Studying the behavior of calcium **sulfate: bioactivity and solubility in simulated body fluid**

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Abstract / Introduction: *several types of synthetic material have been used to correct bone defects, among which is calcium sulfate.* ***Objective:*** *the present in vitro study aimed at assessing the bioactivity of calcium sulfate in simulated body fluid (SBF).* ***Methods:*** *four specimens were prepared in polyvinyl chloride (PVC) circle matrices by mixing calcium sulfate with distilled water, as recommended by the manufacturer. Samples were immersed in 50 ml of SBF, at 36.5 ºC, for no longer than 21 days. The solution was renewed every three days. Bioactivity was assessed by means of Fourier transform infrared spectroscopy (FTIR).* ***Results:*** *The in vitro bioactivity test, carried out by means of FTIR analysis, revealed the presence of apatite formation over calcium sulfate substrate, thereby proving it to be a bioactive material. In addition, there was significant reduction in the size of the sample, which was associated with the process of resorption.* ***Conclusion:*** *within the limitations of the present study, it is reasonable to conclude that calcium sulfate is a bioactive material which is quickly absorbed.*

Keywords: *Calcium sulfate. Biocompatible material. Fourier transform infrared spectroscopy.*

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INTRODUCTION

Treatment with osseointegrated implants is an alternative to rehabilitate completely or partially edentulous areas, with consistent success rates[1,2] and survival rates ranging from 93.75 to 100%.[1,3] Whenever a tooth is extracted, a process of bone regeneration and remodeling is triggered; as a result, implant placement in adequate position might be hindered, thereby affecting esthetic and functional outcomes of implant-supported prostheses. This corroborates the claim that esthetic and functional outcomes yielded by implant treatment are intimately associated with the maintenance of bone tissue tridimensional structure.[4,5]

With a view to correcting defects resulting from resorption of bone tissue surrounding the extraction site, several types of biomaterial and techniques, such as guided bone regeneration,[6,7] as well as autograft,[8] allogeneic[9] and xenograft material,[10,11] all of which present with different success rates, can be used by the general dentist. The ideal bone substitute should provide an unlimited amount of material without causing damage to the donor site; promote osteogenesis; do not cause immune response from the host; promote fast revascularization; promote osteoinduction and osteoconduction; and be completely replaced by bone in amount and quality similar to the host.[12,13]

Other types of bone substitutes have been studied, among which synthetic and alloplastic material can be highlighted.[14] They are an interesting alternative due to leading to a decrease in morbidity, being unlimited in terms of the amount of material to be grafted,[15,16] and minimizing the potential for disease transmission.[17] Among the several types of synthetic material available, calcium sulfate stands out for presenting quick resorption, biocompatibility and ability to promote osteogenesis[18] and bioactivity.[19,20] In addition to that, it does not induce significant inflammatory response, promotes bone regeneration,[21] and presents with a matrix with osteoconductive properties that allow the proliferation of blood vessels, collagen fibers and bone.[22]

The increasing demand for biomaterial leads to a greater interest in research with a view to finding a bone substitute with excellent physicochemical properties, in addition to providing good success rates. Hence, the present study aimed at assessing the bioactivity of calcium sulfate, a type of material that can be used in bone grafting and repair, as a mechanical barrier or hemostasis agent.

MATERIAL AND METHODS

Preparing the specimens

The matrices used to prepare the specimens were manufactured by making a cross section in a PVC tube 10 mm in diameter and 1-mm high. Subsequently, another matrix, 10 mm in diameter and 3-mm high, was manufactured so as to allow the outcomes to be better assessed.

The matrices were manually filled with small portions of type IV gypsum by the same operator. The gypsum paste was obtained by mixing type IV gypsum powder with distilled water with the aid of a #24 spatula, under vibration produced by a benchtop vibration table (Odonto Larcon, Maringá, Paraná, Brazil). Following the manufacturer's instructions, every 100 g of type IV gypsum (Durone IV, Dentsply, Petrópolis, Rio de Janeiro, Brazil), measured by a digital balance with 0.00001 g precision (Shimadzu AUD220D, Kyoto, Japan), were mixed with 19 mL of water measured in a volumetric pipette.

After final setting time was achieved, water sandpaper was used to make the sample edges plane, so as to obtain regular, plane and parallel surfaces. Soon after removing the specimens from the PVC matrices, the former were cut into two equal parts, so as to obtain semicircle specimens (n = 4). Samples were cleaned by means of the ultrasonic technique and with distilled water for five minutes, stored in a desiccator (Pyrex 200 MM, Charleroi, USA) at 23 ± 2 °C and relative humidity of 50 ± 10%, and controlled by a saturate solution of hexahydrate magnesium nitrate $(Mg(NO_3)_2)*6H_2O$ at 50 ± 10%.

IN VITRO BIOACTIVITY TESTS

Bioactivity tests were carried out with simulated body fluid (SBF) developed by Kokubo et al.[23] This fluid is a synthetic solution of which ionic concentration is similar to blood plasma. Table 1 shows all reagents and respective amount used to prepare 1,000 mL of SBF.

For SBF preparation, all reagents were weighed by an analytical balance (Shimadzu AUW220D) and dissolved in deionized water at 36.5 °C (human body mean temperature) in a polypropylene beaker. This procedure is basically divided into three steps. In the first step, reagents, from the 1st to the 8th (Table 1), are dissolved each one at a time into the solution. In the second step, tris (hydroxymethyl) aminomethane (TRIS) and hydrochloric acid (HCl) are used to adjust the solution to pH 7.4 (blood pH). In the third step, SBF is filtered in a 0.2-μm-pore membrane with the aid of a syringe. After SBF is prepared, it must be stored in a refrigerator at 5-10 °C for no longer than 28 days.

Samples were immersed in 50 mL of SBF, in a polypropylene vial, and kept in thermal bath at 36.5 °C. The solution was renewed every three days (Fig 1).

After 8 and 21 days, the sample with the greatest volume (10.0 x 3.0 mm) while immersed in SBF was removed from the solution and stored in an incubator at 40 °C for future analysis, so as to assess the material bioactivity.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR measurements were carried out in a Vertex 70 V spectrometer (Bruker, Billerica, USA) within a 400 to 4,000 cm^{-1} interval and under 4 cm^{-1} resolution. Each spectrum corresponds to a mean of 64 collected spectra. The method used for measurement taking was transmittance spectrum of KBr pellets (potassium bromide). To this end, the material to be assessed was ground and turned into powder which was mixed with KBr powder in a 2:100 mg ratio. Subsequently, the powder was pressurized under 10 ton and

Table 1. Reagents and respective amount used for SBF preparation.

Order	Reagent	Chemical formula	SBF
1st	Sodium chloride	$NaCl$	8.035 g
2nd	Sodium bicarbonate	$NaHCO_3$	0.355 g
3rd	Potassium chloride	KCl	0.225 g
4th	Potassium phosphate dibasic trihydrate	$K_2HPO_4 \cdot 3H_2O$	0.231 g
5th	Magnesium chloride	$MgCl_2 \cdot 6H_2O$	0.311 g
6th	Hydrochloric acid	HCl- 1M	39 mL
7th	Calcium chloride	$CaCl_2$	0.292 g
8th	Sodium sulfate	Na_2SO_4	0.072 g
9th	Tris (hydroxymethyl) aminomethane	$((HOCH_2)_3CNH_2)$	6.118 g

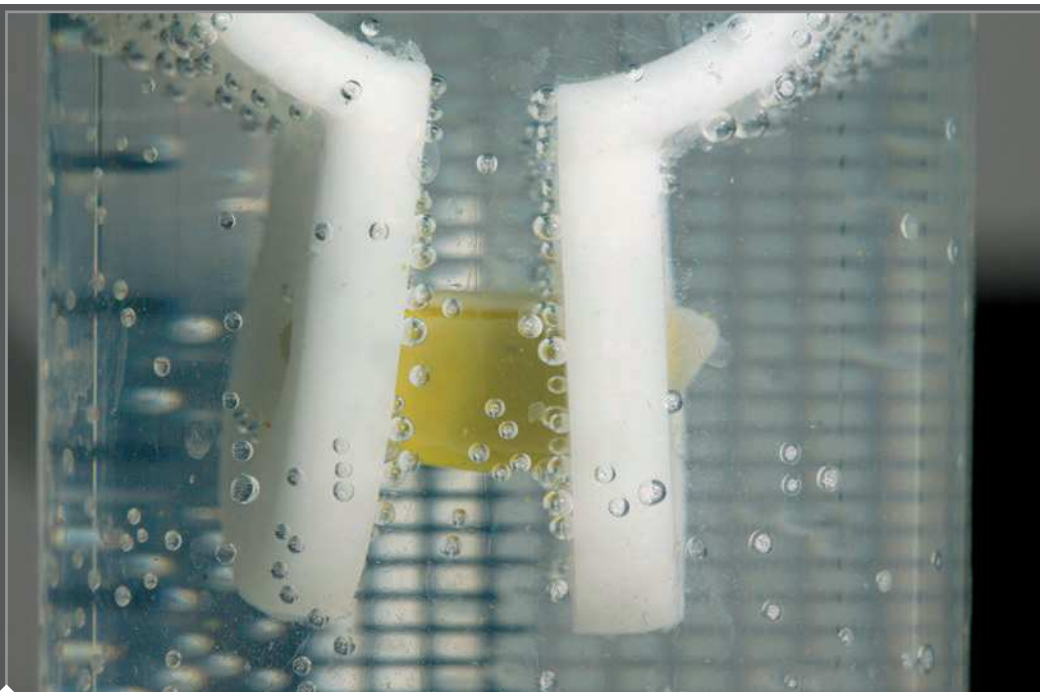

Figure 1. Calcium sulfate sample immersed in SBF at 36.5°C during bioactivity test.

transformed into a compact pellet. The spectrum was obtained by measuring the difference between sample + KBr mixture and KBr spectra. This analysis aimed at assessing the potential for apatite formation after the bioactivity assay.

RESULTS

Eight days after immersion, there was a thick milky layer over the surface of calcium sulfate samples which had been immersed in SBF. Infrared spectroscopy results revealed the presence of bands in the regions of PO_4 vibration modes, thereby suggesting the presence of carbonated apatite over the sample. Figure 3 shows one of the samples that had been immersed in SBF for 21 days. It reveals the presence of apatite and significant reduction in specimen size.

DISCUSSION

Biomaterial has been commonly used in Dentistry; as a result, research has sought to find material with the best physicochemical properties. In bone grafting procedures, the general dentist might use autograft, allogeneic or xenograft material.[9] Synthetic or alloplastic material are also widely available and can be used as bone substitutes, rendering surgical procedures to obtain a donor site unnecessary.[24]

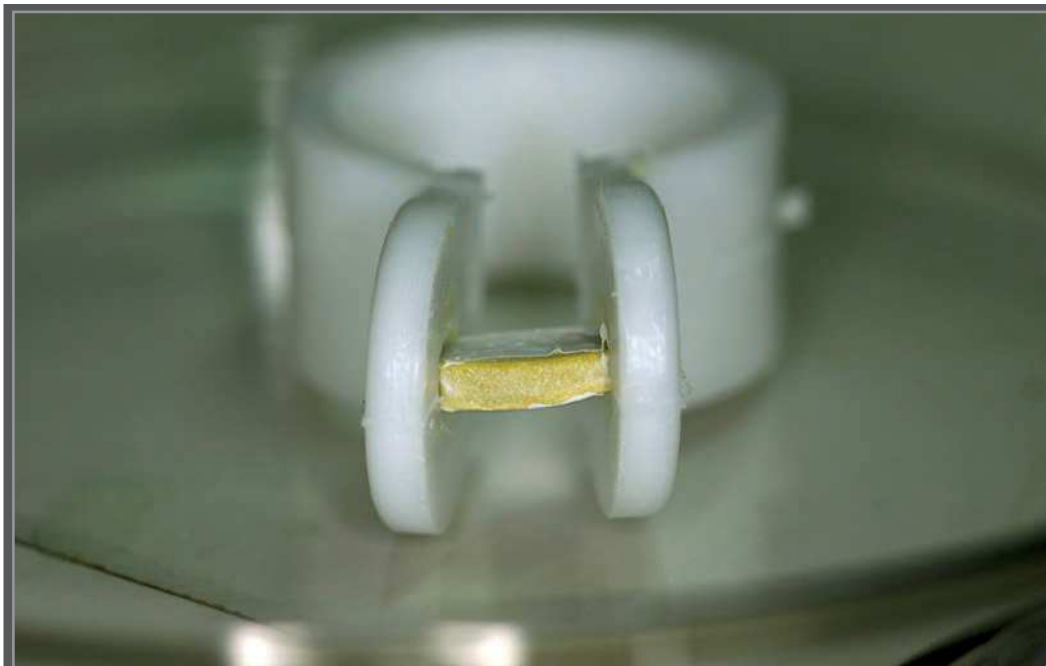

Figure 2. Calcium sulfate sample immersed in SBF for eight days with formation of apatite pellicle.

Figure 3. Calcium sulfate sample and apatite removed from SBF after 21 days of immersion.

Biomaterial should be biocompatible, osteoinductive, osteoconductive and osteogenic, in addition to being able to remain inside one's organism for a sufficient amount of time, so as to be replaced by bone tissue. Moreover, it should be easily manipulated, sterilizable, easily obtained, hydrophilic and inexpensive; it should not act as a substrate for pathogens proliferation, in addition to not being carcinogenic, teratogenic or antigenic.[12,13]

The aim of the present study was to assess the feasibility of using calcium sulfate in bone grafting procedures, such as in fresh sockets, maxillary sinus and filling of other cavities and/or bone defects, as a membrane used to prevent graft material loss. This is because calcium sulfate does not interfere in the healing process.[25] To this end, bioactivity tests were carried out with calcium sulfate combined with water. Calcium sulfate is an easy-to-handle and biocompatible material capable of inducing first intention bone regeneration and facilitating the migration and adhesion of gingival fibroblasts.[26] It can also be associated with other types of material, such as autogenous bone, freeze-dried allogeneic bone, polymers or hydroxyapatite. Importantly, positive effects on its potential for bone regeneration have been reported.[25]

In the present study, a thick apatite layer formed over the surface of calcium sulfate samples, which, therefore, confirmed the high bioactivity of this type of material. In 1991, Kokubo et al[23] reported that for a given type of material to be able to connect to living bone, it essentially requires apatite formation in its surface when in contact with living tissue. In addition, the authors asserted that apatite can be reproduced in simulated body fluid (SBF). Moreover, in the present study, there was significant reduction in the size of specimens, which suggests calcium sulfate to be a highly resorbable material in the assessed means. This claim is proved by the fact that the first samples, with reduced dimensions (10.0 x 1.0 mm), manufactured during a pilot study, were completely absorbed within eight days, with a significant amount of apatite setting down at the bottom of the vial. Bonadio[27] conducted a study with hydroxyapatite-TiO_2 bioactive compound immersed in SBF. The author concluded that the assessed material was bioactive due to having an apatite 13-µm layer formed over its surface. In the present study, the apatite layer formed over calcium sulfate surface, after the material had been immersed in SBF for eight days, was significantly greater, given that it could be seen with the naked eye.

Despite yielding promising results, the present study requires new laboratory and far-reaching studies associated with experimental animal and clinical studies to assess the complete feasibility of calcium sulfate to be used as proposed herein. Additionally, it seems interesting to associate this material with other types of biomaterial, such as hydroxyapatite, since such an association decreases its resorption time within one's organism.

CONCLUSION

The present study evinced that calcium sulfate is bioactive and quickly resorbed in simulated body fluid. It suggests that further studies be conducted in order to assess calcium sulfate *in vitro* and *in vivo* behavior, so as to render its clinical use feasible.

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