Original Research

Chemical Preparation of Beta Calcium Sulfate Hemihydrate Granules with a Special Particle Size as Bone Graft Material

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Abstract

Introduction: Beta calcium sulfate hemihydrate (BCSH), which is commonly known as "Gypsum plaster" has long been used as bone graft material because of its excellent biocompatibility and the ability for bone regeneration. Several methods have been used for the preparation of BCSH, including heat treatment of calcium sulfate dihydrate with water, application of inorganic acids or condensed inorganic salt solutions under high or atmospheric pressure. As a bone graft, it is preferred that the BCSH powder has a granular form for the purpose of manipulation, bio-mechanical properties and ease of injection. **Methods:** For this study granules of BCSH in the size of 500 to 700 micrometers were manufactured and sterilized using gamma ray. For assessing the regeneration of thismaterial, six rabbits were selected and granules were injected in the bone defects that were made using diamond bur in their skull under general anesthesia. Biopsies for histological evaluations were done 3, 6, 9, 12, 14 and 16 months following surgery. **Results:** At the third month time-point, remodeling of the BCSH was evident, and complete newly formed bone was seen in the region of the defect. **Conclusion:** The results of this study demonstrated that BCSH had a good biocompatibility without inducing an inflammatory response and promoted bone healing.

Key words: Beta calcium sulfate hemihydrate, bone graft, granule.

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Introduction

As bone lesions occur in dentoalveolar system in human due to trauma, illness, and surgery, the application of the appropriate material(s) is mandatory in these situations (1,2).

Various techniques that use different types of grafts such as autologous bone graft, allograft, alloplast, and xenograft were proposed. The most success in the field of bone graft is obtained from autografts; as autograft is a living tissue with intact cells and no immune reaction. However, it has two disadvantages (3): first, it is obtained from the secondary region of the body that can make problems due to surgery in the donor region (4) and second, the limitation that exists in the amount of graft material.

Using allograft materials would avoid performing of second surgery. However, the concern about this kind of material is the possibility of infection and disease (5,6).

Allograft materials that are routinely used are demineralized freeze-dried bone, freeze-dried bone allograft, and bovine deproteinized bone (7). In comparison with autograft materials, allograft materials have fewer capacities for osteogenesis, higher rate of absorption, immune response and also more concerns about disease transmission (8). These problems led to the expansion and creation of alternative materials (9). Calcium sulphate has the longest clinical use in

comparison with other materials that are currently available (10).

Calcium sulfate used in bone defects is well-tolerated, gets rapidly and completely absorbed without leaving any obvious inflammatory response. It is made of materials which are cheap and can be easily found in abundance (11, 12). Calcium sulfate is tissue compatible and when used as a binder, it expedites the healing process and avoids loss of the grafting material (13).

On the other hand, the main limitations of calcium sulfate as bone filler are poor handling characteristics, poor mechanical properties and its quick absorption (14). Therefore, in order to avoid or reduce these limitations, this study was focused on changing calcium sulfate into the granulated form with a size of 500 to 700 microns and higher hardness. So the aim of current study was to combine granulated calcium sulfate with a new binder in order to improve its mechanical and handling characteristics and then to evaluate it clinically and histologically.

Materials and Methods

Preparation of Calcium Sulphate

At first calcium sulfate anhydrous crystals were mixed with enough water and a special binder to form a paste (this binder was prepared and designed in the Department of Chemical Engineering of Ferdowsi University of Mashhad). After several minutes, the paste dehydrated and hardened. The solidified paste was ground into the desired particle size range 500-750 microns. Then the dehydrated calcium sulfate was heated in order to lose water in a process known as calcinations. Due to the presence of binder in the mixture the intensity of heating and the final temperature is critical therefore; it should be heated slowly with a specified ramp. The resulted product was calcium sulfate hemi-hydrate. Finally, this product was sterilized using gamma ray.

Hardness Test

The hardness of the specimen was measured by the Rockwell test. The depth of the indentor penetration into the specimen surface was measured. The indentor may be either a hardened $CaCo_3$ ball with the diameter of ¼ inch or a spherical diamond cone with 120 angles.

PH Evaluation

After dissolving $caco_3$ in water, the PH was measured with digital PH meter.

Surgical Procedures

Six New-Zealand rabbits with similar weight (2.5-2.8 kg) and size were participated in this study. Protocols were approved by the ethical committee for animal study at Mashhad Dental University.

The animals were anesthetized preoperatively with an intramuscular injection of ketamine (40mg/kg Rotexamedica, Trittau, Germany) and xylazine (6-8 mg/kg Alfsan, Woerden, Holland). In addition, 1ml of local anesthetic of lidocaine without vasoconstrictor (Daroupakhsh, Tehran, Iran) was injected.

Parietal bone was chosen as the surgical site; surgical area was shaved and disinfected with povidone-iodine (Najo, Tehran, Iran).

A full-thickness incision was performed to expose the parietal bone, a slow-speed dental drill equipped with a carbide round bur was used to create one 6-mmwide defect in bone under irrigation with a large amount of normal saline. The defect was filled with calcium sulphate the surgical site was sutured with stainless-still monofilament wire 3-0 (Supa Medical Devices, Tehran, Iran).

All rabbits were given water and a standard rabbit food; no postoperative complications or death occurred. Rabbits were sacrificed at months 3, 6, 9, 12, 14, 16 and a total of six defects were retrieved.

Histological Processing

All specimens were decalcified in EDTA for two months. The process of decalcification was controlled radiographically and after decalcification, all of them were fixated in paraffin. The specimens were sectioned with the micrometer set at 5 mm.

The parameters used for evaluation were: inflammatory infiltration with probable abscess formation or necrosis and new bone formation with bone typing.

Each of the parameters was scored from 0 to 3, with 0 = normal, 1 = mild increase, 2 = moderate increase, 3 = marked increase. Sparsely scattered neutrophils, plasma cells, macrophages, eosinophils and mast cells arranged in a random fashion were considered normal. Localization of 3-10, 11-30, or 31 or more cells in the wound tissue per × 400 magnification fields were considered as mild, moderate, and marked increased respectively.

Sparsely scattered necrotic cell debris, extravasated erythrocytes (indicative of acute hemorrhage), and hemosiderin-laden macrophages (indicative of chronic hemorrhage) were considered as a mild increase for each of these parameters. Focal dense accumulations of these components in the wound tissue were considered as a moderate increase. Extensive tissue necrosis, massive hemorrhage involving the surrounding tissue, and the presence of greater than 30 hemosiderin-laden macrophages per × 400 magnification fields were considered as marked increase for each of these parameters respectively (15).

New bone formation was considered as inter membranous bone development in which it was highly cellular with rim of active plump osteoblasts. The type of osseous was diagnosed according to histological structure.

Results

Mechanical Hardness

Hardness was measured in scale A and its value was equal 120 shore.

PH Measurement

The PH of caco3 was measured 7.2. The solubility of calcium sulphate in water at this PH is Ksp=2.23g/lit.

Clinical Observation

The rabbits were carefully monitored and examined, during the first 16 months after putting the matter. Throughout the monitoring period, any problems, including death, weight loss and clinical signs of swelling did not occur and flap adaptation and retention of wound closure was achieved in all rabbits. At the time of biopsy, the skin of all the animals was healthy. The exposed bone was carefully studied, and their skin was fully touched. The surgical procedure was well tolerated by the animals; healing was uneventful.

Histological Evaluation

After Three months: one of the rabbits was sacrificed and histologically examination showed normal trabeculation of bone containing Haversian canals and lacunar spaces contained osteocytes, as well as osteoblastic rim. Inflammatory cells and fibrous tissue existed in the sample. Chronic diffuse inflammatory cells and infiltration was seen around the collagen fibers and blood cells and the giant cells, also reaction to foreign body was observed (Figs. 1 and 2).

At sixth months: in this sample the same trabeculation containing haversian canals and lacunar spaces with osteocytes were observed, osteoblast cells existed as a marginal rim. Also, focal infiltration of chronic inflammatory cells without foreign body reaction was visible. Fibrosis was more prominent in this sample (Fig. 3).

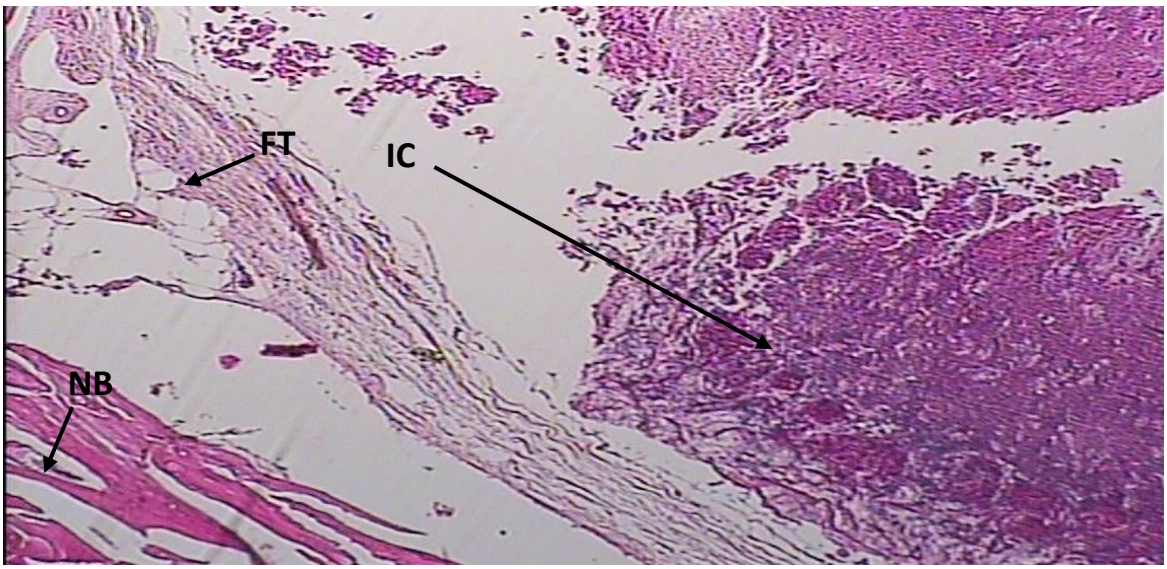

Figure 1. The defect at 3 months. NB: normal bone; FT: fibrous tissue; IC: Inflammatory cells. 100 X and H & E staining

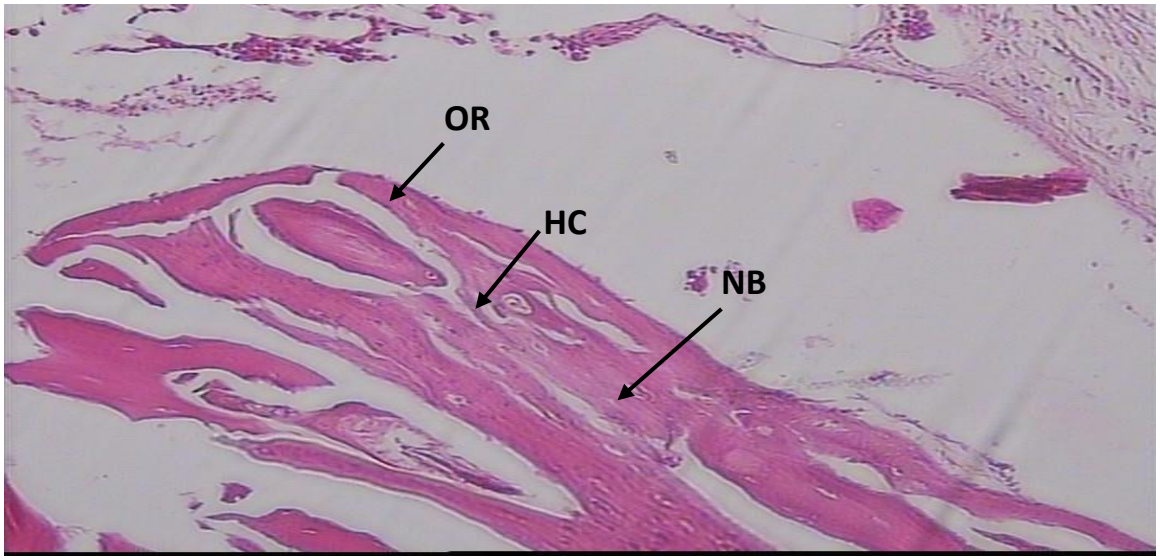

Figure 2. The defect at 3 months. NB: normal bone; HC: haversian canal; OR: osteoblastic rim.400 X and H & E staining

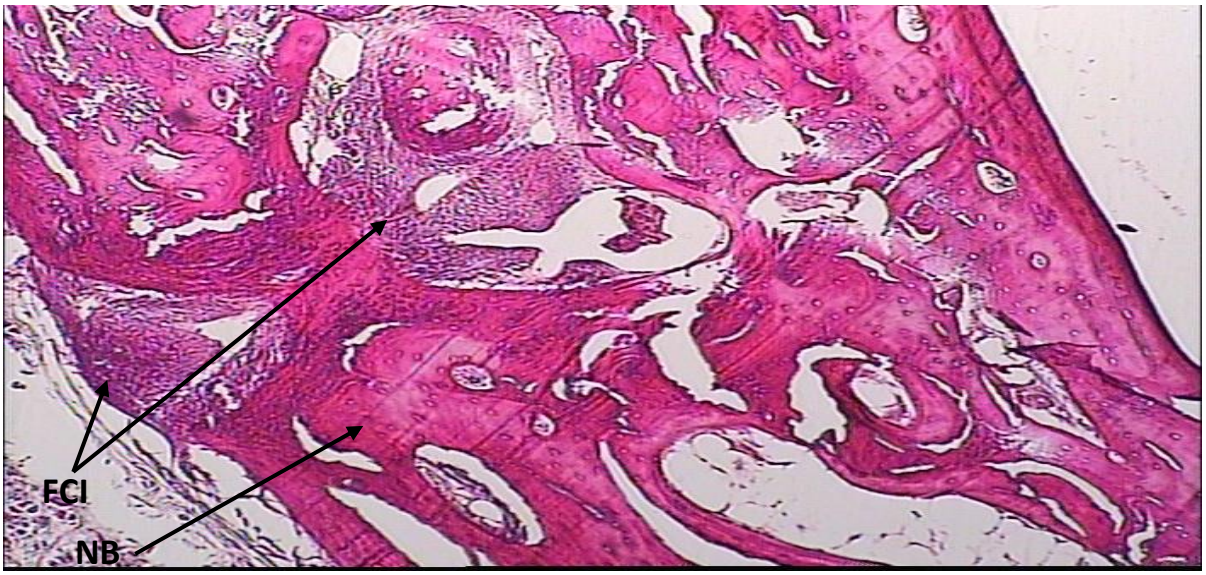

Figure 3. The defect at 6 months. FCI: focal infiltration of chronic inflammatory cells; NB: normal bone. 400 X and H & E staining

After nine months: In 3rd sample the normal bony structure similar to the previous samples was observed; likewise, in this case no sign of inflammation could be seen. However, fibrous tissue was also observed. Bone marrow fibrosis was also present and was advanced (Fig. 4).

At twelfth month, in the 4th sample, normal bony structure was observed. Cartilaginous tissue and inflammation were not found. Reversal lines were easily seen in this sample. In some instances, severe fibrous tissue of bone marrow was also observed.

At the fourteenth month: normal bony structure and osteoblastic rim were observed. Focal infiltration of chronic inflammatory cells was found surrounding the fibrous tissue of the bone marrow and in the periphery may be done after an accidental trauma or rest of previous inflammatory response (Fig. 5).

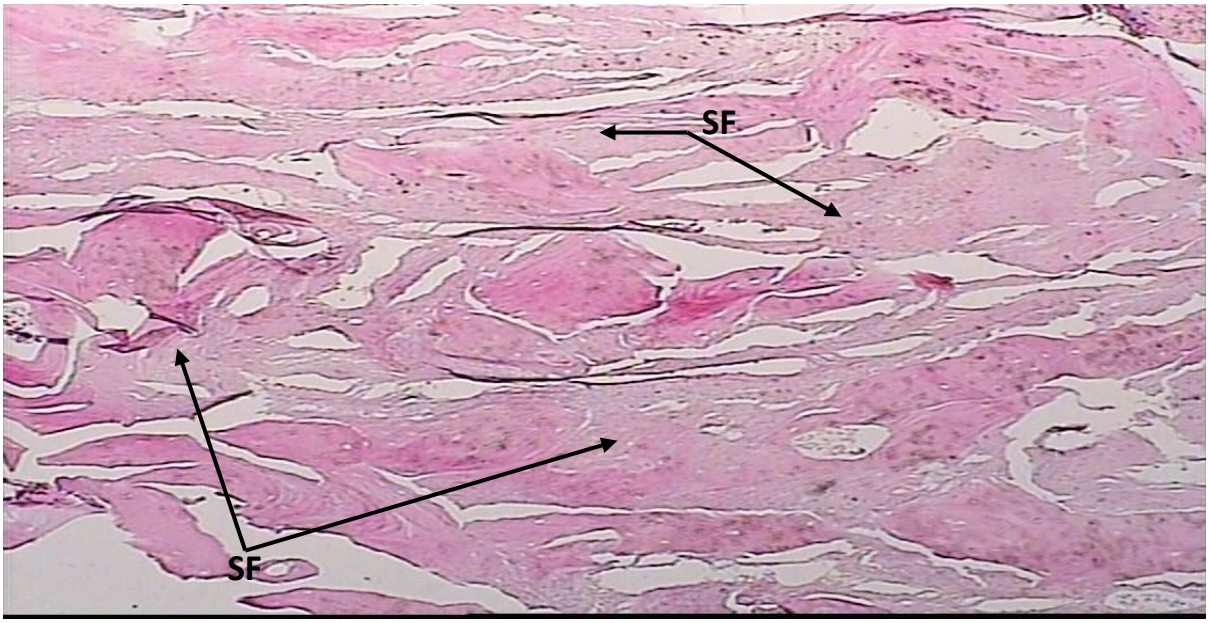

Figure 4. The defect at 9 months. SF: severe fibrous.400 X and H& E staining

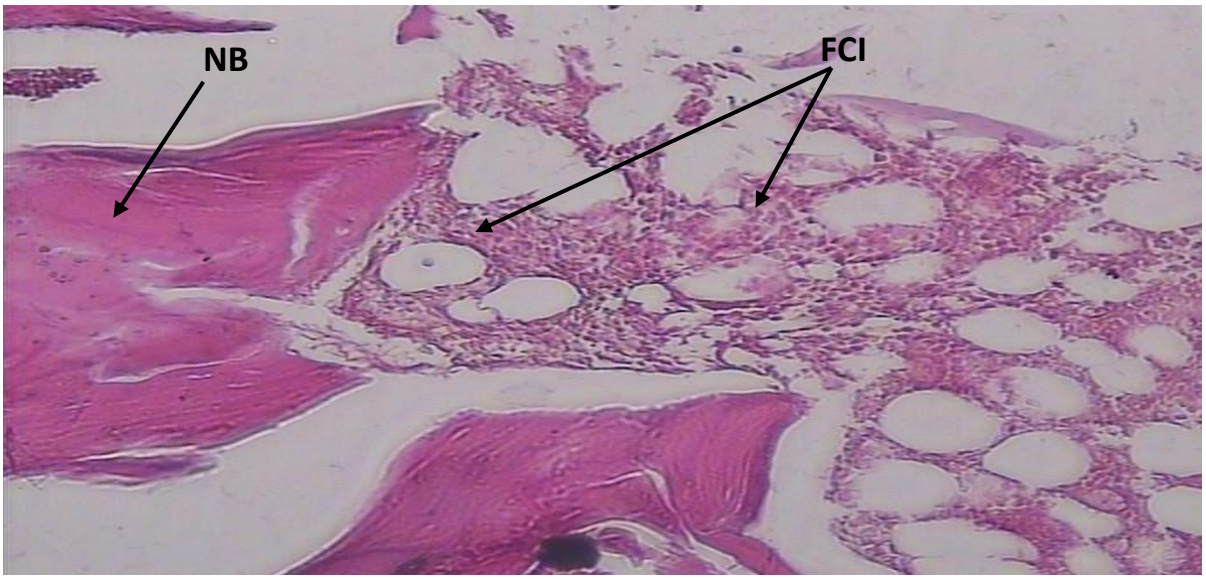

Figure 5. The defect at 14 months. FCI: focal infiltration of chronic inflammatory cells; NB: normal bone.

At the sixteenth month: the new bone can be seen clearly. Normal bone with normal trabeculation that contains Haversian canal and lacunar space with osteocytes and osteoblastic rim beside new bone that shows more cellularity than trabeculation was observed and fibrous tissue without infiltration of inflammatory cells was also found in the bone marrow space (Fig. 6).

One of the most important findings in analysis of cases was that even in the presence of infiltration; no sign of sequestrum was observed (Table 1).

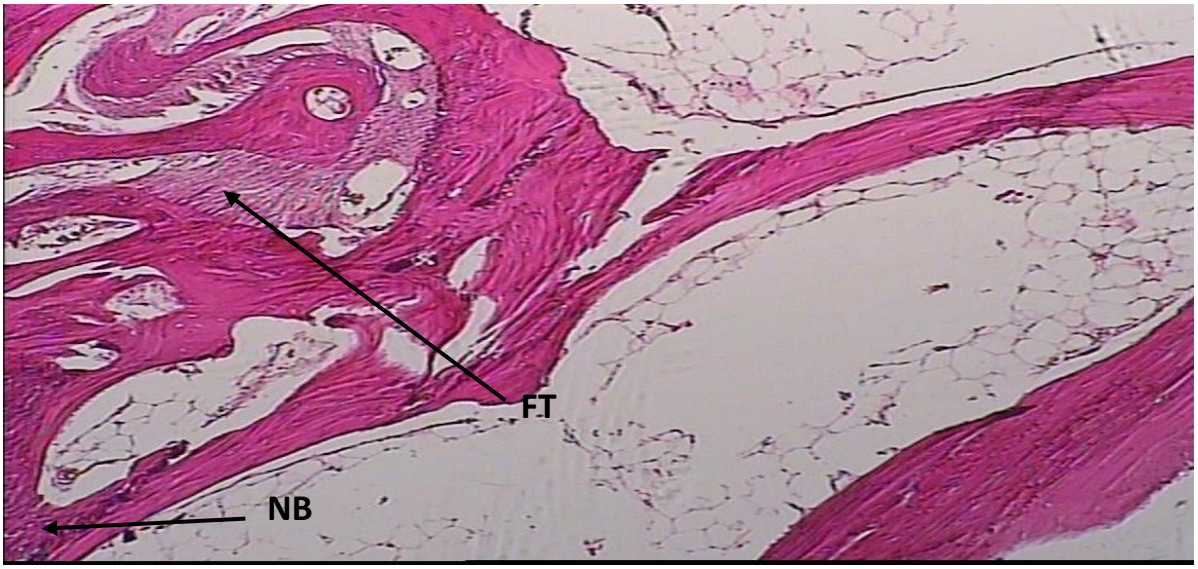

Figure 6. The defect at 16 months. FT, fibrous tissue; NB, new bone.400 X and H& E staining

Table 1. Histological data in different months

	Third month	Sixth month	Ninth month	Twelfth month	Fourteenth month	Sixteenth month
Inflammation	+	+	-	-	+	-
Fibrous tissue	+	+	+	+	+	+
Normal bone	+	+	+	+	+	+
Bone marrow space	-	-	+	+	+	+

Discussion

Various methods are discussed in implant dentistry. One of the most important methods is autograft, but this type of graft has some drawbacks such as additional surgical site and limitation on the amount of graft material. As a result, the other hybrid materials were introduced, which were characterized as being safe, inexpensive and capable of absorbing. One of these materials is calcium sulphate. In addition to the above characteristics, it is biocompatible and can be easily synthesized. Calcium sulfate is used in endodontic surgery, periodontal defect, filling cysts, filling defect remaining after wisdom tooth extraction, regenerative processes and also as a barrier membrane.

Yashikawa et al. (3) used e-PTFE and calcium sulfate in dogs and found that osteogenesis in calcium sulfate was identical to e-PTFE, and it was higher than control group. Pecora et al. (4) in a study using calcium sulfate in through and through lesions and found that the use of calcium sulfate in these lesions is associated with improved clinical results. The results of our study are in agreement with mentioned studies, that bone formation and improvement of the lesions were obvious.

One of the major concerns about calcium sulfate is its rapid resorption. It seems that calcium sulfate gets absorbed completely within 4-10 weeks depending on the blood supply (16). Yashikawa et al. (3) found that calcium sulfate is absorbed completely within 16 weeks. Kelly et al. (17) reported that at six months after surgery, radiographic results showed 99% calcium sulfate resorption. Kelly et al. (17) reported that calcium sulfate cement resorbed completely within 8 weeks. Kim et al. (2) have investigated the injectable calcium sulfate and they reported that the average duration of calcium sulfate resorption is 17.3 weeks. Pecora et al. (18) reported that after 9 months, calcium sulfate was

fully resorbed. The results of our study, was in line with Yashikawa et al. (3), Kelly et al. (17), and Kim's studies (2), but did not agree with the result of Pecora because in our study in the third month, there was no foreign material and it was fully resorbed.

One of the important issues of chemical materials used as bone graft materials or scaffolds is hardness. With the higher degree of hardness, solubility and rate of resorption is reduced. La Gatta et al. (14) in a study to overcome the high resorption rate activated calcium sulfate powder with light and examined solubility of the material. They reported that it caused an increase in the degree of hardness, reduced the absorption rate of calcium sulfate up to five times. Also D'ayala et al. (19) in a study on calcium sulfate granules, concluded that the high degree of hardness increase stability, strength and ductility of the graft material. As stability increases, the absorption time increases and therefore provides enough time for acting as a scaffold (19). Our results for the degree of calcium sulfate granules hardness indicated grade A that is considered as a high and appropriate degree of hardness in engineering and materials science. Synthesis and bone formation in the defect in the third month, not be washed away during surgery, and be easy to pack make our study consistent with the study by other studies (19).

Other issues discussed in the graft materials, are PH and solubility of the materials and their effect on osteogenesis. Guan et al. (20) evaluated the relation between of PH and the solubility of calcium sulfate powder and concluded that reducing PH from 8 to 1.2 increases the solubility. The reason is the effect of PH on increasing the degree of oxygen saturation and probably is due to deposition of calcium sulfate. According to this study, increase of PH, increases size of class calcium sulfate crystals and decreases its solubility (20). Thomas et al. (10) concluded that PH could have a major role in bone formation, so that lower PH related to the solubility of calcium sulfate powder and demineralization of bone. Increase of acidity interferes in the release of growth factors such as BMPs in the lesion and caused difficulty in bone formation (10). The results of our study showed that the solubility decreased with increase of PH that it is consistent with studies by Guan et al. (20) and Thomas et al. (10). Calcium sulfate synthesized in our study, had low solubility in PH range of blood and body fluids (7.35 to 7.45). The solubility, compared with the higher PH (about 10) would change slightly, and it is less than 0.02 mol/lit.

Recently published studies on rabbits showed that in the first month, the lesions which were filled with calcium sulfate, in comparison with lesions that were closed without any substance inside it, showed significant improvements and new bone formation (21). Kim et al. (2) examined calcium sulfate powder and DFDBA for osteogenesis process in the alveolar lesions of dogs. Bone formations were examined after 8 weeks and they concluded there was no significant difference between the two materials. Also, a study by Macneil et al. (22) that compared calcium sulfate powder, HA and bioactive glass (BG) as graft materials in rabbit bone, showed that after one week, the rate of bone formation in calcium sulfate was significantly higher than the other materials investigated. Hing et al. (23) compared beta-3 calcium sulfate, calcium sulfate powder and Si-Cap in rabbit skull, and they found that after 12 weeks, Si-Cap had the best results but calcium sulfate could not attend in bone formation. This could be due to lack of ability in fast bone formation in defect and getting it washed away from the defect during the implant process.

Results of the current study about bone formation by calcium sulfate were consistent with studies by Kim et al. (2) and Macneil et al. (22) and it was against Hing's study (23) which indicated that calcium sulfate bone formation is weak. This is because in our study, histological evaluation showed a positive trend in osteogenesis from the third month. Histological examination showed that the mineral structure of the bone structure was formed well, and extensive areas of bone formation could be observed in the early months, Moreover, in the last specimen healthy and perfect bones were observed.

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