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Research Article

Response of Commercially Available Bone Substitutes When in Contact with Blood - A Scanning Electron Microscopic Study

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Abstract

Introduction: The success of bone grafts relies on a complex sequence of events with a major dependence on vascular ingrowth, differentiation of osteoprogenitor cells, bone remodeling and graft resorption occurring together with host bone ingrowth into the porous coralline microstructure or voids left behind during resorption Clearly, an ideal bone graft substitute should resorb fully and at a predictable rate but also provide a three-dimensional matrix to support bone ingrowth and on growth during resorption. The rationale behind more rapid resorption of alloplasts is related, in part, to new bone formation and decreases the load-sharing environment. The ultimate replacement with the body's own tissue while the implant resorbs needs to be titrated with the rate of new bone ingrowth diagnostic purpose so that regenerative or new bone formation can be assessed radiographically. The degradation of the implant also allows for additional space.

Methods: Nine alloplasts namely Osteogen® (Impladent, USA), Osseomold® (Advanced Biotech, Chennai, India) Pepgen P-15® (Dentsply, USA), Biogran® (Bioactive glass), BioResorb® (Oraltronics, USA), Ortograf-Ld® (HA and Beta TCP), Periobone G® (Calcium HA porous granules), ProRoot® (Dentsply, USA) were selected for the study. The samples were sputter-coated with gold in an ion coater, the morphology was observed and particle size was measured under vacuum by scanning electron microscopy (SEM). SEM analysis provided visual evidence that all examined materials have irregular shape and particle sizes larger than those informed by the manufacturer. EDS microanalysis detected the presence of sodium, calcium and phosphorus that are usual elements of the bone tissue.

However, mineral elements were detected in all analyzed particles of organic bovine bone except for macro cancellous organic bovine bone. These results suggest that the examined organic bovine bone cannot be considered as a pure organic material.

Keywords: Scanning Electron Microscopy; X-Ray Microanalysis; Bone Substitute; Bovine Bone; Human Bone; Hydroxyapatite

Introduction

Regeneration of the lost periodontal structures due to periodontal disease has been a continuing challenge to the dental profession. The goal of periodontal therapy has been eloquently stated as providing a dentition that will function in health and comfort for the life of a patient. The shift in therapeutic concepts from resection to regeneration has also significantly impacted the practice of periodontics since the last quarter of this century. This has led to the development of different approach to therapy to preserve or augment the periodontium. This is an achievable goal and that the dentition can be maintained in a healthy and functional state by using alloplastic materials. Tremendous interest in commercially available alloplasts has emerged from the desire to fill an intrabony or furcation defect rather than radically resect surrounding intact tissue**.** The assumption that their application would manipulate the biological response into a regenerative one rather than a predominantly reparative pattern has rendered the use of these materials an attractive choice in periodontal defects [1]. Alloplastic materials used to reconstruct osseous periodontal defects include ceramics, collagen and polymers. The ideal bone replacement ceramic should be able to trigger osteogenesis. The resorbability and the ability of ceramic to enhance osteoconduction is the impetus behind the development of new substitutes available today [2]. It is imperative that dentists have a secure knowledge of surface properties of alloplasts to be contacted with flowing blood when implanted onto the defect [3]. Adhesion and activation of platelets are important in the thrombosis of blood after contact with ceramic surface and are governed in part by wettability of surface. Since most implanted devices are in contact with blood under flow, it is important to study the effect of wettability of ceramics on the behavior of blood also under flow [4]. The classical estimation of "wettability of alloplasts" by simple contact angle measurements can document their real surface qualities in condition and environment of their intended application.[3] Wettability of a ceramic with blood is influenced by: particle size, interparticulate distance, contact angle, pore size. Smaller particles may be preferable from the standpoint of rapid resorption, greater surface area and enhanced osteogenesis [5-8]. Particles too small in size may induce inflammation, be readily resorbed and result in interparticulate space of reduced dimension that would not be conducive to cellular migration and ingrowth. Particles too large in size will resorb at slower rate leading to reduced surface area [9]. While it is recognized that both the rate of integration and final volume of regenerated bone are dependent on macro porosity there seems to be dispute regarding porosity. The rate and quality of bone integration have been related to pore size, porosity volume fraction, interconnection size and interconnection density [10]. Smaller the contact angle, better able is the blood to fill in the irregularities in surface of ceramic particle. A greater surface to volume ratio could potentially expose more of growth and differentiation factors from blood into matrix of graft and enhance early stages of healing.

Aim

The aim of this scanning electron microscope study was to evaluate the response of nine commercially available ceramics when they come in contact with blood.

Material and Methods

Nine systemically healthy patients were selected who donated blood for the study. These patients had to be free from any systemic disease. An informed consent was taken from all these patients before the start of the study. Nine alloplasts namely Osteogen® (Impladent, USA), Osseomold® (Advanced Biotech, Chennai, India) Pepgen P-15® (Dentsply, USA), Biogran® (Bioactive glass), BioResorb® (Oraltronics, USA), Ortograf-Ld® (HA and Beta TCP), Periobone G® (Calcium HA porous granules), ProRoot® (Dentsply, USA) were selected for the study . All these nine ceramics were placed on nine separate slides. 2 ml of human blood was withdrawn from these patients and was placed in the centre of these slides. Coverslip was placed on all these slides. Then all these slides were placed in silica gel crystals overnight for desiccation of all the slides. All the slides were taken to Department of Metallurgy, Indian Institute of Sciences, Bangalore. and results were analyzed by Scanning Electron Microscope (SEM).

SEM analysis

The materials' particles were fixed on stubs with carbon tape containing powdered graphite (Ceil, São Paulo, SP, Brazil) and sputter-coated with gold in an ion coater (Denton Desk II, Denton Vacuum LLC, Moorestown, NJ, USA). Particle size and morphology were examined under vacuum with a scanning electron microscope (JSM 5600LV, Jeol, Tokyo, Japan). Visual morphological analysis was done using specific software (SEM Control User Interface, version 1.27). Particle size measurements were undertaken in ten particles of each bone substitute, according to the highest longitudinal dimension and were expressed using descriptive statistics.

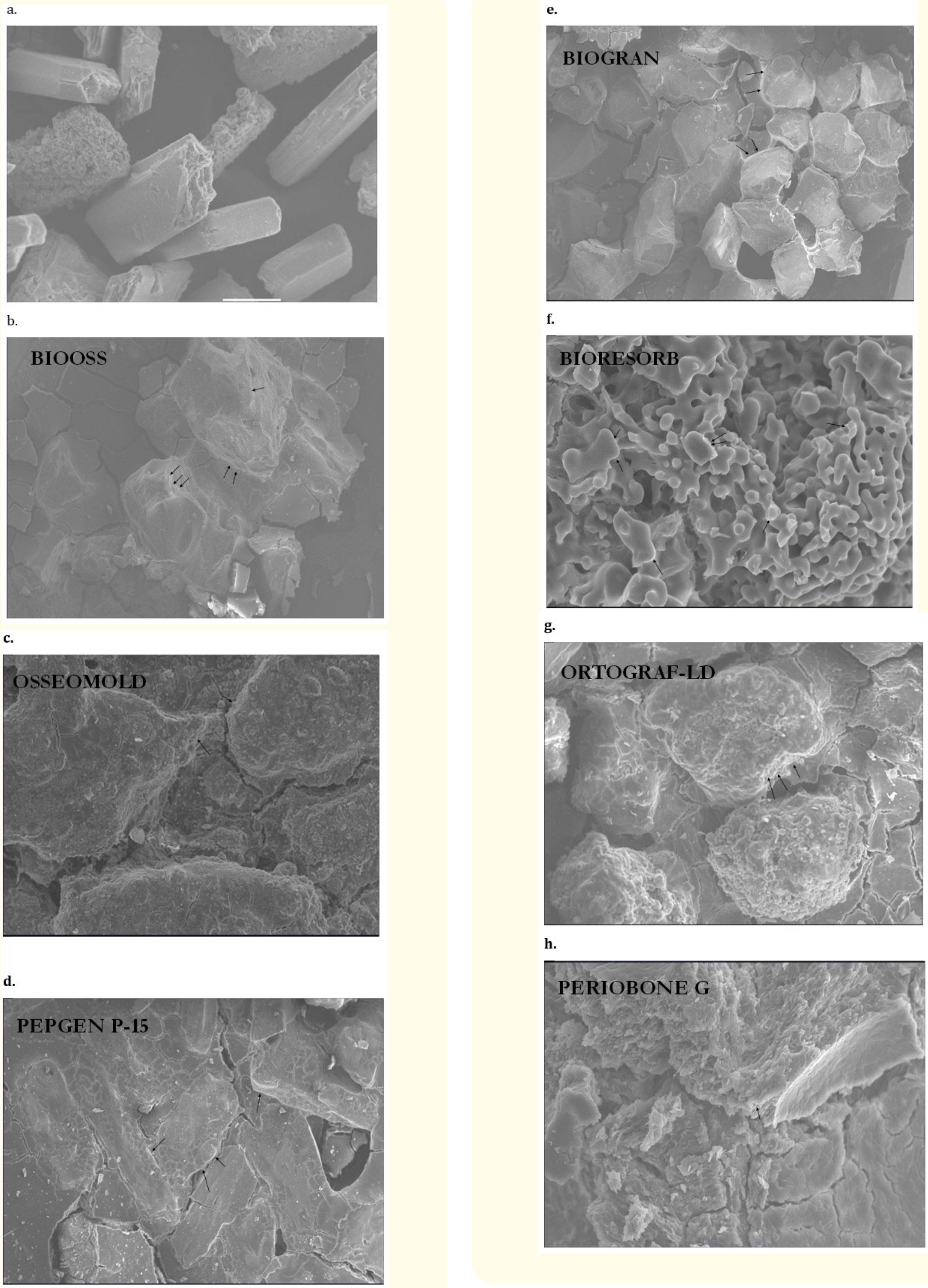
Results

Scanning electron microscope (SEM) analysis

The purpose of this study was to evaluate the response of alloplasts to blood. The analysis was done by three masked examiners. Osteogen bone graft on SEM displayed a lot of mounds and depressions. The interparticulate distance was wide. Bioss displayed lot of cracks and fissures whereas fewer pores were seen. Even Osseomold showed few cracks and grooves. The osseomold surface was not uniform. The Pepgen surface showed smoothness throughout the surface. Cracks and depressions were visible throughout the graft surface. The Biogran surface showed translucency because of the glass particles in the graft. Bioresorb® was shown to be the most wettable amongst all ceramics. Ortograf-Ld® and Bioss® followed next in terms of wettability. Osseomold®, Osteogen®, Biogran® was moderately wettable. Pepgen P-15® showed intermingling of particles. Periobone G® exhibited minimal wettability. Interparticulate distance in Osteogen® reduced.

Discussion

The present study stressed on wettability of blood with various bone allopasts. BioResorb® did exhibit the maximum wettability compared to Bioss® which contains Hydroxyapatite and is supposed to exhibit maximum wettability. Athough the factors that determine ceramic resorbability are still unclear, the chemical compositions of ceramic implant as well as its structural characteristics have been attributed key roles. Depending upon the chemical composition and crystal material structure, ceramic materials may exhibit differential rates of resoption. After implantation; ceramics appear to resorb by one of two different biologic pathways. One pathway is a solution mediated process in which the implant dissolves in physiologic solution, whereas the other is a cell mediated


a.
b.
BIOOSS
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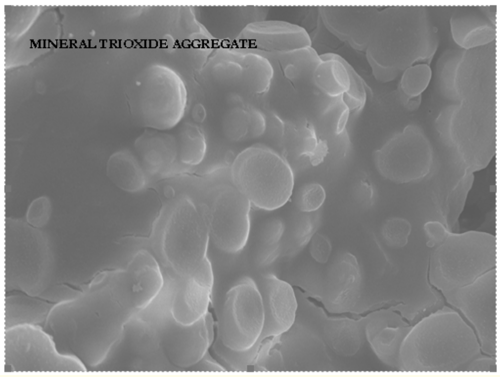

Figure 1: Scanning electron microscopy (SEM) samples of: (a) Osteogen, (b) Bio-oss, (c) Osseomold, (d) PEPGEN P-15, (e) Biogran, (f) BioResorb, (g) Ortograf-Ld, (h) Periobone G (Calcium HA porous granules), (i) ProRoot (Mineral Trioxide Aggregate). The SEM images show different surface texture, particle size and morphology.

process in which phagocytosis of small particle of ceramics occurs [11,12]. Which of these two phenomena is the dominant factor in biodegradation has not yet been determined. Jarcho suggested that the bioresorption rate is directly proportional to their tricalcium phosphate content of the ceramics and that the higher the hydroxyapatite content, the slower the degradation.[12] The resorption of tricalcium phosphate is believed to be mediated primarily by phagocytic mesenchymal cells [13,14]. Concomitant ingrowth of regenerating bone leads to eventual replacement of tricalcium phosphate by reparative osseous tissue [15]. Conversion of ceramic is pivotal to periodontal regeneration first serving as a scaffold for bone formation and then permitting replacement with new bone [16]. The morphology of ceramics have been postulated to their osteoconductive capacity mainly due to the influence of particle size and shape on resorption phenomenon as well as influence of interparticulate space on infiltration of vascular cellular elements and bone formation [1]. Therefore, more the wettability of ceramic with blood more faster blood will penetrate into pores of the ceramic and more faster will be the osteoclastic activity and hence faster the alloplast is replaced by bone. So the ability of blood to wet and adherence of the fibrin clot to the ceramic together has an overall effect on the host bone formation. Regarding the wettability the classical change was seen with Osteogen where the interparticular distance reduced considerably. The principle of interparticular space requirement presumes that that the particles are densely packed together and contact each other on all sides [17]. Even Mazratian., *et al.* have clearly mentioned that evaluated bone replacement grafts seem to yield an interparticulate space large enough for osteoid cells to migrate and for bone to form [18-21]. Though the reduction in the distance between two graft particles can be seen with any other vehicle like saline or water but blood being an autologous source and biological properties of blood makes it superior since it has growth factors responsible for regeneration. At the same time blood will flow into the irregularities of the ceramic particle and provide contact over greater part of surface of the particle. Wettability Studies on Biogran under Scanning Electron Microscope (SEM) are difficult since these are glass particles and the Biogran glass is covered by silica gel layer and calcium apatite layer so the intermingling of particles is not clearly visible. Even Mineral Trioxide Aggregate® a widely used material in endodontics acts like smooth paste on SEM. The main limitations of this Scanning Electron Microscope study were that the pore size, interparticulate pore distance and interparticulate pore size of each ceramic were not assessed. Assessment of interconnected porosity is also important as they act like an organization of vascular channels which can also ensure blood and nutrition supply for bone. At the same time a single ceramic particle can be taken and fractured at its surface and assessed for the strength of fibrin clot using advanced techniques like Transmission electron microscopy and Confocal microscope. Finally, the concept of using porous ceramics to facilitate ingrowth of bone is not without controversy. This is attributable in part to the absence of definitive information about the ultimate fate of bone growing inside the alloplast pores. It is well known that during the remodeling process bone in the area away from the stress is resorbed, whereas bone in an area of abnormally high stress becomes necrotic due to excessive pressure.

Conclusion

To conclude from this study, it was found that BioResorb is the most wettable amongst the nine ceramics tested for their wettability characteristics. This study is a novel study because wettability studies with blood have not been tried and tested. Further studies are needed on this line to assess the efficacy of these ceramics.

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