.

Score	Description
0	Defect remains open; no filling observed
1	Filling ≤25%; defect contours are disrupted
2	Filling 25–50%; partial restoration of shape
3	Filling 50–75%; overall geometry restored
4	Complete defect closure; full structural restoration

Time point	BOSS	A-OSS	Osteon II	Control
15 days	3,0 ± 0,2*#	3,0 ± 0,2*#	2,0 ± 0,3*	1,0 ± 0,2
30 days	3,0 ± 0,2*^	3,0 ± 0,2*^	2,0 ± 0,3*^	1,0 ± 0,2
60 days	4,0 ± 0,0*#^	3,0 ± 0,3*^	3,0 ± 0,3*^	2,0 ± 0,3
90 days	4,0 ± 0,0*^	4,0 ± 0,0*^	3,0 ± 0,3*	2,0 ± 0,3

* — statistically significant difference compared to control group; # — statistically significant difference compared to Osteon II group; ^ — statistically significant difference compared to previous time point (within-group dynamics);
No symbol — differences not statistically significant; Statistical analysis was performed using Student's t-test, significance level $p < 0.05$

Table 5

Dynamics of Inflammatory Response in Rabbits.

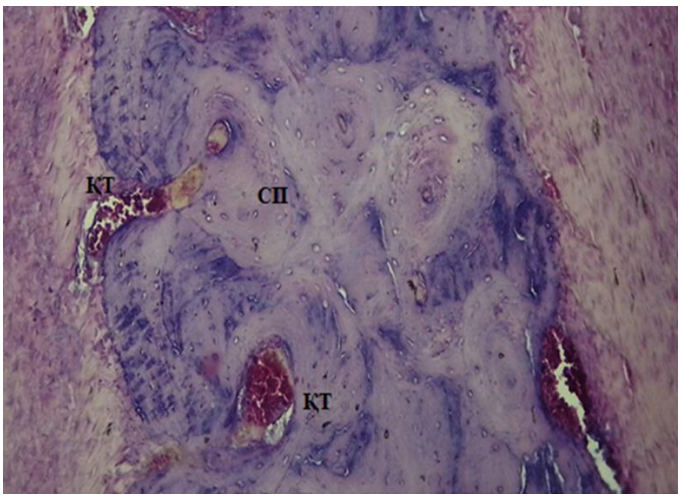
Score	Description
0	No signs of inflammation
1	Single lymphocytes and macrophages present
2	Moderate lymphohistiocytic infiltration
3	Pronounced inflammation with signs of microscopic necrosis
4	Purulent inflammation with abscess formation and cellular destruction

Time point	BOSS	A-OSS	Osteon II	Control
15 days	2,0 ± 0,2*#	2,0 ± 0,2*#	2,0 ± 0,3*	3,0 ± 0,3
30 days	1,0 ± 0,2*#^	1,0 ± 0,2*#^	2,0 ± 0,3*^	3,0 ± 0,2
60 days	1,0 ± 0,0*^	1,0 ± 0,0*^	1,0 ± 0,2*^	2,0 ± 0,3
90 days	0,0 ± 0,0*^	0,0 ± 0,0*^	1,0 ± 0,2*	2,0 ± 0,2

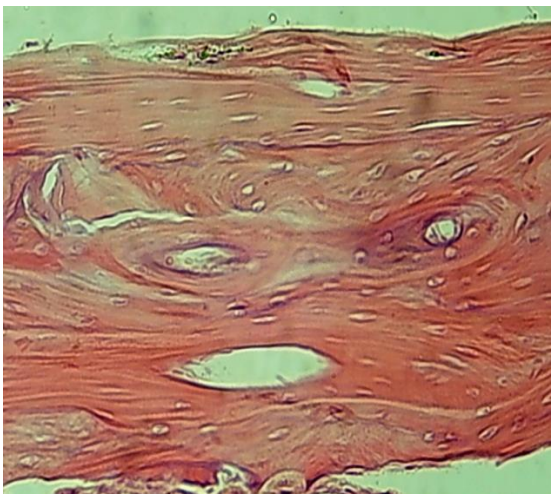
* — statistically significant difference compared to control group; # — statistically significant difference compared to Osteon II group; ^ — statistically significant difference compared to previous time point (within-group dynamics);
No symbol — differences not statistically significant; Statistical analysis was performed using Student's t-test, significance level $p < 0.05$

Histopathological examinations performed at various time points allowed us to trace the sequential dynamics of reparative processes in the bone defect area following the application of different bone graft materials. As early as day 7, in the

BOSS group, the formation of connective tissue was observed between the implanted material and the surrounding bone, indicating an early stage of graft integration. At the same time point, in the A-OSS group, the ingrowth of blood vessels into the graft structure was noted, which can be interpreted as the initial phase of angiogenesis, essential for the subsequent onset of osteogenesis (Fig. 2).



BOSS. On day 30 of the experiment, in the experimental group, the bone graft material exhibited the formation of concentrically arranged bone lamellae around blood vessels. A reduction in the volume of the graft material was observed, corresponding with an increase in vascularization and the intensity of osteogenesis. Ingrowth of blood vessels (BV) into the graft was accompanied by the formation of concentric bone lamellae (BL) surrounding the vessels. Staining: Hematoxylin and eosin; magnification ×100.



A-OSS. On day 30 after the start of the experiment, the formation of compact bone tissue in the form of thin bone trabeculae was observed. Staining: Hematoxylin and eosin; magnification ×400.

Figure 2. **Histopathological findings at various observation time points.**

By day 14 of the experiment, active ingrowth of blood vessels from the surrounding connective tissue into the bone graft material was observed in the BOSS group, while in the A-OSS group, the appearance of bone trabeculae between vessels was noted. By day 30, concentric bone lamellae were forming around the vessels in the BOSS material, indicating active osteon development and maturation of bone tissue. At this stage, a reduction in graft volume was evident, corresponding to its replacement by newly formed bone. In the A-OSS group at the same time point, compact bone tissue was forming in the shape of thin trabeculae, suggesting a continuing but slower process of osteogenesis.

By day 60, the BOSS group exhibited fully formed lamellar bone tissue, with a marked increase in the number of osteons and bone lamellae. In the A-OSS group, a

bone plate infiltrated by blood vessels and covered with loose connective tissue was observed, indicating completion of vascularization and stabilization of the regenerative process. At high magnification, the histological sections showed bone beams with multiple osteons, characterized by a mosaic-like structure and close contact with the bone graft material, confirming successful osteointegration. These morphological findings confirm the high osteogenic potential of BOSS and its ability to accelerate the formation of mature bone tissue.

Discussion. The results of the present study demonstrated that all three bone graft materials—BOSS, A-OSS, and Osteon II Collagen—promoted bone tissue regeneration in the treatment of standardized mandibular defects in rabbits. However, the most pronounced osteogenic and angiogenic activity, along with the lowest inflammatory response, was observed with the use of BOSS. By day 30, the formation of mature bone structures, a dense vascular network, and near-complete replacement of the graft with newly formed bone tissue were noted, leading to full defect restoration by days 60–90. These findings confirm the high osteointegration capacity of BOSS and its ability to accelerate reparative processes through effective biodegradation and stimulation of the cellular response.

Comparative analysis showed that A-OSS exhibited good osteoconductive properties, although the bone remodeling process was slower in this group. Osteon II Collagen demonstrated adequate angiogenic activity but was inferior to the other materials in terms of osteogenesis and extent of defect closure. These differences in biological performance highlight the need for an individualized approach to selecting bone graft materials based on the clinical context. The data obtained expand our understanding of the biological efficacy of different bone substitutes and may be useful in planning reconstructive interventions in the maxillofacial region.

Conclusion. Based on the obtained morphological and quantitative data, it can be concluded that all tested bone graft materials contributed to bone regeneration. However, the most pronounced osteogenic, angiogenic, and anti-inflammatory effects were observed with BOSS, as evidenced by more rapid formation of mature bone structures, development of a dense vascular network, minimal inflammation, and complete defect closure by days 60–90. While A-OSS and Osteon II Collagen also showed positive effects, their regenerative dynamics were less prominent. These findings suggest that BOSS may be considered the most effective graft material among those studied for reconstructing large mandibular bone defects in an experimental model.

REFERENCES

1. Titsinides S, Agrogiannis G, Karatzas T. Bone grafting materials in dentoalveolar reconstruction: A comprehensive review. Jpn Dent Sci Rev. 2019 Nov;55(1):26-32. doi: 10.1016/j.jdsr.2018.09.003.

2. Lee S, Jung J, Lee J, Ko YC, Lee D, Koo KT, Seol YJ, Lee YM. Bone regeneration and biosorption patterns of different bone substitutes: an in vivo study in rabbit skulls. J Periodontal Implant Sci. 2025 Jun;55(3):191-205. doi: 10.5051/jpis.2402840142.

3. Lim HC, Paeng KW, Jung UW, Benic GI. Effectiveness of xenogeneic and synthetic bone-block substitute materials with/without recombinant human bone morphogenetic protein-2: A preclinical study using a rabbit calvarium model. J Clin Periodontol. 2021 Aug;48(8):1126-1136. doi: 10.1111/jcpe.13480.

4. Kudoh K, Fukuda N, Akita K, Kudoh T, Takamaru N, Kurio N, Hayashi K, Ishikawa K, Miyamoto Y. Reconstruction of rabbit mandibular bone defects using carbonate apatite honeycomb blocks with an interconnected porous structure. J Mater Sci Mater Med. 2022 Dec 31;34(1):2. doi: 10.1007/s10856-022-06710-2.

5. Hendawy A, Atiba A, Abdo W, Osman A, Sadakah A, Abou ElReash A, Elsayad G, Xie X. The Effect of Mineralized Plasmatic Matrix and Chitosan on the Healing of Critical-Sized Mandibular Bone Defects in a Rabbit Model. Processes. 2022; 10(9):1890. doi: 10.3390/pr10091890.

6. Jain G, Blaauw D, Chang S. A Comparative Study of Two Bone Graft Substitutes—InterOss® Collagen and OCS-B Collagen®. Journal of Functional Biomaterials. 2022; 13(1):28. doi: 10.3390/jfb13010028

7. Kenkre JS, Bassett J. The bone remodelling cycle. Ann Clin Biochem. 2018;55:308–27. doi: 10.1177/0004563218759371.

8. Marechek A, AlShare A, Pack S, Demko C, Quereshy FA, Baur D. Nonvascularized bone grafts for reconstruction of segmental mandibular defects: is length of graft a factor of success. J Oral Maxillofac Surg. 2019;77:2557–66. doi: 10.1016/j.joms.2019.05.008.

9. Bobbert FSL, Zadpoor AA. Effects of bone substitute architecture and surface properties on cell response, angiogenesis, and structure of new bone. J Mater Chem B. 2017;5:6175–92. doi: 10.1039/c7tb00741h.