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HISTOLOGICAL ASSESSMENT OF INJECTABLE MACRO-POROUS CALCIUM PHOSPHATE CEMENT (CPC) VERSUS AUTOGENOUS BONE GRAFT ON HEALING OF OSSEOUS DEFECT (EXPERIMENTAL ANIMAL STUDY)

Hosam El Din Mostafa *, Mostafa Ezz**, Hosam Sayur *** *and* Hassan Abdel-Ghany ****

ABSTRACT

Objective: The aim of this study is to evaluate histologically the healing potential of injectable macro-porous Calcium Phosphate Cement and autogenous bone. (normal bone healing) on surgically induced bone defect on femur bone of rabbit.

Materials and Methods: Twelve adult white male New Zealand rabbits were selected for this study. Rabbits were divided into two groups and the two groups according to a two evaluation periods divided into 2 subgroups. Rabbits were anesthetized with intramuscular anethesia, and two osseous defects had been created in the distal aspect of the femur bone.one of this defects injected by a grafting material (SI-HPMC CPC) and the other left empty as control (normal healing). Animal were sacrificed after two sacrifice dates and the femur bone excised for histological and statistical evaluation.

Results: Rabbits from each group were sacrificed after 2 and 4 weeks. The femur bone containing the induced defect was dissected. Each femur bone was excised using hard tissue microtome. Each slice was then fixed in 10% neutral buffered formalin. The formalin-fixed bone samples were decalcified in 15% buffered formic acid solution and processed for routine histological examination using hematoxylin and eosin stain under light microscope.

Conclusion: According to our results in the present study, we can consider Si-HPMC CPC a viable alternative to the autogenous bone in the healing of osseous bone defects. This new and simple method to prepare macro-porous CPCs using Si-HPMC a foaming agent in connected syringe, result in a good injectablity and macro porosity of the CPCs which help us to gain our goal from the study.

* BDS, Msc., Faculty of Dentistry, Cairo University
** Professor of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University
*** Senior researcher, Animal Health Research Institute.
**** Associate Professor of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University

INTRODUCTION

Bone is a vital tissue that is capable of repairing itself throughout the host life. Injured bone has the ability to regenerate which lead to complete anatomical and physiological repair. However, with large bone defect as far some cases of trauma, cysts or neoplasm removal, the surgeon usually using bone grafts to restore theses bone defects.[1] Healing of acircumscribed bone cavity has been studied on several experimental animals such as rats, pigs, dogs and rabbits.[2]

Different problems and situations faced the oral-maxillofacial surgeons during their work through the years, such problems increased the demand for the use of different types of grafting materials to restore and reconstruct defects in maxillofacialregion.[3]

Criteria for an ideal bone grafts include the ability to induce bone formation by harvested mesenchymal cells, cell proliferation from live transplanted osteoblasts or osteoconduction of cells along the surface of the graft, and patterning of the primary formed bone with mature lamellar bone, long-term preservation of mature bone without loss of function, low risk of infection, convenient acquisition of materials, low antigenicity, and high reliability[4]

Autologous bone transplantation is still considered the gold standard because it has obvious advantages in terms of osteogenic potential, mechanical properties, and lack of adverse immune responses; however, it has some limitations, for example: additional surgery is required, a sufficient size and shape of the graft. is questionable, as well as risk of morbidity of the donor site.[5] Allogeneic grafts and xenografts have the risk of potential disease transmission or unpredicted immune response of the host, which sometimes leads to complete graft resorption. Therefore, a variety of biomaterials have been evolved as bone substitutes to overcome the shortcomings of these graft materials, such as alumina, hydroxyapatite, zirconia, bioglass and many other polymers.[6]

In this sense, synthetic bone substitutes based on calcium phosphate materials are a viable alternative to tissue transplantation. Calcium phosphate is a synthetic mineral salt, usually sintered at high temperature without steam, and then pressed under high pressure. Calcium phosphate ceramics have received extensive attention and extensive clinical research has been conducted. The use of calcium phosphate cement (CPC) in various fields such as orthopedic surgery, dentistry, maxillofacial surgery and reconstructive surgery has increased.[7]

Due to its good biocompatibility, biological activity and osteoconductivity, CPCs is generally considered a good candidate for bone replacement, which is related to two important advantages: injectability and porosity of calcium phosphate. Some key parameters of CaP ceramics, such as absorption rate and mechanical properties, are closely related to the Ca/P ratio. Crystal and porous structure are very important factors when choosing CaP ceramics.[8] Since Albee 1920, [9] tricalcium phosphate (TCP), especially b-tricalcium phosphate (b-TCP) in the b-diamond form, has been reported for the first time. The Ca/P ratio of B-TCP is 1.5, which is lower than that of hydroxyapatite, which can partially accelerate its decomposition and absorption. Like HAp, TCP has a more interconnected porous structure, which can directly promote fibrovascular infiltration and bone replacement, but at the same time weaken mechanical properties. [10] Biological behavior, the main advantage of CPC is that they can be injected and have the ability to solidify in the body at body temperature.[11,12] After the solid and liquid phases are mixed, CPC forms a viscous paste, which is easy to handle and shape, and can be partially introduced into the defect area to ensure that it can tightly adapt to the surrounding bones even for irregularly shaped cavities which is considered a unique advantage over bioceramics that are difficult to process and shape. [13] Injectability allows the use of minimally invasive surgical techniques to implant bone

cement, allowing the bone cement to perfectly adapt to the defect geometry and accelerate the healing process. [14]

Another important feature of CPC is that they are microporous. Aqueous solution of CPC after hardening and/or intergranular space due to pore size in the micron range. [15] Porosity is important to accelerate CPC absorption and bone growth, but it is also necessary to create large pores of at least 10 microns in the CPC to promote bone colonization in the implant and accelerate the entire process of CPC being replaced by bone, like in CaP bioceramics. [16] A new strategy has recently been proposed, which is to mix the slurry or cement powder phase with the foam obtained using a foaming agent such as albumin, polysorbate 80 [17], gelatin [18], soybean hydrogel [19] or a mixture of the latter two. [20] This method works without affecting the in-situ CPC setting, and can resulted in production of injectable macroporous CPC, which can be implanted using minimally invasive surgical techniques. An important point of this method is to select a foaming agent that should be soluble in water, non-toxic, and biocompatibility, combined with good foam characteristics and foam stability. Considering the work described in the scientific literature, there is still a need for a material that satisfies all the aforementioned characteristics related to simple and safe production. A process that can produce highly stable injection foam.[21]

MATERIALS AND METHODS

I- Selection of the animal samples and housing

The selected samples consisted of New Zealand male adult rabbits had been selected

From a good selection from the Rabbit Unit in Agriculture Collage at Cairo University. Rabbits were selected males with average weight between 3-3.5 kg and an average age of 4 to 4.5 months. All animals were checked by the veterinarian to be clinically healthy checked all animals. They were inspected for any signs of nasal or eye discharge. Pre-vaccinate all animals and pre-treated against scabies, coccidiosis and enteritis. (viral hemorrhage disease).

The animals were acclimatized to the research environment one week before the start of the study. The rabbits are kept in isolated stainless steel cages, one rabbit per cage. All rabbits received a basic diet of fresh hay, pellets and distilled water, designed to meet the nutritional needs of rabbits on the test day.

Study design: Two Femoral defects were induced in all rabbits one defect filled with the intervention material and the other was left empty as control, and rabbits were divided into 2 groups according to sacrifice date as illustrated in table 1:

Group(A):

This group formed of 6 healthy rabbits with induced two femur defects one defect was leaved without intervention (Normal bone healing) and the

TABLE (1): Showing the study design for the selected samples

Groups	Group A	Group B
No. of rabbits	6	6
Date of sacrifice	2 weeks	4 weeks
Day of the surgery	2 Femoral defect induced in all rabbits	
Intervention	3 defect left empty 3 defect filled with Si-HPMC	3 defect left empty 3 defect filled with Si-HPMC
Route	Normal Healing	Intra femoral defect injection

second defect injected by Si-HPMCCPC and the rabbits were sacrificed after 2 weeks.

Group(B):

This group included of 6 healthy rabbits with induction of two femur defects one defect was left without intervention (normal bone healing) and the second defect was injected by Si-HPMCCPC and the rabbits were sacrificed after 4 weeks.

II- Preparation of Graft Material:

All reagents used in this experimental study are of analytical grade and can be used without further purification. ά-TCP powder and Si-HPMC solution are the two main components for the production of Si-HPMC foamed CPC.

Calcium phosphate cement (CPC) preparation:

By heating a mix of dicalcium phosphate anhydrous (CaHPO4) (CaHPO4; Alfa Aesar, Germany) and calcium carbonate (CaCO3) e (CaCO3; VWR, BDH, Prolabo) with a molar quantitative relation 2:1 at 1300ºC for fifteen h employing a Muffle chamber,ά.-TCP powder was shaped. CPCs was processed during a Mortar Grinder for thirty min to induce a fine powder. **B) The silanized-hydroxy propyl methylcellulose (Si-HPMC) powder preparation:** Si-HPMC powder is prepared by silanizing HPMC (Methocel, E4M, Colorcon-Kent-England) with 3-glycidoxypropyl salt (Aldrich, Germany) according to **Bourgesetal and Fatimietal.**[22,23] who described the method of synthesis.

Production of Si-HPMC foamed CPCs (Fig. 1, 2)

The Si-HPMC solution was prepared by dissolving the aforementioned Si-HPMC powder in 0.2 M NaOH solution at 25 C for 48 h, followed by dialysis against NaOH solution (0.09 M) for 16 h using a 6–8 kDa D-Tube Dialyzer (Spectra/Por, UK). The pH value of the resulting Si-HPMC solutions was around 12.8. Si-HPMC solution is stable in a strong alkaline environment (pH> 12.1). When the pH drops, the Si-HPMC solution begins to gel and become a hydrogel. This solution was used to initiate the gelation of Si-HPMC to a final pH of 7-8.

The Si-HPMC solution and NaH2PO4 solution were primarily sealed in the volume of two commercially available syringes: 5ml of 30% by weight NaH2PO4 solution was used to make the gelation of Si-HPMC. Then pump the required volume of air into the syringe. Then connect the two syringes through the connector, and push the two plungers of the syringe alternately in opposite directions for 20 seconds to quickly mix the solution and air until a uniform Si-HPMC foam is formed.

Fig. (1): A photograph showing preparation step of Si-HPMC.

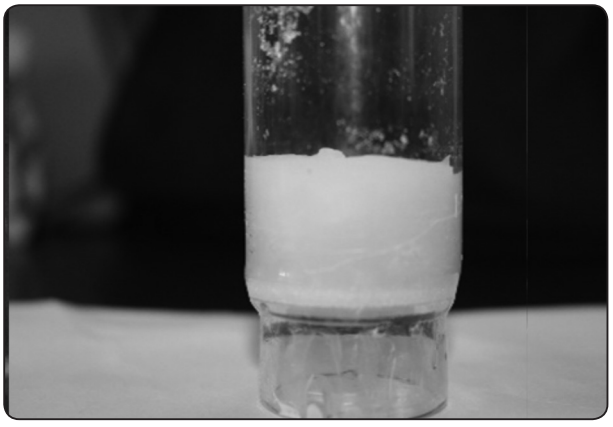

Fig. (2): A photograph showing SI-HPMC foaming agent.

Mix it with 2.5% (weight) Na2HPO4 in a mortar at an L/P ratio of 0.35ml/g for 1 minute, then put the resulting paste into a 5ml syringe, and then remove the entrained air. The HPMC hydrogel foam and CPMC paste were quickly mixed for 30 seconds through the same procedure as the preparation of the Si HPMC foam CPC paste.

III- Surgical procedure:

Pre-operative:

All animals were deprived from food for about 8 hours and from drink for about 3 hours prior to the operative procedures.

Anesthesia:

The rabbits were injected intramuscularly with sedatives, sedative 2% xylazine hydrochloride (Xylazine Injection, Pharmika, India) and anesthetic ketamine 10% solution (ketamine Hydrochloride 10ml, Rootex., Germany). First, xylazine hydrochloride is administered in one dose (2-3 mg per kg of animal body weight). Ten minutes later, the second intramuscular injection of ketamine hydrochloride. The anesthetic is administered in one dose (50 mg per kilogram of animal body weight). This combination of two drugs allows anesthesia for 20 to 30 minutes protocol**.**

Preparation of site of surgery

The site of surgery was prepared by clipping and shaving the skin hair covering femur bone at the thigh region by shaving cream. The region was then washed with soap and water and disinfected with 5% tincture of iodine and betadine antiseptic solution and wrapped with sterile towels.

Intraosseous Grafting procedure

The grafting procedure were performed by injection technique according to those described by **(Tassery et al., 1997).**[24]

An incision 5-7 cm long just below the head of the femur from the great trochanter to stifle joint was made using a Bardparker blade No. 11 through the skin and subcutaneous tissues; exposing the underlying muscles, vastus lateralis and biceps femoral muscles of scissors. Then use scissors to perform a blunt dissection between the two muscles, exposing the outside of the femur. At low speed (25,000 rpm) and manual angular momentum, use the standard sterile rotary drill #2 in the handpiece to create two bone defects (3 x 5 mm). (Fig. 3) One of the bone defects of the femur was implanted with the test material, the second defect was kept empty as a control, and the material was injected into the defect with a 5 ml plastic syringe.. (Fig 4)

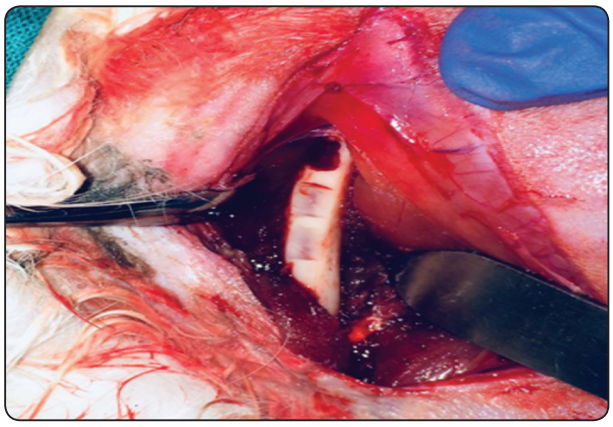

Fig. (3): A photograph showing the two bone defects placed at nearly equal distance from each other.

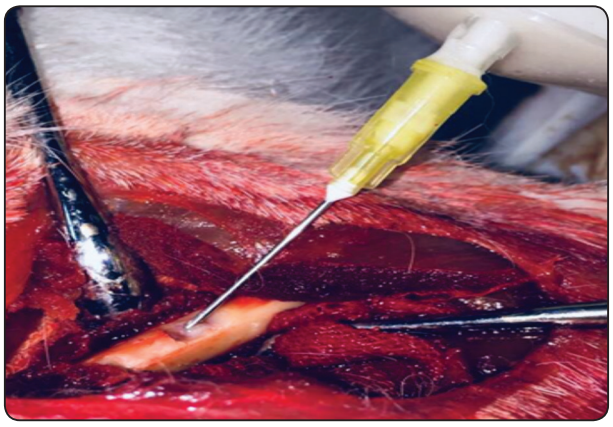

Fig. (4): A photograph showing injection of grafting material in one defect using plastic syringe while the other defect left empty.

Closure

With interrupted suture patterns, suturing of the muscles and subcutaneous tissues **was** carried out using 2/0 vicryl sutures. While, the skin incision was sutured using 3/0 black silk sutures. The graft material is fixed in place by the surrounding soft tissue.

Postoperative care

The rabbits were clinically examined and were subjected to postoperative care and observation of wound healing if there is any sepsis, swelling, rejection of grafted materials, irritability and dehiscence They were given a daily intramuscular injection of pain medication 25 mg diclofenac sodium during the first 3 days after surgery (Declophen amp., Pharco, Egypt) and an intramuscular subcutaneous injection; 1ml Enrofloxacin 10% antibiotic (Enrofloxacin 10% 100ml, Choosing, China) to prevent infection for a week.

Methods of evaluation

A: Clinical evaluation

Animals were observed by macroscopic examination along the three experimental periods; the skin at the site of operation was inspected regularly to record any surface changes or any signs of irritation such as infection, ulceration and discoloration.

B: Histopathological evaluation

After experimental period of 2 and 4 weeks, the animals were sacrificed by decapitation. Immediately after animal sacrifice, the site of the operation, were removed carfully. The femur containing the bone defect is dissected free of soft tissue, leaving the femoral condyle to facilitate the identification of the hole for each defect. The bone sample was then immediately placed in a similar container labeled with the number of animals and the duration of the study.

Preparation of bone samples for histological assessment

The tissues were fixed in 10% natural formalin for 4 weeks, and all samples were labeled with 12.5% ethylenediaminetetraacetic acid (EDTA) for decalcification. , Dehydrate until the ethanol concentration gradually increases, from 70% to 100% soluble alcohol, then add methyl benzoate for one day, then add paraffin benzene for two hours, wash in xylene for two hours, and then immerse in paraffin wax at 55°C. Place the sample in a wax block of appropriate size to be sectioned, and use a microtome to make serial sections through the entire depth of the resulting defect to obtain a 4 μm-thick serial section mounted on a glass slide. Hydrate and stain with hematoxylin and eosin (H&E) for general histological examination.

Data image analysis: The pathologist blindly interprets all micro-histopathological sections without knowing the filling material or the time interval associated with each slide. Histological evaluation is performed 3 times to determine the percentage of newly formed bone area. According to established standards and rating systems (Tassery et al., 1997 [24] and Tassery et al., 1999 [25]), the average score is recorded and tabulated for statistical analysis.

C: Statistical analysis

For each Si-HPMC-CPC and control group, calculate the average and standard deviation (SD) value of the percentage of newly formed bone. A method of analysis of variance (ANOVA), Tukeys, Friedman's test to check all variables in this study. In all statistical tests, the significance level is set to $p \leq 0.05$. Microsoft Office 2013 (Excel) and Social Science Statistical Package (SPSS) version 20 are used for statistical analysis.

RESULTS

I-Surgical results

Healing of the defect was uneventful throughout the follow-up periods, no rejection of materials, no pus or inflammatory reaction was observed.

II-Histological interpretation at two periods of investigation

Microscopic findings: Most of the implanted material was lost during the histological processing. Most of control and SI-HPMCCPC specimens showed moderate inflammatory reaction after two weeks. These reactions subside to become mild along the 4 weeks period of observation. Histopathological evaluations were presented as follow.

i) Control specimen

The tissue response in the control specimen showed an apparent improvement over two observation periods.

A. Evaluation after 2 weeks period: Histological examination revealed filling of the surgical defect by granulation tissue. Specimens showed a mild acute inflammatory reactions. Some of the created defects showed a thick fibrous connective with polymorph nuclear leukocytes as well as few lymphocytes and macrophages. No evidence of any bone resorption. (Fig.5)

B. Evolution after 4 weeks period: The femoral osseous defect showed numerous small blood vessels, fibrous connective tissue network with few lymphocytes and macrophages. No evidence of any bone resorption. Few osteoblasts were also showed at the bone surface.(Fig.6)

ii) SI-HPMCCPC Specimen

The tissue response in the grafting specimen showed an apparent improvement over two observation periods.

A. Evaluation after 2 weeks period: Histological examination revealed filling of the surgical defect by newly formed compact bone trabeculae with large osteonal canals containing high number of osteoblasts. (Fig.7)

B. Evolution after 4 weeks period: The femoral osseous defect showed the newly formed bone filling the defect with uneven surface; the bony tissue became more compact with smaller osteonal canals.(Fig.8)

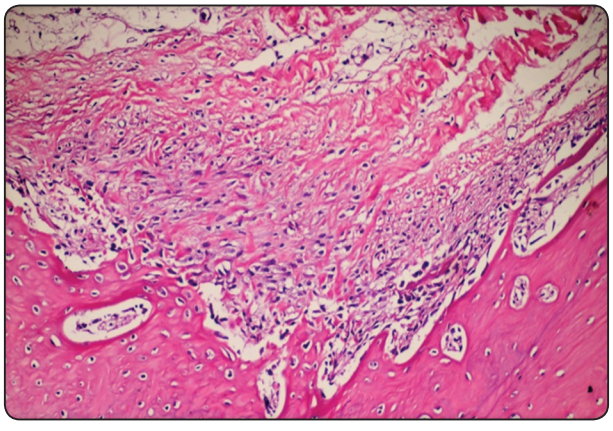

Fig. (5): A photomicrograph of the histological changes after 2 weeks from creating the defect (control group) showing mild acute inflammatory reaction in the form of thick fibrous connective tissue formation with few inflammatory cells. (H&E 200X).

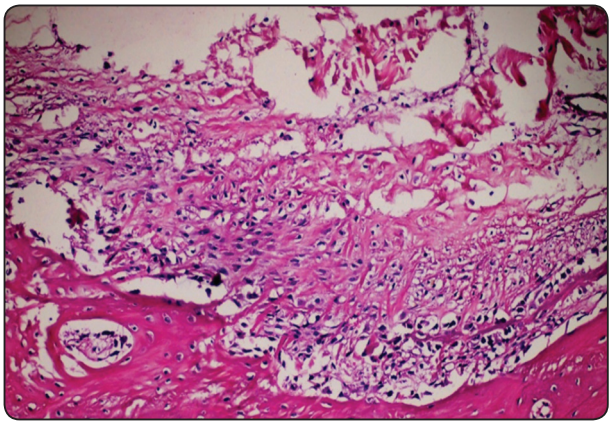

Fig. (6): A photomicrograph of the histological changes after 4 weeks from creating the defect (control group). Mild chronic inflammatory reaction shows thin fibrous connective formation. Areas of newly formed bone (H&E 200X).

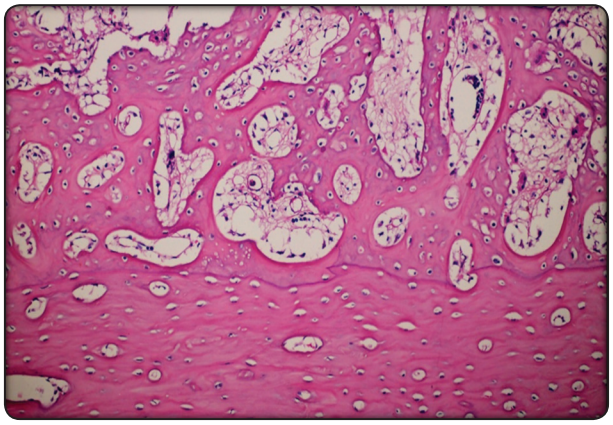

Fig. (7): A photomicrograph of the histological changes after 2 weeks from intraosseous injection of the grafting material (SI-HPMC CPC) . Showing newly formed compact bone trabeculae with large osteonal canals containing high number of osteoblasts. (H&E 200X).

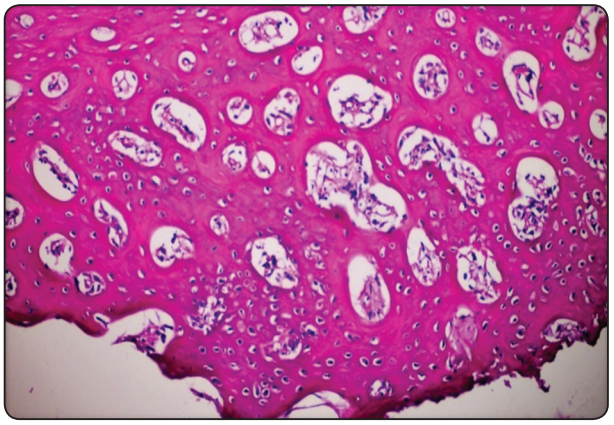

Fig. (8): A photomicrograph of the histological changes after 4 weeks from intraosseous injection of the grafting material (SI-HPMC CPC). Showing the newly formed bone filling the defect with uneven surface, the bony tissue became more compact with smaller osteonal canals. (H&E 200X).

III-Statistical analysis :

New bone area percentage:

i) Comparison between the 2 groups to study the effect of the filling material on the area percent of newly formed bone:

Table (2) represents the mean, standard deviation (SD)values and results of One way ANOVA and and Tukey pair wise comparison test for comparison between the mean new bone area percentages in the control, and Si-HPMC CPC groups at two evaluation periods.

A) After two weeks

One-way ANOVA test showed that the difference between the studied groups was statistically significant ($p<0.001$).

B) After four weeks

One-way ANOVA test showed that the difference between the studied groups was statistically significant ($P<0.001$).

TABLE (2): The recorded means and standard deviation (SD) values of the new bone area percentages for the control and Si-HPMC CPC after 2 weeks, 4 weeks

Group / Period	Group A (Control)		Group B (Si-HPMC CPC)		P-value
	Mean (n=6)	SD	Mean (n=6)	SD	
2 weeks	1.90[a]	0.9	3.25[a]	0.9	< 0.001*
4 weeks	9.05	3.5	14.9	4.3	< 0.001*

SD: Standard Deviation. ***P: Probability level.*** ****: Significant at P ≤ 0.05***

Means with similar super script letters indicate no statistical significant difference.

ii) Effect of time on the area percent of newly formed bone within each group:

A) Control Group:

Table (3) represents the means, standard deviation (SD) values and results of ANOVA for repeated measures for new bone area percentages within control group during the two evaluation periods.

ANOVA for repeated measures test showed that there was a statistically significant difference between new bone area percentage at the two follow-up periods (p=0.001).

Pair wise comparison with Bonferroni correction showed that there was a statistically significant difference between 2 weeks and 4 weeks.

Pairwise comparison with Bonferroni correction stated that there was a difference between 2 weeks & 4weeks which was statistically significant.

B) Si-HPMC group:

Table (4) represents the means, standard deviation (SD) values and results of ANOVA for repeated measures for new bone area percentages within SI-HPMC during the two evaluation periods.

ANOVA for repeated measures test showed that there was a statistically significant difference between new bone area percentages at the two follow-up periods (p=0.001

Pair wise comparison with bonferrioni correction showed that there was a statistically significant difference between 2 weeks and 4 weeks.

TABLE (3): The recorded means and standard deviation (SD) values of new bone area percentages within control group during two evaluation periods:

	2 weeks		4 weeks		P - value
Control	Mean (n=6)	SD	Mean (n=6)	SD	0.001*
	1.9	0.9	9.05	3.5	

SD: Standard Deviation. P: Probability level. *: Significant at P ≤ 0.05.

TABLE (4): The recorded means and standard deviation (SD) values of new bone area percentages within intervention groups during two evaluation periods

Intervention	**2 weeks**		**4 weeks**		**P - value**
	Mean (n=6)	**SD**	**Mean (n=6)**	**SD**	
	3.25	**0.9**	**14.9**	**4.3**	**< 0.001***

SD: Standard Deviation. P: Probability level. *: Significant at P ≤ 0.05.

DISCUSSION

Bone defects in the oral cavity and jaw area caused by the removal of tumors, cysts, etc. should be repaired with bone grafts to prevent functional as well as aesthetic problems to the patient. Due to the improvement in the field of maxillofacial surgery, the importance of developing an ideal synthetic bone graft is very important.[1]

The ideal non-human bone graft substitute have to sterile, non-toxic, immunologically acceptable, and available in ample quantities. The alternative substitute can induce local cell differentiation in bone-forming cells while providing a gradually absorbable conductive scaffold for new bone formation. In addition, the material must prevent fibrous tissue ingrowth mechanically as well as prevent muscle penetration into bone defects. It would be more advantageous to have materials that not only stop bleeding but also promote bone regeneration while resorbing.[5]

Autologous bone has proven to be the best or ideal and most widely used substitute for maxillofacial reconstruction and support. However, it has many limitations such as donor site morbidity and need of second site surgery. Beside if the defect is large, it may be difficult to utilize enough bone, and it may take a long time for the graft to form and delimit the location of its future bone shape.[24, 26]

To counteract these limitations, synthetic bone substitute products have provided many alternatives in the past few decades. Bone substitution procedures have gradually shifted from natural grafts to synthetic bone substitutes and bioactive agents. One of the most important synthetic bone substitutes and biological factors, is calcium phosphate which is considered the most often used substitute either alone or in combination with others. Calcium phosphate cements (CPC) are appealing as bone substitutes because they are injectable and self-setting under physiological conditions, and are more similar to biological appetites. Beside that, from a biological point of view, CPC also has the following excellent characteristics: they are proven to have biocompatibility, absorbability and osteoconductivity, as well as, they also contain internal micropores, which give channels for nutrients and metabolic wastes to pass and be transported alongside the entire implant site promotes bone regeneration. In addition, macropores as well as micropores also seem to be necessary for enhancing CPC reabsorption and bone growth.

In this work, the process of foaming CPC with a syringe and the process of preparing macroporous CPC for injection using crosslinkable hydrogel (Si-HPMC) as a propellant is similar to that of J. Zhang et al. [27]. The author has previously proposed a method for the preparation of CPC-foam, stirring method, where they use a household mixer to mix the liquid CPC stage, and then use a spatula to gently mix the resulting foam with the cement slurry or CPC powder stage to avoid destroying the foam.[28].

Some foaming agents agents require heating to produce foam; in addition, it is difficult to use such techniques to control the amount of air entering a fixed volume of paste. The porous structure is useful for bones reconstruction as it promotes cell adhesion, nutrient transport, blood vessel growth and bone regeneration.[29-31] When the pore size is less than 10 microns, it allows body fluids to circulate. When the pore size is 10 to 75 microns, fibrous tissue penetration is allowed. [32] If the pore size is between 75 and 100 microns, it is conducive to the growth of non-mineralized bone-like tissue. When the pore size exceeds 100 microns, it will stimulate the growth of mineralized bone.[32] In this study, connected syringes are used to foam the viscous liquid phase of CPC, and then the resulting foam is mixed with CPC paste; all the preparation processes can be carried out at room temperature, and the amount of air contained in the cement can be more precise control the large porosity of the final cement. The results are consistent with the work done by Xu Q. et al 2020.[33] In this sense, this method is simpler and easier to replicate for clinical use. The foaming agent selected in this

study is salinized-hydroxypropyl methylcellulose (Si-HPMC), which is reported to have biomedical applications in biomaterials and injectable calcium phosphate. B. Fellah et al,[35] S. Laib et al ,.[36] and C. Vinatier et al[37] showing a good biocompatibility and bioactivity.

The reasons for using SI-HPMC as a foaming agent are as follows: First, the solution can be used as a surfactant to reduce the surface tension of water, so that air is beneficial to the formation and removal of bubbles and combining at room temperature without heating. The solution maintains its shape or position without flowing out of the implantation site, and also shows good injection ability and adhesion, which is necessary for clinical use, as also stated by Vinatier et al.[37]

In the present study, the rabbit femur was chosen because the bone of the rabbit is somewhat similar to the jaw bone. In embryology, it is formed by a precursor membrane; morphologically, it is composed of cortical plates separated by a spongy matrix; physiologically its healing method is the same.

The material used in this study is called Si-HPMC CPC, which is well tolerated by the recipient's femur; in addition, it has been proven to be biocompatible with the natural soft tissue healing of all experimental animals. Allergic reactions during the follow-up period were not observed (4 weeks). The histological observations in this study show that Si-HPMC CPC is a well-tolerated graft material and does not induce inflammation, which is consistent with the work of Zhang et al. [21] who finds that the graft material does not show an inflammatory response clinically, and there is no evidence of a foreign body reaction.

During the operation, the bone defect is completely filled with the graft material, and the flap is put back in close contact with the graft and sutured. In terms of materials, the bone cavity is surrounded by healthy bleeding bone.

In this study, 4 weeks postoperatively was chosen as the end time of the study, because the rabbit femoral defect caused by the operation healed quickly. In group A, the histological samples observed at the control defect two weeks later showed that the defect surface was full of fibrous connective tissue and inflammatory cells, while the sample treated with Si-HPMC-CPC showed that the defect surface was full of fibrous connective tissue and inflammatory cells and newly formed bones and some osteoblasts.

In group B, the histological observation of the control defect specimens for 4 weeks showed some osteoblasts with fibrous connective tissue, while in Si-HPMC-CPC treatment, it showed denser bone formation with more bone canals and osteoblasts. The defective area is covered with SI-HPMC. After 4 weeks, the CPC was almost reorganized and ossified, while the control site still showed fibrous tissue infiltration, which was similar to the situation of J. Zhang et al. [21] in their in vivo study of rabbits. The analysis showed that the control group had the least amount of bone remodeling after 2 weeks, and the percentage of new bone area of Si-HPMC was higher than that of the control group. The statistical significance of the percentage of new bone formation between the control group and the study group showed that SI-HPMC CPC improved the quality of bone healing in a short period of time, similar to the results obtained by Schmidt et al. 2019.[38]. It is consistent with all the results obtained, it can be concluded that the calcium phosphate cement for injection is biocompatible, has the biological properties of bone conduction, and can be used to fill the cavity of bone defect.

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