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Histological Study Of The Influence Of Bioactive Glass On Bone Healing (An Experimental Study On Rat Femur)

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Abstract:

The objective is to study the histological influence of the bioactive glass on bone healing in surgically created holes in rat's femur.

Methods: The two wall holes were made in the right femurs by 2-mm drill, filled with bioactive glass in the examined rats. Another two wall holes were made similarly in the right femurs of other group of rats without filling with bioactive glass and used as the control group. Animals were regularly examined over a period of five weeks for bone healing.

Result: Histological examination of both control and treated sites showed newly formed bone. The newly formed ostoid tissue was significantly increased in the treated holes in the form of foci of newly formed bone around and within the glass particles. In the control group, the junctional epithelium migrated up to the base of the hole.

Conclusion: The treated animals with bioactive glass had better healing than control. The bioactive glass particles have osteo-conductive property as well as osteo-stimulatory capacity. The graft material showed a promising inhibition of greater cementum deposition in the bone holes. We can use it in Osteosurgery as it can promote bone healing.

Key words: Bioactive glass, bone hole, bone healing, histological changes.

Introduction

Bone is the most common organ showing replacement in the body. Currently, both biological and synthetic grafts have been used for bone repair. The autogenous material is still the best choice for reconstruction of bone holes. Several materials have been introduced for bone grafts, i.e., autografts, allografts, xenografts and alloplastic grafts, but the currently available materials have not shown the predictable bony regenerative effect (de Macedo *et al*., 2004). Ideally, a bone graft should be biocompatible, able to support abundant bone formation (osteoconduc-tive), able to induce bone formation (osteoinductive), able to form a continuous interface with surrounding bone tissue (osteointegrative), able to support angiogenesis, and able to be structurally and mechanically compatible with bone tissue (Lu *et al*., 2003).

The interest in the development and use of a synthetic material has led to the development of several studies evaluating the results of the utilization of hydroxyapatite (HA) as a bone tissue substitute. 45S5 bioactive glass is the most bone bioactive material known to date. In addition to being osteointegrative, the biocompatibility, osteoconductive, and osteoinductive nature of 45S5 bioactive glass has been well documented (de Macedo *et al.*, 2004). Several *in vitro* studies have shown the non-toxicity of bioactive glass, its positive influence on osteoblast culture, inhibitory capacity on fibroblast proliferation and ability to form calcified foci in periodontal ligament fibroblasts. The first studies on bioactive glass and the possibility of its application as a bone filling material were published early in the 1970's and 1980's. This material has a granular shape and composed of 45% SiO2, 24.5% CaO, 24.5% Na2O and 6% P2O5 (Hench, 1991).

Biomaterials, such as bioactive glass, glass-ceramics and calcium phosphates (Ca-Ps) have been widely studied for orthopedic and dental applications. It has been relies on their ability to induce

hydroxyapatite (HA) in the physiological environment (Drury and Wallington, 1980). The implantation of bioactive glass *in vivo* forms a quick bond with bone through the hydroxyapatite layer that formed on the material surface (Hattara *et al*, 2005). Briefly these involve cation release from the glass with consequential increase of the matrix PH, formation of silica – rich layer and then precipitation of a Ca-P rich layer that further crystallizes as HCA (Hydroxy carbonate apatite).

Histological studies, in experimental animals, showed that bioactive glass implanted in non-periodontal sites is biocompatible and incorporates into the bone tissue thus producing an alkaline media at the implantation site (Villaca *et al*, 2005). However, despite its osteoconductive potential and superior ability to bind to bone, the direct application of bioactive glass in load-bearing situations has been limited. Although existing bioactive materials possess high compressive strength, they are unfortunately very brittle and have inherently poor tensile and torsional properties (Yaszemski *et al*., 1996).

The appropriate selection of the biomaterial component of the tissue-engineered scaffold is a critical step in determining the ultimate success of the engineered graft. Scaffold surface chemistry and physical properties will direct biological response such as cell adhesion and differentiation. Material selection is especially important in bone tissue engineering because a supporting substrate is critical for maintaining mechanical strength, structural support, and providing the optimal culturing environment for bone formation during the early stages of the regenerative process. Because no single existing material possesses all the necessary properties required in an ideal bone graft, there is a growing interest in composite materials. Composites are formed to improve the properties of existing materials, resulting in a superior material for the intended application (Lu *et al*., 2003).

Material and Method

1- Animals: In the present study, 20 male albino rats weighing 200-250g were used, which were fed a solid diet before and during the experimental period and received ordinary tap water. The entire experimental study was carried out in the anatomy and histology departments, Al-Azhar University, Cairo, Egypt. Then animals were divided into two groups, ten animals each; the control group (group 1), and test group (group 2).

2- Material preparations: Bioactive glass 45**S**5 silica- based glass was prepared by mixing 45% SiO, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5 (weight percentages). This mixed glass was melted in platinum crucible at 1400 ^oC for one hour. The melting glass was poured into ice water at 0 ^OC to quenches as glass frit, dried and ground according to Hench formula (1980). The bioactive glass was cleaned in an aceton- filled ultarasonic cleaner for about 20 minutes and then sterilized conventionally with autoclave for 1 hour.

3- Surgical procedures: Animals were anesthetized with sodium pento-barbital injection in a dose of 40mg/kg of body weight. After shaving, disinfection and sterile draping of the operation site, the femoral shaft was exposed by means of medical longitudinal incision. Initially, a friction bone hole was created by a 2-mm drill. The drill holes were carefully rinsed with Ringer's solution and cleaned out, so that any abraded particles formed during drilling were removed.

The holes in group 1 were then left and covered with the periostium, but in group 2 the holes were completely filled with small fragments of bioactive glass after saline irrigation of cavities. The wound was sutured in two layers with 3.0 nylon and 6.0 chromic catgut brow.

4- Histological preparation: When the bioactive glass was retrieved after 5 weeks, animals were sacrificed; femurs were excised, and then fixed in 10% formol/saline. Decalcification was carried out in 5% EDTA for 10 days. Specimens were then embedded in paraffin; sections were cut out at 8 micrometers thickness and stained with H&E, Masson Trichrome and PAS.

Results were interpreted on the basis of quantitative analysis using OPTIMAS 6.5 software for image analysis and laboratory automation.

Results

Femur bone samples in all operated animals with implanted bioactive glass material showed normal bone surface and fortunately, remnants of the bioactive glass material was present at the periosteum of healed bone holes demarcating out the site of operation.

Histological study of the implanted site showed gradual transformation of the fibrous callus, which occupied the bone hole, into ostoid tissue that gradually changed into mature cortical bone. Early, the bone hole was filled with large heterogeneous pleomorphic cells scattered randomly and new blood capillaries started to appear (fig. 6). Two weeks later, the collagen bundles oriented in the field (Fig. 3, 4), where the central part of the gap was occupied with cellular infiltration, while the healing area looked relatively avascular (Fig.7). After that, proliferated elongated cells arranged at the peripheries of the callus and started to deposit ostoid matrix (fig. 10). By the fourth week, the histological sections showed increased amount of bone lamellae that are oriented the normal direction of bone. The central part of the gap remained the same (Fig.9). By the end of 5th week, the formed bone lamellae started to be arranged concentrically around Harversian canals to form osteons (Fig.11).

In control group (group 2) healing with the developed bone callus was noticed with similar stages of differentiation, but with slower rate, than that occurred within the treated group. Table 2 and chart 2 showing that the mean areas of bone formation were higher in the bioactive glass implanted samples (group 1) than in the non implanted group (group 2) along the time of healing.

The mean optical density value of PAS positive materials in the callus of bioactive glass implanted samples (group 1) was higher in the bioactive glass implanted samples than in the non implanted group (group 2) along the time of healing. (Table 1); (Fig.8)

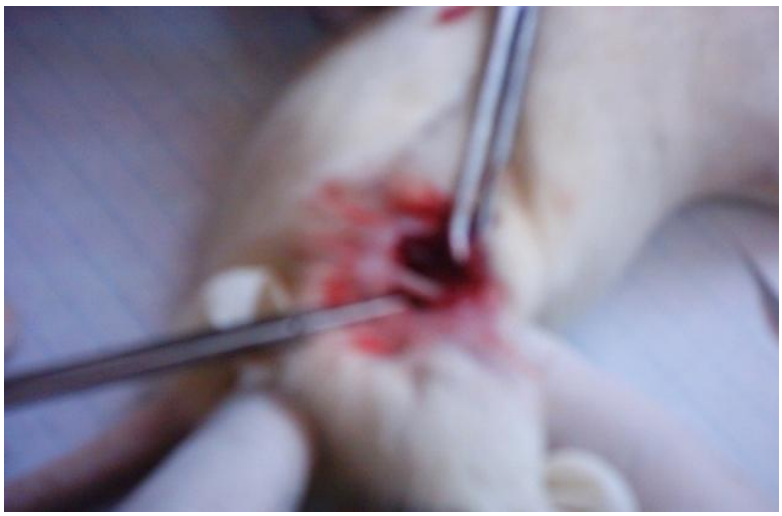

Fig. (1): Femur bone exposure and formation of a hole to implant bioactive glass samples.

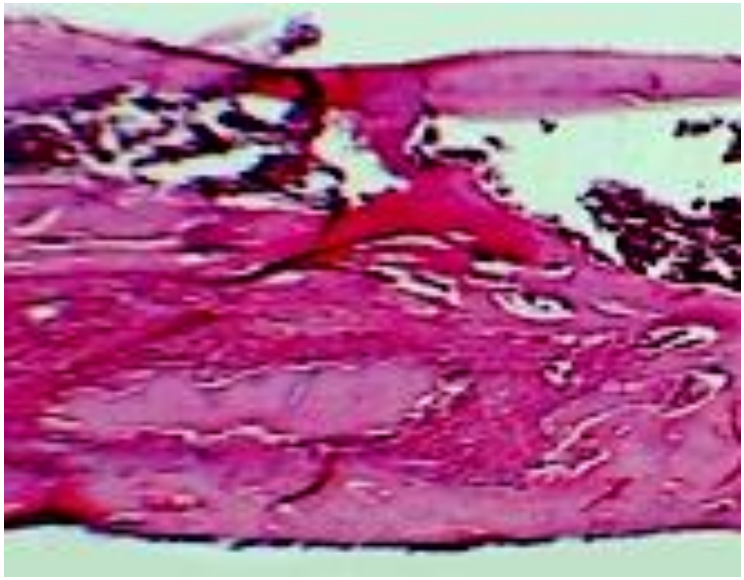

Fig. (2): Longitudinal section in the femur bone of control rat (one week after the operation) showing the small callus at the site of healing with minimal cellular proliferation. Hx & E x 100

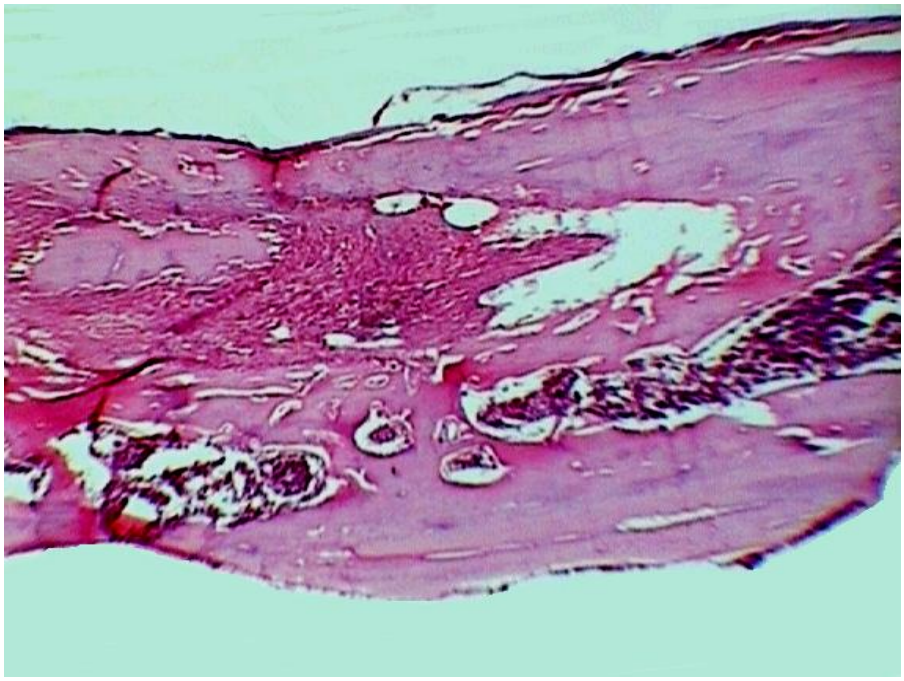

Fig. (3): Longitudinal section in a rat femur bone with bioactive glass implants at the site of healing (one week after the operation) showing the activated large sized callus. Hx & E x 100

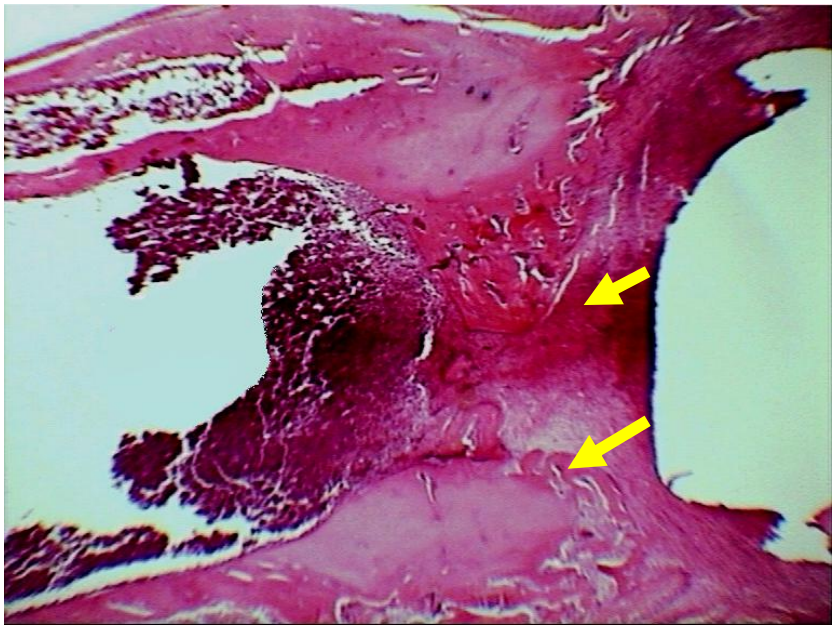

Fig. (4): Longitudinal section in the femur bone of control rat at the site of healing (two weeks after the operation) showing approximation of newly formed bone (arrows) within the callus at the site of healing. Hx & E x 200

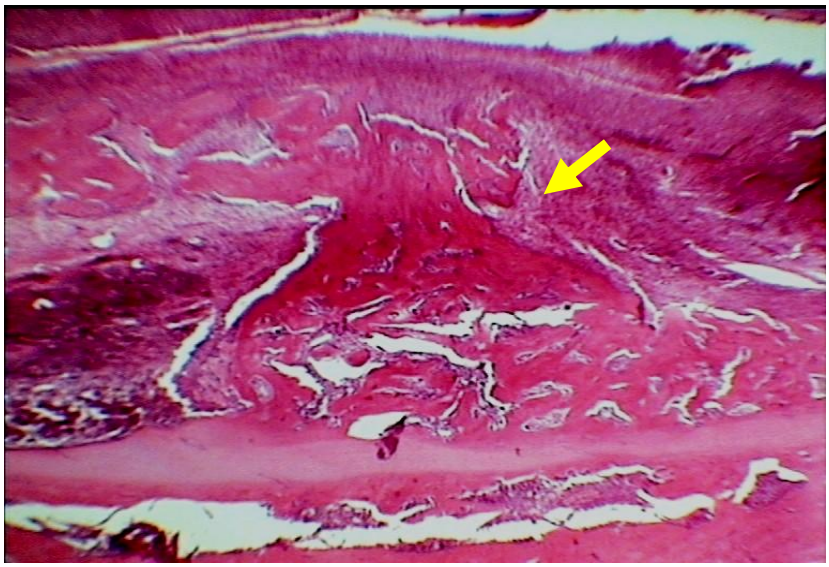

Fig. (5): Longitudinal section in a rat femur bone with bioactive glasses implanted at the site of healing (two weeks after the operation) showing the activated large sized callus surrounded with cellular proliferation. Hx & E x 200

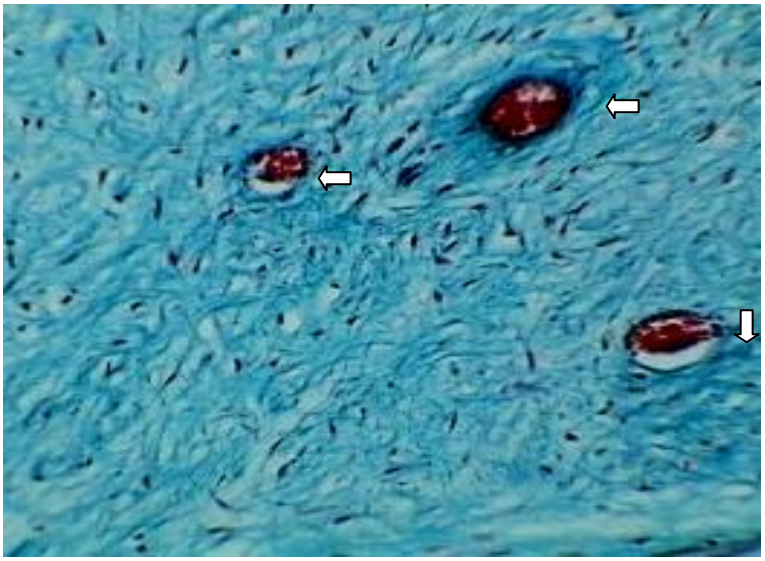

Fig. (6): Histological section in a rat femur bone with bioactive glass implants at the site of healing (one week after the operation) showing cellular proliferation, with fibrous tissue deposition invaded by blood vessels (arrows).

Masson trichrom x 400

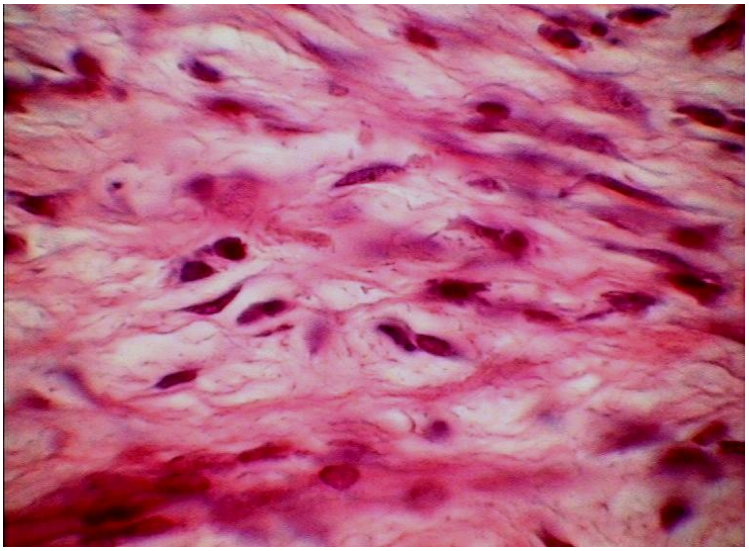

Fig. (7): Histological section in a rat femur bone with bioactive glass implants at the site of healing (two weeks after the operation) showing cellular proliferation with fibrous deposition in between forming the healing callus.

Hx & E x 1000

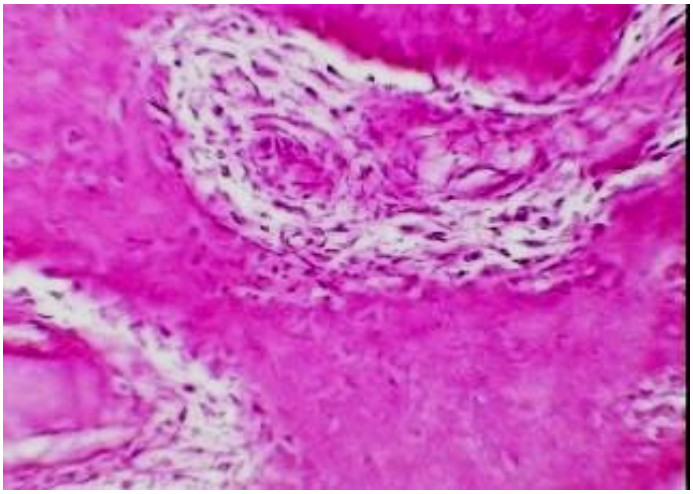

Fig. (8): Histological section in a rat femur bone with bioactive glass implants at the site of healing (four weeks after the operation) showing PAS positive material in the healing callus. PAS x 400

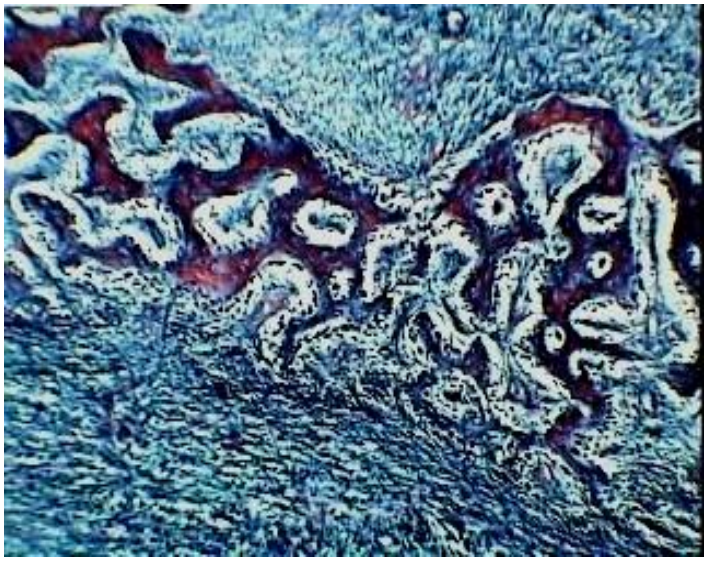

Fig. (9): Histological section in a rat femur bone with bioactive glass implants at the site of healing (three weeks after the operation). Masson trichrom x 250

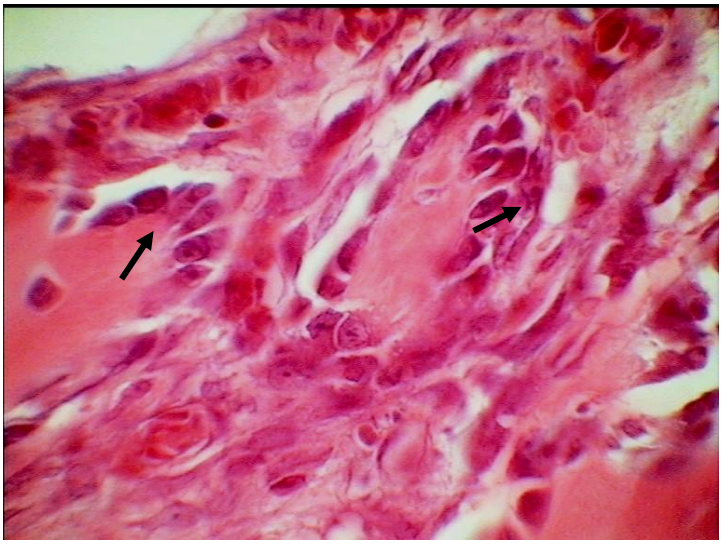

Fig. (10): Histological section in a rat femur bone with bioactive glass implants (three weeks after the operation) showing proliferated osteoblasts at the periphery of developing bone. Hx & E x 1000

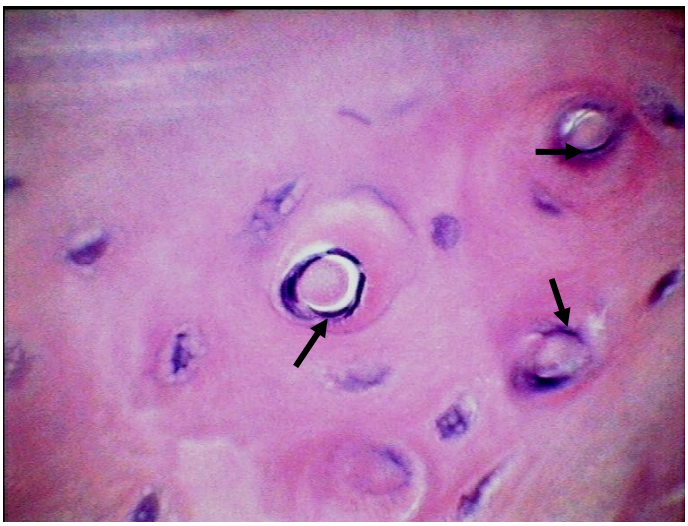

Fig. (11): Histological section in a rat femur bone with bioactive glass implants at the site of healing (at the end of the 5th week) showing the formed bone lamellae arranged concentrically around Harversian canals (arrows) to form the characteristic osteons. Hx & E x 1000

Table (1) showing the mean values of the optical density of PAS positive material in the callus regions of the examined rats.

	1week	2weeks	3weeks	4weeks	5weeks
GROUP 1	**0.133163**	**0.146274**	**0.15647**	**0.186881**	**0.21471**
GROUP 2	**0.118203**	**0.126677**	**0.133366**	**0.176191**	**0.197365**

GROUP 1: The mean values of PAS positive material in bioactive glass implanted group.
GROUP 2: The mean values of PAS positive material in control group.

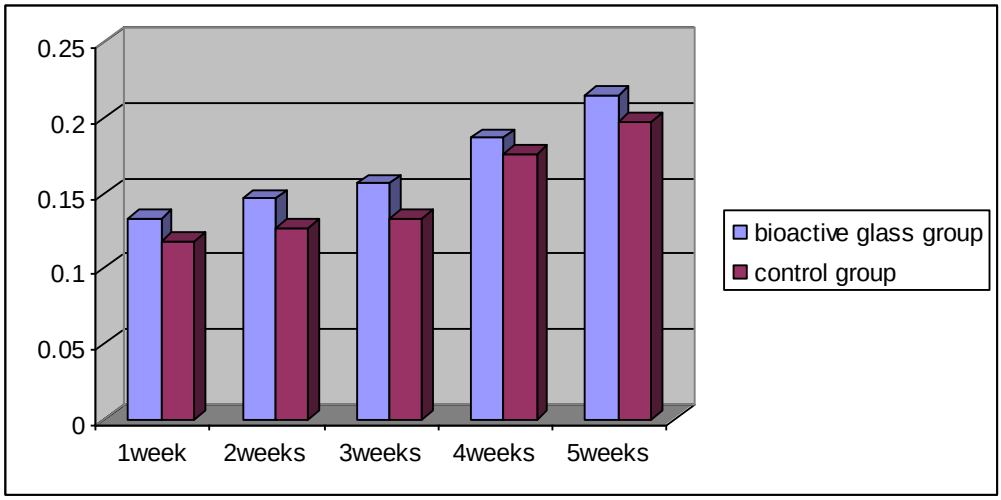

Chart (1): Histogram showing a comparison between the mean optical density values between bioactive glasses implanted group and control groups.

Table (2) showing the mean areas occupied by ossified tissue in both bioactive glass implanted and control groups, within the same period.

	Week 1	Week 2	Week 3	Week 4	Week 5
GROUP 1	**6411±0.2**	**100803±0.2**	**113771±0.2**	**185219±0.2**	**236929±0.2**
GROUP 2	**5501±0.2**	**236506±0.2**	**392895±0.2**	**425656±0.2**	**496647±0.2**

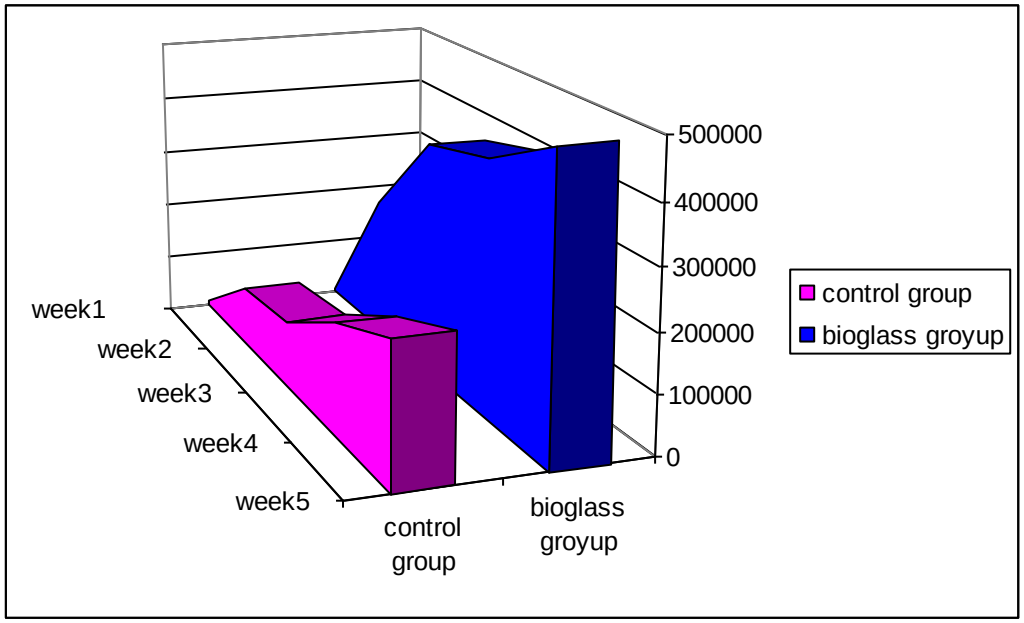

Chart (2): Histogram showing the mean areas occupied by ossified tissue in both bioactive glasses implanted and control groups, within the same period.

Discussion

Bone is the most commonly replaced organ of the body. The main goal of treating bone defect is regeneration of the bone tissue destroyed by diseases, traumas, etc; where, biological and synthetic grafts used to enhance bone regeneration. Frequently, guided bone regeneration techniques have been used as well as autogenous, xenogenic and allogenic bone grafts; and alloplastic materials with good osteoinductive potential, which causes minimal inflammatory reactions, rapid vascularization, affinity with host tissues, and easy accessibility (Schepers et al. 1991; Park *et al*. 2001). Several *in vitro* studies have shown the non-toxicity of bioactive glass, its positive influence on osteoblast culture, inhibitory capacity on fibroblast proliferation and ability to form calcification foci.

Bioactive glasses stimulate bone marrow stem cells to differentiate into osteoblast-like cells with a large amount of mineralized tissue formation, but inhibit the formation of osteoclast-like cells (Bosetti and Cannas 2005). According to Moore, *et al*. (2001) bioactive glass granules are quickly reabsorbed, thus allowing more precocious new bone formation within bone defect. Where, osteoblasts show several cytoplasmic processes, pseudopodia, compact appearance, and disorderly dorsal surface, which characterize a high cell membrane activity.

Histologically, our results confirmed the osteoconductive and osteointegrative properties of the bioactive glass particles, which are documented by the close contact between the glass material and the newly formed bone, as well as enhanced bone growth around them. The histological examination showed areas of osteoid tissue (bone tissue being formed), which no longer changed into mature bone and complete resorption of the glass material. Early; bone gap showed increased collagen deposition with elongated cell layer at the edges of bone cavity, with increased mean of the optical density of PAS reaction in the callus that suggest the cellular activity. This result was parallel with the findings conducted by Oonish *et al*. (1997). Additionally, the same researchers compared the effect of bioactive glass and synthetic hydroxyapatite on bone healing process. However, they had demonstrated

that synthetic hydroxyapatite presented bone formation with little density around granules, whereas greater bone formation was noticed by using bioactive glass granules (Oonish *et al*. 1997).

Turunen *et al* (1997) also demonstrated the effectiveness of bioactive glasses as they improve the osteointegration of coated implants, provide better bone support, and present increased stability when compared to machined implants. Glass particles, when come in contact with body fluids, trigger three reactions; diffusion, dissolution, and precipitation. Initially, an ionic exchange occurs between glass particles and the solution, where sodium separates from glass and then replaced by protons from the environment. This way, the pH of the wound rises to neutral, creating good conditions for repair. Together with the diffusion reaction, there is dissolution of the glass silica weave, making up silica gel (Shapoff *et al*, 1997). The silica gel will provide the main property of the material and bonding both to hard tissues and soft tissues. The bond occurs through bioactive fixation involving collagen fibers inside the polycrystalline matrix on the implanted material surface induced by the carbonated apatite layer. The quick surface reaction of the material, and the formation of a calcium phosphate hydrated layer biologically active on its surface, is responsible for the fast bone formation. The gel layer, rich in silica, has a wide surface area, negatively charged, which works as a nucleation site to the formation of a calcium phosphate layer. This stage is initially amorphous, but it soon crystallizes to form an apatite hydroxycarbonate organized structure. This compound attracts collagen fibers, condroitin sulphate, and glycosamino-glycans, which works as a nucleation site to the formation of a calcium phosphate layer. This stage is initially amorphous, but it soon crystallizes to form an apatite hydroxycarbonate organized structure. This compound attracts collagen fibers, condroitin sulphate, and glycosamin-oglycans, which are incorporated into that gel layer. Osteoblasts are also attracted to this layer; organic constituents are released, followed by mineralization (Yilmaz *et al*. 1998; Hamadouche and Sedel. 2000).

The osteogenic characteristic of bioactive glass particles may be related to the activation of an autocrine mechanism in osteoblasts, mediated by the induction of secretion of transforming growth factor-b, as occurs with the mitogenic effect of soluble silica on osteoblast-like cells in cultures. (Elena *et al*. 2006; Cordioli, et al., 2001). The bioactive glass particles release a substantial concentration of soluble silica, as well as Ca and P, during the first few days in contact with the body fluids on the site healing, which may be responsible for the osteogenic effects observed in this study. These particles are then incorporated to the growing bone as a component, and are used to build new bone (de Macedo *et al*. 2004).

Conclusions

Considering the results obtained, the following can be concluded:

- The bioactive glass promoted comparable bone formation over the entire extension of the hole, independently of their granules size, thus confirming their biological osteoconductive property.
- No inflammatory reaction was observed due to the presence of the implanted materials.
- As a consequence of its osteoconductive and osteointegration properties, bioactive glass can be recommended for the treatment of bone holes, either separately or in combination with other techniques, or in composition with other bone substitute materials.

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دراسة هستولوجية لتأثير الزجاج الحيوي النشط على التئام العظام (دراسة تجريبية علي عظمة الفخذ في الفار)

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أقسام الفيزياء بكلية العلوم و الهستولوجي و التشريح بكلية الطب - جامعة الأزهر- بنين القاهرة

الزجاج الحيوي النشط ،احد المواد المخلقة التي تلاقي استحسانا كبيرا لدي الباحثين حيث تبين انها متعددة الفوائد خاصة في مجال الطب . وقد أجريت الدراسة الحالية لتقييم تأثير هذه المادة علي تكون العظام أثناء الالتام. وتمت التجربة علي عظمة الفخذ في الفئران البيضاء وذلك بعمل تجاويف في جدار العظمة وغرس عينات من المادة في هذه التجاويف وتركها حتى تلتئم وقد أجريت الجراحة تحت تأثير التخدير الكلي وفي ظروف التعقيم المعتادة. وقد تم عمل نفس العملية بنفس الطريقة ولكن بدون غرس المادة في مجموعة فئران للمقارنة وبعد أسبوع تم ذبح الحيوانات في أوقات متدرجة وتم أخذ مكان الالتام لدراسته هستولوجيا وتم ذلك بعمل شرائح لدراسة طبيعة الأنسجة التي تكونت مكان الالتام باستعمال صبغات الهيماتوكسيلين وماسون الثلاثية وتفاعل باص الهستوكيميائي . وقد لوحظ سرعة تكون العظام في المجموعة التي استعمل فيها غرس عينات مادة الزجاج الحيوي عنه في المجموعة الضابطة وقد أظهرت النتائج الهستولوجية أن عملية تكون العظام فى المجموعتين تمت بنفس الطريقة ولكن كانت اسرع فى المجموعة المعالجة بالزجاج النشط عنها فى المجموعة الضابطة وقد تم التام العظام علي ثلاثة مراحل:

- المرحلة الأولي تميزت بتزاحم الخلايا الحبيبية الناتجة من الانقسام السريع للخلايا حول العظام حيث ملأت التجويف.
- المرحلة الثانية تميزت بكثرة ألياف الكولاجين حيث بدأت الخلايا الحبيبية بالتحول إلي خلايا ليفية و بدأت بإفراز ألياف الكولاجين بغزارة حتى امتلأ الفراغ بأكمله حيث تركزت الخلايا علي هيئة صف من الخلايا متفاوتة الطول.
- المرحلة الأخيرة تميزت بظهور العظام حيث نشطت الخلايا الطرفية مكونة خلايا العظام وبدأت في ترسيب الكالسيوم فوق ألياف الكولاجين المتكونة ، وظهرت العظام في البداية علي هيئة شعاب طرفية امتدت نحو الوسط ثم ازدادت تدريجياً والتحمت ببعضها فيما عدا بعض الفراغات البينية التي سرعان ما امتلأت ببعض الخلايا المتحولة إلي خلايا ليفية وخلايا نخاع. وبعد مرور ستة أسابيع من تمام التجربة أظهرت النتائج تكون نسيج عظمي مماثل تماماً للعظام المجاورة, وقد تكون النسيج العظمي بطريقة بناء العظام داخل الأغشية فى البداية ثم تحول الى نسيج مماثل للعظام المجاور.وهذه التجربة تعضد استعمال هذه المادة بأمان تام في مساعدة التام العظام الملتئمة خاصة في كبار السن كما تفتح أملا جديدا في دراسة تعويض العظام المتآكلة في الأسنان بل ولمدي ابعد حيث يوصي بدراسة إمكانية استخدام هذه المادة في الحصول علي نسيج عظمى مزروع خارج الجسم.