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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THE PHYSICAL AND CHEMICAL PROPERTIES OF THREE DIFFERENT ALLOPLASTIC BONE GRAFT MATERIALS USED IN PERIODONTAL REGENERATION

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ABSTRACT

Objective: The aim of the present study was to analyse the physicochemical characteristics of three different types of alloplastic bone graft materials used in periodontal regeneration.

Materials and Methods: The materials used were Monophasic Hydroxyapatite – Biograft HA, Bioactive Glass- Perioglas and Tricalcium phosphate- Biograft TCP. The physicochemical properties of the materials were tested using Scanning Electron Microscopy, Thermogravimetry and Dissolution rates.

Results: Perioglas and Hydroxyapatite showed smaller particle size while Tricalcium Phosphate showed larger particle size. Biograft HA and Biograft TCP showed rough and irregular surface while Perioglas showed a smooth surface. As the three materials were synthetic or alloplastic, there was the absence of lattice water or organic content. The tested materials were stable upto 1000^0C, which shows that they were thermally stable and phase pure and without any impurities. In the dissolution tests done for calcium, all the materials showed positive results. Bioactive glass had more dissolution compared to Hydroxyapatite and Tricalcium phosphate. In the dissolution test for silica, Perioglas showed increased dissolution of silica upto 24 hours.

Conclusion: The alloplastic biomaterials belonged to calcium phosphate and bioactive glass group. It was found that even for those with similar chemical characteristics, significant differences were noted with regard to particle size, surface roughness and dissolution properties. Calcium phosphate based ceramic showed porous surface architecture conducive to cellular and vascular proliferation. All the tested materials were phase pure with no impurities. Increased dissolution of calcium and silica were seen in Perioglas when compared to calcium phosphate.

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INTRODUCTION

Researchers have long been trying to find an ideal biomaterial for use as a bone substitute (Kaufmann, 2000). Autogenous bone grafts are still considered the "gold standard" therapy for bone repair because they contain viable cells which induce osteogenesis, osteoinduction and osteoconduction. But, the main disadvantages of autogenous bone grafts are donor site morbidity, limitations on the quantity of grafted materials and high cost (Al Ruhaimi, 2001 and Conz *et al*., 2005). Allografts and xenografts have complications such as viral transmission and immunogenicity. Hence there is a critical need for the development of bone substitutes that match the properties of bone but without the drawbacks of autografts or allografts, being available in any amount and at a lower cost (Jones *et al*. 2006 and Turhani *et al*., 2005).

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Alloplastic bone substitutes represent a large group of chemically diverse synthetic calcium – based biomaterials like calcium phosphate, calcium sulphate and bioactive glasses (Mats Hallman and Andreas Thor, 2008). Bioactive ceramics include hydroxyapatite, bioglas and tricalcium phosphate. They may be porous or non porous (Bernard, 1991). Depending on how they are manufactured they may be resorbable or non- resorbable. Several alloplastic materials have been used in an attempt to improve clinical conditions and regenerate bone in periodontal infrabony defects. The effectiveness of alloplasts is mainly dependent on the morphology, chemical composition, porosity and particle size of the material. The alloplasts used in the present study were Monophasic Hydroxyapatite (Biograft HA), Tri Calcium Phosphate (Biograft TCP) and Bioactive Glass (PerioGlas). The present study was designed to compare the physicochemical properties of the three alloplastic bone graft materials used in periodontal regenerative procedures in order to acquire a full interpretation of the in vivo performance of these materials.

MATERIALS AND METHODS

Materials Used

The three different alloplastic bone graft materials used for this study were:

- Monophasic Hydroxyapatite- Biograft HA
- Tricalcium phosphate- Biograft TCP
- Bioactive Glass- PerioGlas.

All samples were obtained in sealed vials as provided by the manufacturer and was used without any further treatment in the present study, except for grinding or cutting to a size appropriate for each analysis. All the three materials were tested for chemical contents and trace element levels. The materials satisfied the toxicological safety criteria which have been established through animal studies.

Methods

The physicochemical characterization of the materials were tested at Sree Chitra Institute of Medical Science and Technology, Poojapura, Trivandrum under the following headings:

- Scanning Electron Microscopy (SEM) – to assess the samples' surface topography and particle size.
- Thermogravimetry (TG) – reflects the divergent physical properties and chemical composition among the specimens resulting from their diverse thermal histories.
- Dissolution rates – to find the solubility rate of calcium and silica from the materials tested.

Scanning Electron Microscopy

In the present study, granules were washed with acetone and deionised distilled water, air dried and critically point dried to remove moisture. The topography of the samples were viewed with SEM (FEI Quanta 200 Scanning Electron Microscope).

Thermogravimetry

Thermogravimetric analysis (TGA) uses heat to drive reactions and physical changes in materials. In the present study, thermogravimetry was conducted using SDTQ 600. Platinum or alumina pans were used for weighing the samples. The change in sample weight was recorded while the sample was maintained isothermally at a temperature of interest or subjected to a programmed heating. Here, the temperature was increased from room temperature upto 1000^0C at a heating rate of 20^0C/minute.

Dissolution Rates

Firstly Tris buffer (Hydroxy Ethyl Amino Methane- Sisco Research Lab) was prepared using a magnetic stirrer with hot plate. The pH was 7.3 and the temperature 37^0C. The test materials were ground to a fine powder using a agate mortar and pestle. 150ml of Tris was mixed with 1gm powder and maintained at 37^0C. The measurements were made in a thermodynamically closed system. Aliquots were taken at 10, 30 and 60 minutes. Immediately after taking each aliquot, it was filtered through Millipore Millex-VV filter. Spectroscopic elemental analyses were performed with a Spectro EOS combination sequential coupled plasma ICP spectrometer.

RESULTS AND OBSERVATIONS

Scanning Electron Microscopic Analysis

Monophasic hydroxyl apatite – Biograft HA: Under low magnification (100X), each particle had a size of approximately 100-300 microns (Table 1). Under high magnification (6000X), definite pores were visible. The pores were micro and nano sized and the particles were composed of nano sized crystallites and appeared roughened. The pores had an approximate size of 1µm.

Tricalcium Phosphate – Biograft TCP: Under low magnification, the particle size was approximately 200-300 microns. Under high magnification, pore size was approximately 0.5-1 µm (Table 2). The surface of the particle was almost smooth and sintered in nature.

Bioactive Glass – Perioglas: Under low magnification, the particle size ranged between 100-200 microns and has a smooth surface. Under high magnification (6000X), pores were not visible and the surface of the particles were smooth with less roughness. The sharp surfaces of the particle may be due to the presence of glass particles in it.

Table 1. Particle size by SEM – at low magnification of 100X

Material tested	Size of the particle(nm)
HA	100-300
TCP	200-500
Perioglas	100-200

Table 2. Pore size by SEM – at high magnification of 6000X

Material tested	Size of the pore(nm)
HA	0.1
TCP	0.5-1
Perioglas	Dense

Thermogravimetric Analysis

Monophasic hydroxyl apatite – Biograft HA: At 988.43^0C, weight was 4.899mg and weight% was 97.17%. Under thermogravimetric analysis, HA does not contain adsorbed water/lattice water or any organic phases present in it. So there was a weight loss of 2.83%.

Tricalcium Phosphate – Biograft TCP: TCP did not contain adsorbed water/lattice water or any organic phases present in it. There was a weight loss of 1.95%. At 583.83^0C, weight was 5.044 mg and weight% was 99.66%. At 988.21^0C, weight was 4.962 mg and weight% was 98.05%.

Bioactive Glass – Perioglas: Perioglas did not contain adsorbed water/ lattice water or any organic phases present in it. So there was a weight loss of 0.55% when heated up to1000^0C.

Dissolution Rates

Dissolution rate of Calcium: Dissolution rate of calcium was seen to increase in the three graft materials tested. HT showed dissolution which was time dependent. TCP had increased dissolution than HT. Dissolution was seen to increase up to 4 hours but remains a constant after that. Perioglas showed dissolution to increase up to 24 hours but decreased after that.

Dissolution of Silica: Prioglas showed increased dissolution rate for 24 hours and remained a constant after that.

DISCUSSION

The purpose of this study was to analyse and compare the physical and chemical characteristics of three commonly available alloplastic bone grafts calcium phosphates and bioactive glass. The three fundamental parameters on which the biological performance of a biomaterial depends on are chemical composition, morphology and biodegradability. The analytical methods used to analyze these properties were scanning electron microscopy, thermogravimetry and dissolution tests. Scanning electron microscopy of the biomaterial helps to assess grain size, porosity and surface irregularities. A sufficient pore size and an inter connecting pore structure is necessary for osteoblasts to grow into the graft. It was seen in this study that PG and HA show smaller particles (100-300μm) while TCP show larger particles (200-500μm). The particle sizes of the studied alloplasts are in accordance with studies that have shown that smaller particles (around 300μm) are the basis for better performance when implanted into bone (Cruz, 2006). Surface roughness is known to influence the cell attachment in vitro and in vivo. Studies have shown that irregularly shaped particles produce a greater response than spherical particles (Jui, 1998). Rough apatitic structures enhance osteoclastic attachment compared to smooth ones (Gomi, 1993). In the present study Biograft HA and Biograft TCP showed rough and irregular surface while Perioglas showed a smooth surface. Studies have shown that nanoporous structure improves cell adhesion, proliferation and differentiation (Karageorgiou, 2005). SEM data of the pore size of the tested materials showed that with the exception of PG the other two alloplasts had porous architecture. In the present study as the other two grafts except Perioglas were in granular form, pore interconnectivity is naturally ensured.

The results confirm that the materials tested except Perioglas are within the recommended range for pore size in literature. Thermogravimetric analysis is used to determine the content of water, organic material and mineral (Calcium phosphate). For all the alloplasts tested in the study there was only a minimal weight loss up to 2% which could be attributed to the presence of moisture or due to oxidation of the samples. The tested materials were stable up to 1000^0C, which renders them thermally stable and phase pure without any impurities. In the dissolution tests done for calcium in the 3 alloplastic biomaterials, all the materials showed positive results. Perioglas had more dissolution compared to HA and TCP. This could be explained by the fact that bioactive glass contains silica which shows increased dissolution which was time dependent. The factors during manufacture that affect dissolution include sintering temperature, duration of sintering and the Ca/P ratio in the feed material (Joschek, 2000). In the dissolution test for silica, Perioglas showed increased dissolution of silica up to 24 hours. The dissolution of silica for bioactive glasses is an important step in its increased bioactivity properties. Dissolution affects the osteoconductivity of a material. In a healthy site where rapid regeneration is expected, it is advisable to use a silica based material where as in a pathogenic condition, a material with slow resorption can be used. From the present study it can be concluded that hydroxyapatite based materials like HA and TCP can be used in the treatment of pathogenic conditions. The results are in accordance with the studies by Tadic *et al*. (Tadic, 2004), who did a physicochemical characterization of 14 different calcium phosphate based bone substitution materials. It can be concluded from the present study that next generation biomaterials should combine bioactive and bioresorbable properties to activate in vivo mechanisms of tissue regeneration, stimulating the body to heal itself and leading to replacement of the scaffold by the regenerating tissue.

Conflicts of Interest: The authors report no conflicts of interest in the study.

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