

Research Article

# Immunohistochemical evaluation for integrin binding sialoprotein on healing process of intrabony defect treated by bone sialoprotein

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**Abstract:** Background: Bone defect healing is a multidimensional procedure with an overlapping timeline that involves the regeneration of bone tissue. Due to bone's ability to regenerate, the vast majority of bone abnormalities can be restored intuitively under the right physiological conditions. The goal of this study is to examine the immunohistochemistry of bone sialoprotein in order to determine the effect of local application of bone sialoprotein on the healing of a rat tibia generated bone defect. Materials and Methods: In this experiment, 48 albino male rats weighing 300-400 grams and aged 6-8 months will be employed under controlled temperature, drinking, and food consumption settings. The animals will be subjected to a surgical procedure on the medial side of the tibiae bone, with the bone defect repaired with absorbable hemostatic material in the control group (12 rats). The experimental group (12 rats) will be treated with local administration of 30 µl bone sialoprotein fixed by absorbable hemostatic sponge. After surgery, the rats will be slaughtered at 7, 14, and 28 days (four rats for each period). Results: Immunohistochemical analysis of bone sialoprotein by stromal cells reveal a substantial difference between the bone sialoprotein group and the control group.

Conclusion: The study concludes that local application of bone sialoprotein could be a successful therapeutic treatment for bone injuries; these findings are encouraging for future clinical use.

**Keywords:** Bone defect, bone sialoprotein, bone.

## Introduction

The organic matrix of the bone, of which type-I collagen is the main component, which is largely responsible for bone repair. The involvement of non-collagenous proteins (NCPs) in bone deformation, on the other hand, is not well understood. The majority of NCPs are found in far lower concentrations than collagen and are known to influence bone mineralization, participate in cell signaling, and they have hormonal roles <sup>(1,2)</sup>. NCPs such as bone sialoprotein (BSP), osteopontin (OPN) and osteocalcin (OC) have recently been shown to improve bone fracture resistance. Diffuse damage arises in rat and human bone, allowing bone to disperse energy without producing overt fracture<sup>(3)</sup>.

Non-collagenous extracellular bone-matrix proteins like bone sialoprotein are synthesized and secreted by osteoblasts. These non-collagenous matrix proteins are recognized to play a key role in the mineralization of the bone <sup>(4,5)</sup>. These proteins found in the bone matrix have proven to be particularly valuable as osteogenic indicators<sup>(6,7)</sup>.

Bone sialoprotein is a non-collagenous extracellular matrix protein that is highly expressed by osteoclasts, osteoblasts and hypertrophic chondrocytes in bone, and is especially abundant in primary bone formation locations<sup>(8,9)</sup>.

Bone sialoprotein is a powerful mineralization nucleator as well as a matrix-associated signal that promotes osteoblast development and enhanced mineralized matrix synthesis<sup>(10,11)</sup>

It is abundantly expressed in bone by osteoblasts, osteocytes, osteoclasts, and chondrocytes<sup>(12,10)</sup>. BSP overexpression boosts osteoblast-related gene expression and accelerates mineralized nodule formation in culture, despite the fact that it has long been thought to be a hallmark of late osteoblastic differentiation. In contrast, inhibiting the expression of osteoblast markers by reducing BSP expression in osteoblasts with particular shRNA leads to a considerable reduction in matrix mineralization<sup>(10)</sup>.

### Materials and methods

All experimental procedures are carried out in conformity with the Baghdad College of Dentistry's ethical principles. In this investigation, 48 albino male rats weighing 300-400 grams and aged 6-8 months will be employed under strict temperature, drinking, and food consumption controls. The animals are given an intrabony defect in the medial side of the tibiae bone, which is treated with absorbable hemostatic sponge in the control group (12 rats) and local application of 30  $\mu$ l bone sialoprotein fixed by absorbable hemostatic sponge in the experimental group (12 rats). The rats were slaughtered seven, fourteen, and twenty-eight days following operation (four rats for each period in each group).

The following materials were used in this study:

- 1- Bone sialoprotein (Recombinant Mouse Bone Sialoprotein 2/IBSP Protein (HisTag) Elabscience company .
- 2-IBSP Polyclonal Antibody Elabscience company

### Surgical technique

A surgical procedure was performed on the animals. The procedure was done in a sterilized environment with a gentle technique. The dose of general anesthesia given to each animal was calculated based on its weight. Intramuscular injections of xylazine 2% (0.4 mg/kg B.W.) and ketamine HCL 50 mg (40 mg/kg B.W.) were used to induce general anesthesia, as well as an antibiotic treatment with oxytetracycline 20% (0.7ml/kg) intramuscular injection. Tibiae were shaved, and the skin was washed with a solution of ethanol and iodine, followed by an alcohol-soaked piece of cotton. An incision was created, and the skin was removed. The skin and fascia flap were mirrored after an incision was made. A hole of 1.8mm was drilled with a tiny spherical bur at a rotational speed of 1500 rpm using instrument drilling and continuous cooling with irrigated saline.

The operation site was cleaned with saline solution after the hole preparation to eliminate debris from the drilling site. After the operation, the area was air dried, and then 30  $\mu$ l bone sialoprotein was applied to the experimental group. The muscles were sutured using absorbable cat gut, then the skin was sutured. A local antibiotic was applied on the surgical site (tetracycline spray).

### Results

The presence of a brown cytoplasmic stain indicated a positive reading, whereas the absence of immunological reactions suggested a negative reading, depending on the positive and negative controls.

#### Analytical statistics

Mean, S.D., Min., Max., F-test, P-value were used to assess bone cell count as well as osteoblast, osteocyte, and stromal cells expressed by bone sialoprotein.

#### Results of immunohistochemistry

##### In the control group, bone sialoprotein expression was as follows:

At 7 days, immunohistochemical analysis of the control group revealed positive expression in osteoid tissue, osteoblast, and osteocyte. Basal bone expression is negative (Figure 1). Bone sialoprotein expression is positive in osteoblast, osteocyte, and bone marrow stromal cells after 14 days (Figure. 2). Positive expression was detected in osteoblasts, osteocytes, and osteoclasts after 28 days. The new bone has a negative BSP expression (Figure 3).

##### Bone sialoprotein expression in the bone sialoprotein-treated group:

Positive staining in the osteoid tissue is after 7 days. Basal bone expression is negative (Figure 4). A positive DAB stain for BSP was seen in stromal, osteoblast, and osteocyte cells after 14 days (Figure 5).

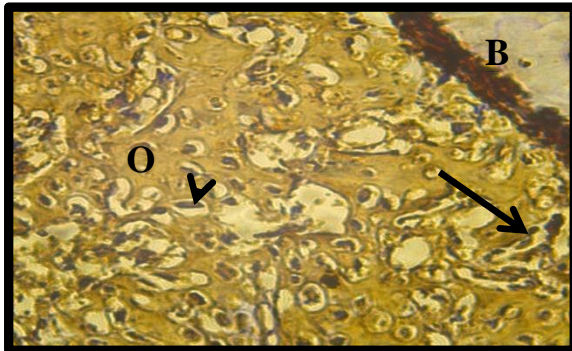
BSP immunohistochemistry expression in osteoblasts and osteocytes after 28 days. BSP expression was negative in mature bone, which contain several tiny Haversian canals (Figure. 6).

Statistical analysis revealed a high significant value for bone sialoprotein group in comparison to control and in periods (7, 14, and 28 days) as indicated in Table (1). for positive expression of bone sialoprotein by stromal cells in study groups.

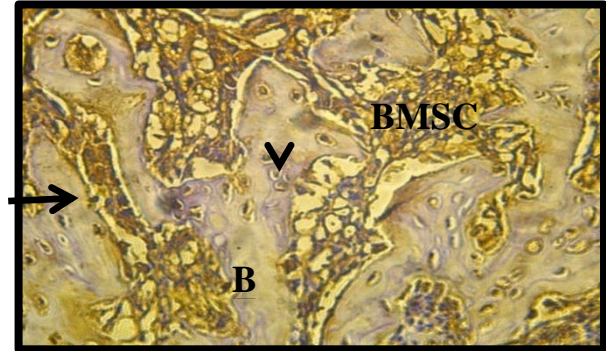
Positive expression of bone sialoprotein by osteoblasts, osteocytes, and osteoclasts, as determined by the following statistical analysis are as follow: (See Table (2).

- In 7 days, osteoblast and osteocyte levels were highly significant, while osteoclast levels were non-significant.

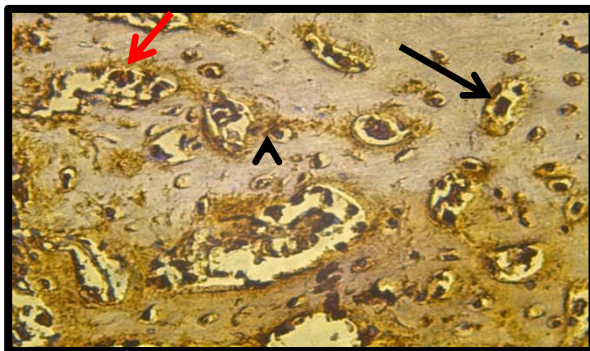
- In 14 and 28 days, osteoblast ,osteocyte and osteoclast were highly significant.



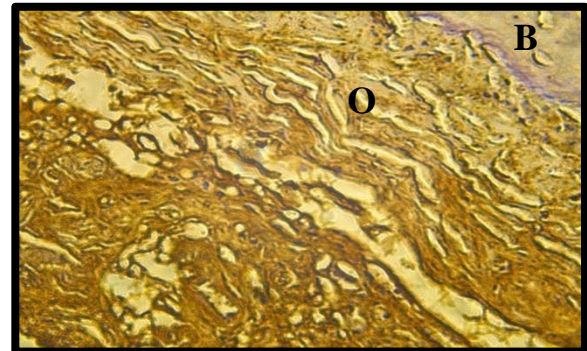
**Figure 1:** View of 7 days interval of control group shows positive expression of bone sialoprotein in osteoid tissue (OT), osteoblast ( arrow), osteocyte cells (arrow heads) and shows negative expression in basal bone (BB). DAB stain X40.



**Figure 2:** Osteocyte (arrow heads) and osteoblast (arrows), bone marrow stromal cells (BMSCs) show positive BSP expression in control group 14 days. Negative expression in trabecular bone (BT) DAB stainX40



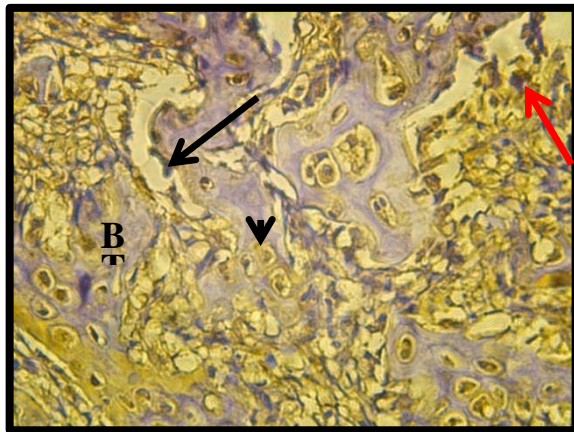
**Figure 3:** View of 4 weeks duration of control group showed mature bone contain positive expressed of osteocytes(arrow heads), osteoblast (arrow) and osteoclast ( red arrow).DAB stain with counter stain hematoxvlin x40



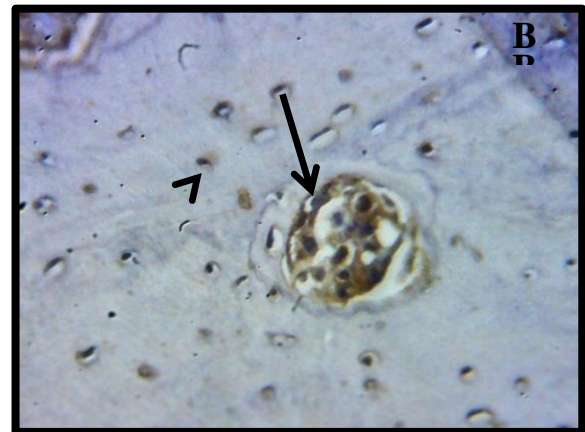
**Figure 4:** Immunohistochemical view shows positive BSP expression in osteoid tissue(OT) in bone sialoprotein group 7 days with negative expression in basal bone (BB) . DAB stainX40

**Table 1 :** Descriptive statistics of the positive stromal cells expressed by bone sialoprotein and groups' difference in each duration.

Duration	Groups	Descriptive statistics				Comparison	
		Mean	S.D.	Min.	Max.	F-test	p-value
7 days	Cont	17.915	1.799	15.33	19.5	36.963	0.000
	B	25.958	1.159	24.33	27		
14 days	Cont	8.500	1.443	7.25	9.75	84.990	0.000
	B	17.375	0.924	16	18		
28 days	Cont	4.625	0.750	4	5.5	14.678	0.000
	B	8.125	0.854	7	9		



**Figure 5:** Immunohistochemical view for bone sialoprotein group 14 days show positive expression to BSP in osteoblast (arrows), osteocyte (arrow head) and osteoclast (red arrow). Negative expression in bone trabeculae (BT). DAB stain X40



**Figure 6:** View after 28 days duration of bone sialoprotein with positive expression of osteocytes (arrow head), and osteoblast (arrows). DAB stain with counter stain hematoxylin x40.

**Table 2 :** Descriptive statistics of the positive bone cells expressed by bone sialoprotein and groups' difference in each duration.

Duration	Cells	Groups	Descriptive Statistics				Comparison	
			Mean	S.D.	Min.	Max.	F-test	p-value
7 days	OB	Cont	21.250	1.936	18.5	23	81.111	0.000
		B	31.395	0.966	30.5	32.75		
	OC	Cont	19.408	1.045	18.33	20.8	41.176	0.000
		B	24.458	1.030	23	25.33		
	OCL	Cont	0.173	0.022	0.15	0.2	0.358	0.785
		B	0.200	0.115	0.1	0.3		
14 days	OB	Cont	20.188	1.841	18.25	22	10.378	0.001
		B	15.208	1.567	13.33	16.5		
	OC	Cont	20.500	1.620	18.75	22.5	26.256	0.000
		B	12.625	1.109	11.5	14		
	OCL	Cont	1.148	0.108	1	1.25	5.188	0.016
		B	0.700	0.216	0.5	1		
28 days	OB	Cont	10.313	1.463	8.75	12	7.313	0.005
		B	6.500	1.080	5	7.5		
	OC	Cont	10.208	2.347	7.5	13	5.683	0.012
		B	6.500	1.225	5	8		
	OCL	Cont	0.450	0.091	0.35	0.55	5.419	0.014
		B	0.200	0.082	0.1	0.3		

**Discussion**

The goal of this study was to see how local application of bone sialoprotein affected experimentally produced bone defects in rats' tibiae. Over the last decade, the rat has been employed in a considerable fraction of animal fracture investigations in major orthopedics research<sup>(13)</sup>.

Proliferation, apoptosis, adhesion, migration, angiogenesis, and ECM remodeling could all be influenced by bone sialoprotein<sup>(14)</sup>. In a nude rat model, anti-BSP Ab was also found to prevent osteolysis while stimulating bone growth<sup>(15,16)</sup>.

Bone sialoprotein expression and localization were brownish in color in all groups, showing that there was expression of bone sialoprotein; positive cells were found at osteoblast, osteocyte, and osteoclast in all groups. The new bone matrix and the surrounding area of the resorptive lacunae showed high positive expression for bone sialoprotein. This was in line with the findings of<sup>(17,18)</sup>, and<sup>(19)</sup>, who claimed that strong BSP staining in newly produced bone in swine fetuses' mandibular alveolar bone. In this investigation, bone sialoprotein was expressed in a large number of osteoblasts, osteocytes, and a small number of osteoclasts in the space of a bone defect for 1 week in all groups. This conclusion matched with<sup>(20)</sup>, who showed significant staining of osteoblasts and osteocytes associated with newly produced bone during periodontal regeneration in dogs. While disagreeing with<sup>(21)</sup> who claimed that faint BSP staining in osteoblasts present in embryonic rats' mandibles.

In the experimental group, however, there was a decrease in bone sialoprotein expression over time. This finding is consistent with<sup>(22)</sup> who found that newly formed bone had more intense BSP staining at 1 week, whereas mature bone was weakly stained, and osteoblasts and osteocytes had higher BSP positivity at 1 week, which gradually decreased with time when staining was mild and limited to the lacunae. Also<sup>(23)</sup> Found that the bone sialoprotein mRNA signal was strongest in osteoblasts along the surface of woven bone trabeculae in actively growing bone tissue, and that it decreased with bone growth.

These findings suggest that while bone sialoprotein is necessary for de novo creation, it is less important for bone growth because growth is still occurring.

## Conclusion

The findings of this investigation reveal that bone sialoprotein has the potential to aid in the mending of bone abnormalities.

**Conflict of interest:** None.

## References

1. Proding P M, Bürklein D, Foehr P, Kreutzer K, Pilge H, Schmitt A, ... & Tischer T. Improving results in rat fracture models: enhancing the efficacy of biomechanical testing by a modification of the experimental setup. *Bmc musculoskeletal disorders*, 2018; 19(1): 243.
2. Oury F, Khrimian L, Denny C A, Gardin A, Chamouni A, Goeden N, ... & Suyama S. Maternal and offspring pools of osteocalcin influence brain development and functions. *Cell*, 2013; 155(1): 228-241.
3. Nikel O, Poundarik A A, Bailey S, & Vashishth D. Structural role of osteocalcin and osteopontin in energy dissipation in bone. *Journal of biomechanics*. 2018; 80: 45-52.
4. Aubin JE. Osteogenic cell differentiation. In: Davies JE. *Bone engineering*. 2000; 19-30.
5. Haley S, Gulliver K, Baldassarre R, Miller S, Lane R and Moyer-Mileur L. Tactile and Kinesthetic Stimulation (TKS) intervention improves outcomes in weanling rat bone in a neonatal stress model. *J Musculoskelet Neuronal Interact*. 2013; 13(2):157-165.
6. Sodek J and Mckee M. Molecular and cellular biology of alveolar bone. *Periodontol*. 2000; 24:99-126.
7. Jamil B. Role of biomaterial collagen coated titanium implant surface on expression of bone protein markers and osseointegration reaction, in comparison to titanium implant coated with zirconia. PhD thesis, collage of Dentistry, University of Baghdad. 2011.
8. Gorski J P, Wang A, Lovitch D, Law D, Powell K & Midura R J J J O B C. Extracellular bone acidic glycoprotein-75 defines condensed mesenchyme regions to be mineralized and localizes with bone sialoprotein during intramembranous bone formation. 2004; 279: 25455-25463.

9. Bouet G, Boulefour W, Juignet L, Linossier M T, Thomas M, Vanden-Bossche A, Aubin J E, Vico L, Marchat D & Malaval L J P O. The impairment of osteogenesis in bone sialoprotein (BSP) knockout calvaria cell cultures is cell density dependent. 2015; 10: e0117402.
10. Gordon J A, Ttys C E, Ssampaio A V, Underhill T M, Hunter G K & Goldberg H A J B. Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in vitro. 2007; 41: 462-473.
11. Baht G S, Hunter G K & Goldberg H A J M B. Bone sialoprotein–collagen interaction promotes hydroxyapatite nucleation. 2008; 27: 600-608.
12. Fisher L W & Fedarko N S J C T R. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. 2003; 44: 33-40.
13. O'loughlin P F, Morr S, Bogunovic L, Kim A D, Park B & Lane J M. Selection and development of preclinical models in fracture-healing research. 2008; JBJS, 90(Supplement\_1): 79-84.
14. Zhu B, Guo Z, Lin L & Liu Q J E R M P S. Serum BSP, PSADT, and Spondin-2 levels in prostate cancer and the diagnostic significance of their ROC curves in bone metastasis. 2017; 21: 61-67.
15. Bäuerle T, Peterschmitt J, Hilbig H, Kiessling F, Armbruster F P & Berger M R J I J O O. Treatment of bone metastasis induced by MDA-MB-231 breast cancer cells with an antibody against bone sialoprotein. 2006; 28: 573-583.
16. Zepp M, Kovacheva M, Altankhuyag M, Westphal G, Berger I, Gather K S, Hilbig H, Neuhaus J, Hänsch G M & Armbruster F P J T J O P C R. IDK1 is a rat monoclonal antibody against hypoglycosylated bone sialoprotein with application as biomarker and therapeutic agent in breast cancer skeletal metastasis. 2018; 4: 55-68.
17. Chen J, McCulloch C A. & Sodek J J A O O B. Bone sialoprotein in developing porcine dental tissues: cellular expression and comparison of tissue localization with osteopontin and osteonectin. 1993; 38: 241-249.
18. Pinero G J, Farach-carson M C, Devoll R E, Aubin J E, Brunn J C & Butler W T J A O O B. Bone matrix proteins in osteogenesis and remodelling in the neonatal rat mandible as studied by immunolocalization of osteopontin, bone sialoprotein,  $\alpha$ 2HS-glycoprotein and alkaline phosphatase. 1995; 40: 145-155.
19. Arambawatta A K S, Yamamoto T & Wakita M J A O A A. Immunohistochemical characterization of noncollagenous matrix molecules on the alveolar bone surface at the initial principal fiber attachment in rat molars. 2005; 187: 77-87.
20. Ivanovski S, Li H, Daley T & Bartold P J J O P R. An immunohistochemical study of matrix molecules associated with barrier membrane-mediated periodontal wound healing. 2000; 35: 115-126
21. Ishigaki R, Takagi M, Igarashi M & Ito K J T H J. Gene expression and immunohistochemical localization of osteonectin in association with early bone formation in the developing mandible. 2002; 34: 57-66.
22. Tera T D M, Nascimento R D, Prado R F D, Santamaria M P & Jardini M A N J J O A O S. Immunolocalization of markers for bone formation during guided bone regeneration in osteopenic rats. 2014; 22: 541-553.
23. Chen J, Shapiro H S, Sodek J J J O B & research M. 1992. Developmental expression of bone sialoprotein mRNA in rat mineralized connective tissues. 1992; 7: 987-997.

**العنوان:** دراسة كيميائية مناعية نسيجية لبون سيالوبروتين المرتبط بانتجين لتقييم التطبيق الموضعي لبون سيالوبروتين في تعزيز شفاء العظام على الجردان  
**الباحثون:** ميادة كامل جعفر ١ , ايناس فاضل كاظم ٢

#### المستخلص:

الخلفية: شفاء عيب العظام هو إجراء متعدد الأبعاد مع جدول زمني متداخل يتضمن تجديد أنسجة العظام. نظرًا لقدرة العظام على التجدد ، يمكن استعادة الغالبية العظمى من تشوهات العظام بشكل حدسي في ظل الظروف الفسيولوجية الصحيحة. كان الهدف من هذه الدراسة هو فحص التقييم النسيجي المناعي الكيميائي لبون سيالوبروتين من أجل تحديد تأثير التطبيق الموضعي لبون سيالوبروتين على التئام العظام في فجوة عظمية طبقت على الجردان.  
 المواد والطرق: في هذه التجربة ، سيتم استخدام ٤٨ من ذكور الجردان البيضاء التي تزن ٣٠٠-٤٠٠ جرام وتتراوح أعمارهم بين ٦-٨ أشهر خضعت لظروف مماثله من حيث درجة الحرارة واستهلاك الطعام والشراب. الحيوانات خضعت لعمليات جراحية في الجانب الإنسي من عظم القصبة ، الحفرة العظمية عولجت بإسفنجة جراحية قابلة للامتصاص في مجموعة السيطرة (١٢ جردًا). المجموعة التجريبية (١٢ جرد) الحفرة العظمية عولجت ب ٣٠ ميكرو لتر من بون سيالوبروتين مثبت بإسفنجة جراحية قابلة للامتصاص. بعد الجراحة تم ذبح الفئران في ٧ و ١٤ و ٢٨ يومًا (أربعة فئران لكل فترة).

النتائج: أظهر التقييم النسيجي المناعي الكيميائي لبون سيالوبروتين في خلايا انسجة نخاع العظم وجود اختلاف جوهري بين مجموعة البون سيالوبروتين ومجموعة السيطرة. الاستنتاج: خلصت الدراسة إلى أن التطبيق الموضعي لبون سيالوبروتين يمكن أن يكون علاجًا ناجحًا لإصابات العظام. هذه النتائج مشجعة للاستخدام السريري في المستقبل.

الكلمات المفتاحية: عيوب العظام ، بون سيالوبروتين .