ASSESSMENT OF BIODEGRADABLE CALCIUM POLYPHOSPHATE FOR BONE SUBSTITUTE APPLICATIONS IN THE HEALING OF THE RAT CALVARIUM

by

Frank Armand Mauro

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Faculty of Dentistry
University of Toronto

©Copyright by Frank Armand Mauro 1999
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author’s permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-45973-X
ABSTRACT

The purpose of this study was to investigate the osteoconductive properties of calcium polyphosphate in particulate form using the calvarium of the rat. In conjunction with the material, a dense Teflon membrane or barrier was included in some animals to assist with retention of the particulate material at the wound sites. Twenty-eight rats were utilized each with an 8mm diameter defect ("critical size"). The animals were grouped as follows; group 1 consisted of 3 control rats in which the created defects remained empty, group 2 had 8 rats in which only a membrane was placed over each defect; group 3 had 8 rats in which calcium polyphosphate particulate was placed into the defects; and group 4 consisted of 8 rats for which both the particulate and dense teflon membrane were used. After a healing interval of 4 weeks all animals were sacrificed and specimens prepared as non-demineralized sections for light microscopic (LM) and backscattered electron imaging (BSEI). Images from the BSEI analysis were assessed using SigmaScan® software which quantified the amount of new bone growth into the defect, and the amount of residual particulate material. The results were submitted to a repeated measures ANOVA and indicated that the use of the particulate CPP material with or without the membrane resulted in a significant reduction in the amount of new bone in growth into the defect compared with the controls or with the membrane-covered defects. There were no statistically significant differences in the amounts of residual material with or without the use of the membrane. None of the 28 calvarial defects entirely healed in the one month period. It was concluded that calcium polyphosphate in particulate form is not osteoconductive in the rat calvarial model. The validity of testing particulate or granular material in such a large and unsupported defect is questionable and may not truly demonstrate the capabilities of tested
materials. Further studies in a more appropriate animal model should be carried out so as to investigate a more compatible environment in which to test the osteoconductive properties of synthetic particulate materials.
ACKNOWLEDGEMENTS

Having completed this thesis, I would like to thank all those involved in this project. Of the many that have been part of this endeavour I wish to thank the committee members which include Drs. Deporter, Pilliar, Kandel, Grynpas, and Filiaggi. These individuals made themselves readily available throughout my work. A special note to Dr. Deporter and his indefatigable editorial assistance.

Many thanks to Nancy Valiquette for her technical assistance and encouragement from the initial laboratory component to compiling of data. I am also very grateful to Robert Chernecky for all the imaging and data collection and Susan Carter who provided a terrific environment to perform the experiments.

I would also like to thank my family who live both near and afar who provided persistent support and encouragement. And last but not least, a thank-you to my wife Emy. Four years ago she understood why it was necessary for me to return to school and followed me to Toronto to pursue my dream. Thank You!
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables and Graphs</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>viii</td>
</tr>
<tr>
<td>1) INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>2) LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>2.1) Autograft</td>
<td>2</td>
</tr>
<tr>
<td>2.2) Allograft</td>
<td>3</td>
</tr>
<tr>
<td>2.3) Xenograft</td>
<td>5</td>
</tr>
<tr>
<td>2.4) Alloplastic</td>
<td>9</td>
</tr>
<tr>
<td>2.4.1) Characteristics of Synthetic Bone Substitute Material</td>
<td>9</td>
</tr>
<tr>
<td>2.4.2) Synthetic Graft Materials Employed in Dentistry</td>
<td>12</td>
</tr>
<tr>
<td>2.5) Conclusion</td>
<td>37</td>
</tr>
<tr>
<td>3) BACKGROUND ON CALCIUM POLYPHOSPHATE</td>
<td></td>
</tr>
<tr>
<td>3.0) Relevant Studies</td>
<td>41</td>
</tr>
<tr>
<td>3.1) Objectives</td>
<td>47</td>
</tr>
<tr>
<td>3.2) Hypothesis</td>
<td>48</td>
</tr>
<tr>
<td>4) METHODS AND MATERIALS</td>
<td></td>
</tr>
<tr>
<td>4.1) Manufacturing</td>
<td>48</td>
</tr>
<tr>
<td>4.2) Animal Model</td>
<td>49</td>
</tr>
<tr>
<td>4.3) Surgical Procedure</td>
<td>51</td>
</tr>
<tr>
<td>4.4) Histological Preparation</td>
<td>54</td>
</tr>
<tr>
<td>4.5) Quantification Method</td>
<td>56</td>
</tr>
<tr>
<td>4.6) Qualitative Assessment</td>
<td>57</td>
</tr>
<tr>
<td>4.7) Statistical Analysis</td>
<td>58</td>
</tr>
<tr>
<td>5) RESULTS</td>
<td></td>
</tr>
<tr>
<td>5.1) Clinical</td>
<td>58</td>
</tr>
<tr>
<td>5.2) Histological</td>
<td>59</td>
</tr>
</tbody>
</table>
6) DISCUSSION 77

7) CONCLUSIONS 89

8) APPENDICES
   1) Terminology 76
   2) One Way ANOVA & Duncan Test 74

9) REFERENCES 90
List of Tables and Graphs

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Available Xenograft Materials</td>
<td>8</td>
</tr>
<tr>
<td>Table 2</td>
<td>Commercially Available Dense Non-Porous Hydroxyapatite Materials</td>
<td>15</td>
</tr>
<tr>
<td>Table 3</td>
<td>Commercially Available Porous TCP and HA Products</td>
<td>19</td>
</tr>
<tr>
<td>Table 4</td>
<td>Coralline and Non-Calcium Phosphate Derived Materials</td>
<td>25</td>
</tr>
<tr>
<td>Table 5</td>
<td>Calcium Sulfate Available As</td>
<td>26</td>
</tr>
<tr>
<td>Table 6</td>
<td>Commercially Available Bioactive Glasses</td>
<td>32</td>
</tr>
<tr>
<td>Table 7</td>
<td>Polymer Material Available As</td>
<td>35</td>
</tr>
<tr>
<td>Table 8</td>
<td>Properties of Commercially Available Synthetic-Bone Substitute Materials</td>
<td>40</td>
</tr>
<tr>
<td>Table 9</td>
<td>New Bone Growth: Individual Means</td>
<td>72</td>
</tr>
<tr>
<td>Table 10</td>
<td>Histomorphometric Measurements of Each Treatment Group at 28 Days</td>
<td>73</td>
</tr>
</tbody>
</table>

<p>| Graph 1a | Mean New Bone (mm²)                                                          | 75   |
| Graph 1b | Residual Particulate Material (mm²)                                          | 75   |</p>
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Schematic structural representation of Calcium Polyphosphate Structure</td>
<td>41</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Prepared defect in rat calvaria with mid-sagittal sinus venous</td>
<td>62 &amp; 62b</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Particulate CPP placed into defect</td>
<td>62 &amp; 62b</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Dense Teflon Membrane placed over graft</td>
<td>62 &amp; 62b</td>
</tr>
<tr>
<td>Figure 5a</td>
<td>Image generated from BSEI</td>
<td>63 &amp; 63b</td>
</tr>
<tr>
<td>Figure 5b</td>
<td>Cropped SEM image</td>
<td>63 &amp; 63b</td>
</tr>
<tr>
<td>Figure 6a</td>
<td>Photomicrograph of control specimen</td>
<td>64 &amp; 64b</td>
</tr>
<tr>
<td>Figure 6b</td>
<td>Higher magnification of newly formed bone</td>
<td>64 &amp; 64b</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Computer image of control specimen with SEM analysis</td>
<td>65 &amp; 65b</td>
</tr>
<tr>
<td>Figure 8a</td>
<td>Photomicrograph membrane only specimen with distinctive new bone ingrowth</td>
<td>66 &amp; 66b</td>
</tr>
<tr>
<td>Figure 8b</td>
<td>Higher magnification of membrane only specimen</td>
<td>66 &amp; 66b</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Computer image of membrane only specimen with BSEI cropped image</td>
<td>67 &amp; 67b</td>
</tr>
<tr>
<td>Figure 10a</td>
<td>Photomicrograph of particulate only specimen</td>
<td>68 &amp; 68b</td>
</tr>
<tr>
<td>Figure 10b</td>
<td>Higher magnification of particulate only specimen</td>
<td>68 &amp; 68b</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Computer image of particulate only specimen</td>
<td>69 &amp; 69b</td>
</tr>
<tr>
<td>Figure 12a</td>
<td>Photomicrograph of membrane + particulate specimen</td>
<td>70 &amp; 70b</td>
</tr>
<tr>
<td>Figure 12b</td>
<td>Higher magnification of membrane + particulate specimen</td>
<td>70 &amp; 70b</td>
</tr>
<tr>
<td>Figure 13</td>
<td>Computer image of membrane + particulate specimen with BSEI cropped image</td>
<td>71 &amp; 71b</td>
</tr>
</tbody>
</table>
1) INTRODUCTION

The development of materials to promote de novo synthesis or regeneration of osseous tissues around teeth and dental implants represents a major challenge in the biomaterials/tissue engineering field. The following literature review serves as an introduction to the many bone graft materials, both of organic and non-organic origin, that are currently commercially available. Emphasis will be placed on the synthetic non-organic materials as a prelude to better understanding the rationale for the present study designed to investigate the osteoconductivity of a new alloplastic calcium phosphate material in an animal model.

2) LITERATURE REVIEW

Bone grafts and bone substitute materials are commonly used materials in dentoalveolar reconstructive surgery. These materials can be used in conjunction with endosseous dental implants as well as in the more demanding environment of intrabony defects associated with periodontal disease.

Several animal and human histological studies (Holmes, 1979; Kenny et al, 1986; Carranza et al, 1987; Schallhorn and McClain, 1988; Gunterberg et al, 1994) have shown that the regeneration of bone and the other periodontal tissues, cementum and periodontal ligament, is possible although generally unpredictable following the use of a variety of bone graft materials. The clinician also may utilize such a graft to act as a template or scaffold for bone formation in such circumstances as pre-prosthetic ridge augmentation in esthetically-demanding situations prior to bridge placement, to augment inadequate alveolar ridges or pneumatized maxillary sinuses, to permit subsequent placement of dental implants, to retain and augment bone in extraction sockets or to cover exposed dental implant surfaces (especially...
with implants placed immediately at the time of tooth extraction). Consistent, predictable and effective new bone formation and in the case of periodontal defects new cementum and periodontal ligament formation, are the ultimate goals whenever such bone graft materials are used.

There are four different classes of bone graft materials. These include: *autografts* which consist of bone harvested from the same individual’s body and transferred to the site of restoration (e.g. iliac crest bone transferred to the oral cavity); *allografts* which comprise material that is procured and processed from another member of the same species (e.g. demineralized freeze-dried human bone); *xenografts* which constitute material harvested from a different species than the recipient (e.g. bovine-derived used in humans); and, *alloplastic* materials which are derived totally from synthesized components (e.g. synthetic hydroxyapatite or polymer)(Ou hayoun, 1996). In the past, the most extensively used materials for bone replacement applications around teeth and dental implants have been autografts and allografts, despite the fact that their use presents potential problems and concerns as outlined below (see Appendix 1 & Table 8).

2.1) Autograft

Autografts or autogenous bone grafts are considered the most desirable form of grafting materials (Boyne, 1984). Autogenous bone retains viability and is composed of both inorganic and organic components including hydroxyapatite, osteocytes, osteoblasts, and various other mesenchymal cells and osteogenic signaling proteins making it the only osteogenic,
osteoinductive and osteoconductive material available according to some authors (Misch et al, 1993) (see Appendix 1).

Drawbacks associated with autogenous bone however include the need for a second surgical site and associated morbidity. As well, quantity and quality of the harvested bone, be it from an intraoral or extraoral site may also limit its use. For example, iliac crest bone when used for periodontal regeneration has been shown to result in extensive root resorption of the treated teeth in conjunction with the formation of new bone (Dragoo and Sullivan, 1974).

Transplanted autogenous bone also varies in rates of resorption depending on the donor site. For example grafts of mandibular bone show slower resorptive activity than bone harvested from the iliac crest (Koole et al, 1989). However at present most clinicians still favor autogenous bone preferably from an intraoral site for periodontal reconstruction and in conjunction with dental implant placement.

2.2) Allograft

Allograft materials, unlike autogenous bone, are readily available from a variety of sources. The most commonly used allografts in the oral cavity are freeze-dried bone allograft (FDBA) or demineralized, freeze-dried bone allograft (DFDBA) both considered to be useful alternatives to autogenous bone depending on the application. Studies published by Urist (1965) and others (e.g. Glowacki and coworkers, 1981; Mellonig, 1981) showed that demineralized bone in animal models demonstrated significantly increased amounts of new bone formation when compared to non-demineralized allograft. The premise Urist initially promoted was that once the harvested allograft bone is exposed to hydrochloric acid for demineralization, biologically active molecules called bone morphogenetic proteins (BMP’s)
which are osteoinductive, become available to encourage bone cell differentiation and new
bone formation at the implanted site. As a result of this work, the use of freeze-dried non-
demineralized bone allograft (FDBA) went out of favor in dentistry and demineralized freeze-
dried bone allograft became the preferred material.

Deminerlized freeze-dried bone allograft has also been reported to encourage
regeneration of the other periodontal tissues i.e. cementum and periodontal ligament (Mellonig
et al, 1981; Mellonig, 1984; Bowers et al, 1985; Bowers et al, 1989). More recent studies,
however (Schwartz and coworkers, 1998) have revealed the lack of consistent osteoinductive
properties of this allograft material. Reasons for this inconsistency include variable donor age
with material from older donors being less osteoinductive, how carefully the material is
processed, the level of residual calcium and the final particle size of the prepared graft. The
effects of variable residual calcium, particle size, donor age and gender were all investigated
using muscle pouches in athymic mice. The results revealed that DFDBA from females aged
31 to 40 or male donors, age 41 to 51 years appeared to contain the highest osteoinductive
potential, while that from both females and males in the 51 to 60 year age group showed the
least osteoinductive potential. The investigators also suggested that the optimal particle size
was 500 to 710 μm while the worst was <250 μm. In addition, the most osteoconductive graft
appeared to contain ~2% residual calcium (Zhang et al, 1997).

Commercial bone banks with few exceptions do not test or indicate the osteoinductive
potential of their demineralized freeze-dried bone products. These suppliers simply screen the
donor’s health history and test the materials for possible disease transmission. While the
consensus still seems to be that DFDBA is a useful graft material, there should be greater attempts to standardize its BMP content (Schwartz et al, 1996).

2.3) Xenograft

A third category of materials, sometimes incorrectly considered as synthetic, are the Xenograft bone materials (Table 1). These materials are generally of bovine origin and undergo a series of processing steps that generally include extraction of all organic components, exposure to virucidal agents, and sterilization (Mellonig et al, 1992). Xenograft materials obviously no longer have viable cells and because they are deproteinized are non-osteoinductive and minimally immunogenic.

The bovine-derived xenografts BIO-OSS® (Osteohealth Co., Shirley, NY) and OSTEOGRAF/N-300® (Ceramed Dental, Lakewood, CO) currently are in widespread clinical use. Both are provided in large and small granular forms to accommodate different requirements. BIO-OSS® is marketed as either a cancellous or cortical-derived particulate form as well as in block form. This material is said to be derived from highly selected and exclusively bred, young herds usually young calves, free of any known diseases. A chemical low heat processing procedure removes all organic components and preserves mineral structure with a calcium phosphate ratio of 2.1:1 and porosity of 75 to 80%, i.e. similar to natural hydroxyapatite.

Bio-Oss exhibits osteoconductive properties with a crystalline structure similar to human bone and is said to resorb within 12 to 24 months based on human histological sections of sinus core samples (Wallace et al, 1996; Hislop et al, 1993). Schmitt et al (1997) in an animal study using critical-sized defects in the radii of 24 rabbits revealed that this material
resulted in more bone at 4 and 8 months than did bioactive glass-treated sites. Berglundhe and Lindhe (1997) investigated the ability of Bio-Oss to regenerate bone in trough-like defects created in one side of the edentulous dog mandible. After three months of healing, dental implants were placed into both grafted and non-grafted edentulous sites in these dogs. After healing, histological results revealed no difference in implant integration between the grafted and non-grafted sides of the mandible confirming the suitability of Bio-Oss for this application.

Fugazzato (1998) describes the use of Bio-Oss in conjunction with implant placement including site development prior to placing dental implants in humans. Ensuring that primary stabilization of the implant was achieved within the available alveolar bone, any exposed dental implant surfaces were covered with this material and subsequently protected with a ePTFE membrane. If implants could not be placed simultaneously with the bone regeneration procedure, separate preparatory ridge augmentation was done with the material and a barrier in preparation for subsequent dental implant placement. Regardless of the approach used, treated sites appeared to perform well with no failures after a number of years of occlusal loading of the dental implants, presumably because the Bio-Oss had promoted bone formation and remodelling at the grafted sites. This study clearly demonstrated the clinical effectiveness of Bio-Oss for ridge augmentation prior to or in conjunction with implant placement.

Valentini et al (1998) presented a case report in which Bio-Oss was used in a sinus elevation procedure to accommodate the placement of three titanium plasma-sprayed implants. At re-entry, often a 6 month healing interval, it was evident that one implant was non-restorable due to its positioning and a decision was made to remove it along with a small portion of the surrounding peri-implant tissues. Histological assessment indicated that the Bio-Oss had
promoted new bone formation along the implant surface but minimal to no resorption was observed at this time. The authors raised concern that the un-resorbed Bio-Oss might impede natural bone remodelling and maturation. Histological studies by others, however, indicate that this is not the case and that residual particles become incorporated into the matrix of the maturing bone without any deleterious effects and in certain cases increase the density of the bone (Peetz 1997; Hislop et al, 1993).

Periodontal application of Bio-Oss was investigated by Clergeau et al (1996) in a study in which Bio-Oss was incorporated with porcine collagen fibers and grafted into periodontal defects created in the dog. The animals were sacrificed at 6, 18, and 36 weeks after the regenerative surgery. The results indicated that sites implanted with the collagen-Bio-Oss material had greater bone regeneration than the control sites. However, no evidence was provided for the regeneration of periodontal ligament attachment to the root surfaces. The clinical relevance of this study is questionable as the investigators provided no controls such as Bio-Oss alone or collagen alone to differentiate the effectiveness of each component. Camelo et al (1998) performed periodontal regenerative surgery on four individuals with anterior single rooted teeth that had “hopeless” prognoses. All four defects were debrided, the bony walls perforated and the defects filled with Bio-Oss. Two cases received an additional slowly resorbing collagen membrane (Bio-Gide) used to isolate the grafted defect. The sites were clinically and radiographically reassessed at 6 to 9 months and bloc specimens retrieved. All clinical and radiographic results showed evidence of increased attachment and decreased pocket depth. Histological assessment revealed that many particles had become embedded into newly formed bone with the range of height of new bone being from 4.2 to 5.3mm. As well, new
cementum formation with collagen fibers orientated perpendicularly to the root surfaces was observed adjacent to the graft material with the length of new cementum ranging from 5.0 to 7.6mm. The 2 sites with the membrane showed increased amounts of new bone formation compared to the areas without a membrane. Realizing the small sample size, the authors concluded that Bio-Oss has the capacity to stimulate new bone formation and that this capacity is increased when used in combination with the collagen membrane Bio-Gide. Obviously more research with Bio-Oss as a bone graft substitute in human periodontal defect is indicated.

Although use of xenograft materials may preclude the need for human allografts with their possibility of disease transmission, the risk remains that other unknown pathological vectors of bovine origin may exist in this form of graft (Ouhayoun, 1996). Whether it is human allograft or animal xenograft-derived material, the public perceives such materials as foreign and is skeptical of their proclaimed safety.

Table 1  
Available Xenograft materials:

<table>
<thead>
<tr>
<th>Brand</th>
<th>Manufacturer</th>
<th>Available as</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Oss® cortical and cancellous</td>
<td>Osteohealth Co.</td>
<td>granular 25-1.0 or 1.0-2.0mm block</td>
</tr>
<tr>
<td>OsteoGraft®/N-300 Ø/N-700</td>
<td>CeraMed</td>
<td>granular 250-420μm granular 420-1000μm</td>
</tr>
</tbody>
</table>

Bovine derived graft material not used in dentistry: Endobon®, Laddec®
2.4) Alloplast Materials

Alloplastic or totally synthetic bone substitute materials have become increasingly more popular in dentoalveolar reconstruction due to the previously mentioned perceived disease transmission concerns with allograft and xenograft materials. Characteristics such as texture, porosity and resorbability will determine which synthetic material is best suited for a particular application. Understanding these attributes will enable the clinician to select the correct material to achieve an intended outcome. Synthetic bone substitute materials include: calcium-phosphate based ceramics (CaP), coral calcium carbonate, non-ceramic calcium phosphates, bioactive silica-based glass (BG), hard tissue replacement (HTR) in polymer form, calcium sulfate, and recently introduced genetically engineered synthetic inductive factors such as cytokines and BMP's.

2.4.1) Characteristics of Synthetic Bone Substitute Materials

The ideal synthetic material is one that is totally replaced by natural bone over a predictable and desirable period of time (Hench, 1986), during which functional stresses are shared by the resorbing implant and developing natural bone. The resorption or replacement of the product once implanted likely will occur by a combination of solution-mediated processes in which the implant dissolves in physiologic solution and/or via a cell-mediated process in which phagocytosis and elimination of small particles of material occurs over time. Hopefully, for both of these avenues of resorption the waste products are harmlessly eliminated from the body. As a result of either of these processes, the degradative by-products must be biocompatible so as not to elicit an inflammatory response from the host during the
regenerative process. An inflammatory response could lead to fibrous encapsulation and failure of the material to achieve complete bone regeneration (Lindholm, 1995).

The likely method of action of all synthetic alloplastic materials (with the exception of inductive factors like recombinant bone morphogenetic proteins) is by osteoconduction alone (appendix 1). Osteoconduction and ultimately bone regeneration are dependent upon an influx of osteoprogenitor cells migrating from the endosteal and periosteal bone surfaces as well as marrow spaces of the recipient bone. This migration requires that graft materials provide a scaffolding system with interconnecting pores (Gross et al., 1982; Misch et al., 1993; Ouhayoun, 1996). The volume, size and character of the interconnected porosity are the result of the different materials and manufacturing processes employed to fabricate the alloplastic material (Klawitter et al., 1973; Jarcho et al., 1981). A dense material with highly packed particles resulting in minimal porosity will lead to minimal bony ingrowth into the material as well as only minimal surface resorption but will provide immediate strength due to its increased density. Increasing the porosity of a structure will introduce more surface area which in turn will make it more susceptible to dissolution or phagocytic activity; however, the resulting decrease in density will also decrease strength (Ferraro et al., 1979; deGroot, 1980; Osborn et al., 1980). Highly interconnected channels, greater than 150 μm in size, appear to provide an optimal environment for bone cell migration and influx of vascular elements providing nutrients for the developing tissues (Klawitter et al., 1971; Jarcho, 1981).

Responses to implanted materials will also vary according to the recipient site. Variations due to the amount of trauma, vascularity and type of existing bone (i.e. trabecular or cortical), and the presence of connective tissue and other bone marrow elements will affect the
rate of degradation and bony ingrowth. Conditions such as an acidic or highly vascular recipient site may cause the material to undergo increased dissolution rates (Mors et al, 1975; LeGeros, 1991; LeGeros et al, 1983; Glowacki, 1992; Misch et al, 1993).

“Bioactivity” has been defined as the ability of a synthetic substitute material to attach to host tissue in an expeditious manner so as to aid in its stabilization and encourage superior bone ingrowth (Hench and Wilson, 1984). In contrast, a “bioinert material” is said to be one which exhibits minimal bonding capabilities to tissue, relying instead on intimate mechanical contact to permit force transfer and one which is not resorbed over time (Misch et al, 1993).

Bioactivity varies between different synthetic materials and is based on the chemistry of the material (Hench, 1995). Examples are the bioactive glasses and glass-ceramics which have the capability of “bonding” to both soft and hard tissue. This phenomena is possible through the formation of a hydroxy carbonate apatite (HCA) layer on the material’s surface in the recipient site. This apatite layer or interface forms a mechanical bond with the developing irregularities of the implant material as ions dissolve into solution and the host bone surface. There has not been evidence to date that there is any form of chemical bond at this interface. The more rapid a material can “bond” to its implant site the less likely it will experience any movement during healing. Micromotion or macromotion of implant material relative to host bone will have deleterious effects, encouraging the formation of fibrous encapsulation during healing, with the subsequent prevention of osteoconduction or osteoinduction (Shimazaki et al, 1985; Rosen et al, 1990; Lindholm et al, 1994).
2.4.2) Synthetic Graft Materials Employed In Dentistry

2.4.2.1) Calcium Phosphate Ceramics

The use of calcium phosphate ceramics in dentoalveolar surgery was first described by Albee in the early 1920's, in the successful repair of a bony defect. It was not until the early 1970's however that this idea became popular. By this time a number of researchers (Jarcho et al, 1976; Denissen et al, 1979; deGroot et al, 1980; Aoki et al, 1981) had been able to develop and bring to market materials termed 'calcium hydroxyapatite' for use in bone repair and augmentation.

Members of this family of materials that are commercially produced include: β-tricalcium phosphate (β-TCP), tetracalcium phosphate (TTCP), oxyhydroxyapatite (OHA), biphasic Ca-P (BCP) which is a mixture of HA and TCP, Durapatite, and Fluorapatite (Jarcho et al, 1976; Hench et al, 1984; Shetty et al, 1991; Rawlings, 1993;). They are all said to have a calcium phosphate composition and crystallographic structure which is very similar to natural bone thus ostensibly making them the materials of choice to investigate and develop.

The two most commonly used and studied compounds in the group are hydroxyapatite (HA) (Ca₁₀(PO₄)₆(OH)₂), with a calcium to phosphate ratio of 1.67, and tricalcium phosphate (TCP) (Ca₃(PO₄)₂) with a calcium to phosphate ratio of 1.5. TCP's are sometimes referred to incorrectly as HA and while these are chemically similar these materials have distinctly different crystal structures with TCP being less thermodynamically stable than HA in an aqueous environment. (Ferraro, 1979; LeGeros, 1988)
Manufacturing of these ceramic products involves the firing of a mixture of nonmetallic minerals or apatite powders. The variations introduced during the initial preparation of the reagents, followed by compression and finally sintering will result in significant chemical and physical differences. Sintering involves the controlled heating of a material so as to cause a thermally activated process with reduction in surface energy being the driving force (Pilliar, 1999). Ideally, this results in a systematic formation of neck-like attachments to adjacent particles with minimal decomposition of the original material to any secondary, unwanted byproduct (Jarcho, 1981). Manufacturers may label the product as “pure HA” for example during plasma spraying but the high temperature processing inevitably produces other CaP phases such as CaO or biphasic CaP, which become incorporated into the material. Any variation in processing and secondary products generated are of great significance as these may affect the level of osteoconductivity, resorbability and rate of resorption/bone replacement occurring. These effects are a result of these other phases being less thermodynamically stable than HA and thus readily dissolving in an aqueous environment (Jarcho et al, 1976; Lemons, 1996). A brief description of the composition and application of the five (2.4.2.1a to e) calcium phosphate based materials follows in order to clarify the characteristics previously described.

2.4.2.1a) Dense Hydroxyapatite (HA)

Dense or solid HA material is produced by compression of HA powder into a given shape at 10,000-20,000 psi to produce a compact, frequently called a “green” state, which is then sintered at high temperatures ranging from 1100 to 1300 °C resulting in a dense ceramic
material with less than 5% microporosity (Jarcho et al, 1976; Shetty et al, 1991). The minimal porosity imparts significant compressive strength but renders the material non-resorbable.

The replacement or repair of skeletal structures in orthopaedic surgery, which in many cases are load-bearing and demand immediate strength and rigidity, is best suited to use of a dense hydroxyapatite with its characteristics of minimal porosity and slow resorption (Rawlings, 1993; Doyle et al, 1994). This high tensile strength, non-porous material is generally stabilized by rigid fixation with surgical templates or screws. This form of material leads to limited osseous regeneration or bony replacement but initiates no untoward reaction by the host (Johnson et al, 1996).

Non-resorbable HA in crystalline form was initially introduced as a means of filling intrabony defects around teeth including furcation defects (Yuha et al, 1989; Yukna, 1993). Although shown to result in superior clinical results when compared to debridement alone, histological evidence indicated healing by formation of a long junctional epithelium and fibrous encapsulation of the particulate material with minimal new bone and periodontal regeneration. In a report of specimens from 4 patients, Froum et al (1982) used HA particulate placed in three-walled osseous defects exceeding 4 mm which were then examined histopathologically at 2 to 8 months after placement. The investigators concluded that the material served adequately as a defect filler and was well tolerated but resulted in no new attachment to the root surface.

The use of dense HA in block form as well as crystalline form has also been used in attempts at ridge augmentation in the fully edentulous arch with the intention of providing stabilization for full dentures. However, because of its non-resorbability it is not appropriate for the later placement of endosseous dental implants (Froum et al, 1982; Misch et al, 1993). The
material provides minimal bone-bonding or osteoconductivity (Frame, 1987), the latter being restricted to the outer surface due to the lack of porosity which also precludes any significant bone ingrowth (Osborn et al, 1980; Aoki et al, 1992). This HA material will generally become encapsulated by fibrous connective tissue if there is relative movement during healing. A list of dense HA products promoted for this purpose is provided in Table 2. The manufacturers do not reveal the other phases of CaP that may have formed during the fabrication of their product nor the percent of HA unless the product is sold as a mixture of HA and TCP.

Table 2  Commercially-Available Dense Non-Porous Hydroxyapatite Materials:

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Manufacturer</th>
<th>Available as</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCITITE HA 2040 and 4060®</td>
<td>Sulzer</td>
<td>block &amp; granular</td>
</tr>
<tr>
<td>ORTHOMATRIX HA-500 &amp; 1000®</td>
<td>Lifecore</td>
<td>granular</td>
</tr>
<tr>
<td>BIOAPATITE® (Durapatite)</td>
<td>Levallois-Perret</td>
<td>granular</td>
</tr>
<tr>
<td>OSTPROVITE®</td>
<td>Feldmühle</td>
<td>granular &amp; block</td>
</tr>
<tr>
<td>OSTEOGRAF/P ® or /D®</td>
<td>Ceramed Corp.</td>
<td>granular</td>
</tr>
<tr>
<td>PERIOGRAF®</td>
<td>Cooke-Waite</td>
<td>granular</td>
</tr>
<tr>
<td>PERMARIDGE®</td>
<td>Ceramed Corp.</td>
<td>bound matrix of 1000μm granules strung on suture</td>
</tr>
</tbody>
</table>
2.4.2.1b) Porous Hydroxyapatite (HA) & Tricalcium Phosphate (TCP)

Porous hydroxyapatite (HA) and tricalcium phosphate (TCP) are possible ceramic alternatives to dense HA, if bony ingrowth is desirable. These two porous materials are fabricated by combining predetermined proportions of calcium and phosphate salts with water at moderate temperatures of 500-600 °C. The resulting CaP product is made into a slurry with H$_2$O$_2$ and then sintered causing O$_2$ release and creation of interconnecting pores of 100µm and greater. Alternatively, naphthalene particles can be mixed with the CaP during the compacting process and later removed during sintering resulting in a porous structure. The porosity of HA or TCP results in a less dense material which is not as strong and rigid as non-porous HA (Klawitter et al, 1971; Shetty et al, 1991; Rawlings, 1993). The resulting poor fracture resistance and low fracture toughness properties make porous HA and TCP in granular and block form unsuitable for sites where the materials are to be placed into immediate function, e.g. weight-bearing sites (Rawlings, 1993). These materials are generally used in granular form so that they can be easily compacted into irregularly shaped defects such as those associated with periodontally-diseased teeth without having to provide strength. Their porosity provides scaffolding and an interconnecting network of pores for cellular and perivascular ingrowth and subsequent bone development (Metsger et al, 1982; Misch et al, 1993). Regeneration of bone therefore is a possibility making the materials useful in circumstances such as grafting tooth extraction sockets later to receive endosseous dental implants, or in the repair of other small bony defects.

A number of studies have revealed that TCP is more resorptive and osteoconductive than porous HA (Snyder et al, 1984). TCP’s superiority in this regard was shown by Johnson
et al (1996) who compared TCP to porous HA and collagen hydroxyapatite (35% TCP, 65% HA with added collagen). The three different materials were placed in 2.5cm defects created in the dog radius. The results indicated that TCP underwent the most resorption by six months and that this degradation had occurred concurrently with the most bone ingrowth. Other studies have indicated that the combination of porous HA with TCP to form porous biphasic calcium phosphate, BCP, results in a material that is more osteoconductive than either material alone (Ellinger et al, 1986; Daculsi et al, 1990; Nery et al, 1992). It is believed that the slower resorbing HA maintains the scaffolding properties of the material for a longer period of time to accommodate the process of new bone formation. Concurrently, the faster resorbing TCP allows the early influx of cells and initiation of bone formation.

Calcium phosphate ceramics such as TCP have been used as bone graft substitutes in treating periodontal intrabony defects and have produced statistically significant reduction in probing depth when compared to debridement alone. Furthermore, the treated sites have more of a tendency to remain stable over time without relapsing compared to sites treated with debridement alone. The literature reports few complications such as an inflammatory host response or sequestration of material (Meffert et al, 1985; Kenney et al, 1986; Saffar et al, 1990). However, both HA and TCP although well tolerated have minimal to no ability to regenerate the other periodontal tissues essential in re-establishing a complete periodontal attachment apparatus (Baldock et al, 1984; Wilson et al, 1992; Fetner et al, 1994). Since the healing pattern associated with porous HA appears to be by repair only and as the long-term clinical implication of a graft material that does not resorb but remains as a space-filler is unknown, such use of porous HA is limited (Mellonig, 1991).
A list of commercially available porous bone substitute materials containing HA and/or TCP is given in Table 3. While all of these materials have bone-bonding capabilities (Wilson and Low 1992) assisting initial stabilization in the first week of healing and promoting some new bone formation, they are best used in situations other than periodontal regeneration procedures because of their failure to regenerate fully the periodontal attachment apparatus.

TCP has also been investigated as a possible graft material for ridge augmentation prior to implant placement. Fugazzatto et al (1998) incorporated equal amounts of TCP with DFDBA or freeze-dried bone (FDB) for the purpose of ensuring space maintenance should the allograft materials resorb prematurely. Both composites of materials were covered with non-resorbable membranes. Although 6 of 131 implants with the TCP/DFDBA material were deemed failures at re-entry, the majority of alveolar ridges had been successfully augmented. The authors did note that residual TCP particles were evident one year after the site development procedure when the endosseous dental implants were placed. They concluded that the use of either of these composite graft approaches was deemed inferior to the use of Bio-Oss which at 7 months post-operatively revealed no residual material and an acceptable ridge form for dental implantation.

Gauthier et al (1999) examined the possibility of maintaining alveolar ridge integrity by injecting a paste formation of biphasic calcium phosphate (BCP) into fresh canine extraction sockets. At 3 months histomorphometric analysis revealed that approximately 20% of original graft material remained in the socket and that there was no statistically significant difference in the amount of new bone between the control and the experimental extraction sites. The authors
concluded that in this extraction model, although the composite calcium phosphate was osteoconductive, it did not appear to contribute to ridge form during healing.

**Table 3** Commercially-Available Porous TCP and HA Products:

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Available as</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYNTHOGRAFT® TCP</td>
<td>Johnson &amp; Johnson</td>
<td>granular</td>
</tr>
<tr>
<td>CALCIRESORB® TCP</td>
<td>Ceraver Osteal</td>
<td>granular</td>
</tr>
<tr>
<td>AUGMEN® TCP</td>
<td>Miter</td>
<td>granular</td>
</tr>
<tr>
<td>TRIOSITE® BCP</td>
<td>Zimmer Co.</td>
<td>porous block &amp; granular</td>
</tr>
<tr>
<td>CALCITITE® HA</td>
<td>Sulzer Calcitek</td>
<td>two ranges of granules</td>
</tr>
</tbody>
</table>

**2.4.2.1c) Coral-Derived Hydroxyapatite**

A third method of production of calcium phosphate ceramic material involves the transformation of natural marine coral. The coral is treated with ammonium phosphate \([(\text{NH}_4)_2\text{HPO}_4]\) under high pressure and temperature. The original three-dimensional porous structure and consistent interconnectivity of the coral is preserved through this ‘replamineform’ or replacement process in which the original calcium carbonate is chemically converted to hydroxyapatite (White et al, 1986; Lane, 1995). Different types of coral have different inherent pore sizes. INTERPORE 200 (Interpore International, Irving, California), for example, is harvested from the Great Barrier Reef as Porites and results in a porous HA material with pore
size of approximately 200\(\mu\)m (Mora et al, 1995). Although from a natural source, this porous HA resembles synthetic porous HA, in that it has slow and unpredictable resorptive properties (Kenny et al, 1986). Proponents of this material promote its similarity to the trabecular structure of human bone. It is available in both block and granular forms allowing intra-operative modification and manipulation to achieve the desired outcome (White et al, 1986).

Short-term human studies include case reports with histological specimens up to a year after the use of Interpore in periodontal defects. The studies concur that Interpore when placed in close approximation to bone will act as an osteoconductive substrate. However, histological assessment reveals no new periodontal attachment and the persistence of Interpore particles dispersed within new bone as well as fibrous connective tissue (Kenny et al, 1986; Stahl et al, 1986). Long-term human clinical and histological studies supporting the regenerative benefits of the material in periodontal applications are lacking. However, researchers have concluded that coral hydroxyapatite is essentially equivalent to synthetic porous HA in all aspects and as such responds in the same manner. The advantage of using Interpore over any other form of TCP or HA as a bone graft material is not evident in the literature. The convenient packaging of Interpore 200 advances its use in block and/or granule form as an implant graft providing an adequate matrix for bone ingrowth, as an onlay for alveolar ridge augmentation or as an interpositional implant for orthognathic surgery in the mandible (White et al, 1986).

Harvesting a natural resource from the oceans is limited by governmental regulations such as the Convention on International Trade of Endangered Species of Wild Fauna & Flora (CITES). Although existing imposed quotas meet the demand for such products, producers have no discretion to increase quotas regardless of future market needs. Another concern with
this form of HA is that the replamineform process i.e. carbonate to HA conversion, is not complete and contaminants or weaknesses in the bulk form may be introduced (White et al, 1986). These partially converted phases of CaP will increase the degradation rate of the Interpore due to their thermodynamic instability.

2.4.2.1d) Coral Calcium Carbonate

INOTEB produces Biocoral 450® (Saint-Connery, France) which is porous calcium carbonate or natural coral in the form of aragonite. The raw coral has all organic components chemically removed. Unlike Interpore however, Biocoral does not undergo the conversion process but remains as CaCO₃. Ouhayoun et al (1992) used surgically created defects in the swine mandible to compare the resorption of coral calcium carbonate to synthetic porous hydroxyapatite. Following a period of 1 year of healing, the coral graft was totally resorbed and replaced with newly formed bone while the HA remained as particles of varying size surrounded by a bony matrix.

A comparative study by Mora et al (1995) was undertaken to investigate the effectiveness of natural coral skeleton (Biocoral®), coral hydroxyapatite (Interpore 200®) and debridement alone in treating periodontal defects in humans. Clinical and radiographic assessment at one year including re-entry of the surgical sites revealed similar defect fill at just under 60% for both materials and 22% fill with debridement alone. Although no histological specimens were taken to confirm that true periodontal regeneration was achieved, the parameters measured indicated that the grafted sites had significantly decreased probing depth and increased attachment levels (Mora, 1995). Results with Biocoral reveal similar bony defect filling abilities as seen with other synthetic materials, while limited histological data reveal less
soft tissue encapsulation and a more predictable and rapid resorbability (Carranza 1996; Ouhayoun 1996). Due to this rapid resorption, its bioactivity capabilities must be questioned and further investigated. Rapid resorption of the material can preempt its ability to initiate bone-bonding and function as a scaffold. Biocoral also has been investigated as a bone substitute material in maxillofacial surgery. Karaca et al (1993) prepared 3.2mm round defects in the mandibular symphyses of guinea pigs. The two control groups had no material in the defects but one had the periosteum replaced over the defect while the other had the periosteum totally resected. The two experimental groups had similar treatments with the periosteum as the control groups but with the addition of Biocoral in both defects. The results showed bone growth as early as 20 days around the graft particles with the group having the intact periosteum and graft material exhibiting the most bone formation. This study does demonstrate the osteoconductive capabilities of the material but does not incorporate a critical size defect nor does it provide histological detail describing the interface between the Biocoral and the bony walls of the defect or the new bone.

There are no long-term reports in humans on the effectiveness of coral calcium carbonate as a bone graft substitute in guided bone regenerative procedures nor in conjunction with implant placement. Calcium carbonate will require more extensive long-term studies and re-evaluation in terms of its osteoconductive capabilities and performance.

2.4.2.1e) Non-Ceramic Calcium Phosphate

Non-ceramic Ca-P formulations differ from the classic calcium phosphate compounds in their fabrication and resulting chemical structure. OsteoGen® (HA Resorb) from Impladent
Ltd. (Holliswood, NY) and OsteoGraf/LD® from CeraMed Dental (Lakewood, Colorado) are made using a relatively low-temperature proprietary process not involving sintering. The materials consist of angular particles forming spherically shaped crystal clusters with each unit having lengths of no more than 600 μm. The precipitation and/or hydrolysis by which the crystal cluster is grown is judiciously controlled to produce a narrow size range of crystal and to promote an optimal resorption rate of 4 to 6 months.

Wagner (1989) reported on a case involving the use of OsteoGen (HA Resorb) as a bone graft substitute in conjunction with restoring lost alveolar bone at the time of removal of periodontally hopeless incisors. At the time of implant placement, 4 months post-grafting, a bone core specimen was harvested revealing formation of woven cancellous bone in intimate contact with mature bone. The histology indicated no intervening fibrous tissue or inflammatory response with residual graft particles adjacent to newly forming bone. The author however did not describe the geography of the bony defect into which the material was placed nor the amount of material incorporated into the defect.

An in vivo animal study by Ricci et al (1992) involved the use of titanium growth chambers with plasma sprayed HA-coated experimental surfaces and were filled with low-density HA particles (OsteoGen). The control chambers had surfaces of roughened commercially pure titanium and no other added materials. All chambers were surgically inserted into the distal femurs in dog. At 6 and 12 weeks the HA-coated, OsteoGen-filled chambers exhibited significantly enhanced bone ingrowth compared to chambers with a roughened titanium surface only. At 12 weeks, radiographic examination revealed that all the HA-coated/OsteoGen-filled chambers were completely bridged with new bone while less than
half of the control chambers showed this complete fill. Further, histological evidence revealed that new bone had formed directly on both the HA lining and the low density particles and this was in contrast to the titanium surface where there was evidence of a thin layer of loose connective tissue on the chamber walls separating any new bone from the titanium. It was concluded that OsteoGen is a biocompatible, osteoconductive material that conducts bone ingrowth. This investigation would be of more value had the investigators placed the OsteoGen material in titanium-only growth chambers. This would eliminate the osteoconductive effect of the plasma-sprayed coating and demonstrated the true effect of the OsteoGen.

In a human clinical study by Corsair (1990), OsteoGen was used as a bone graft substitute in 24 intraosseous defects involving posterior teeth. Following 4 to 6 months of healing the sites were re-evaluated with bone probing techniques to reveal a mean bone fill of 2.26mm and the amount of bone fill ranging from 1.2 mm to 3.7mm. Although there were no controls in this human study, the results are in accordance with other clinical investigations of synthetic materials (Carranza et al, 1986; Yukna 93; White et al, 1986).

Limited human histological and clinical studies infer that OsteoGen® provides predictable bone ingrowth for ridge augmentation as well as periodontal regenerative procedures with little if any development of intervening soft tissue (Wagner, 1992). More studies are required to investigate the full potential of this low density HA as a bone grafting substitute material.
Table 4  Coralline and Non-Calcium Phosphate Derived Materials

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Manufacturer</th>
<th>Available as</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpore 200®</td>
<td>Interpore International</td>
<td>block and granular</td>
</tr>
<tr>
<td>(Pro-Osteon® non-dental)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biocoral®</td>
<td>Inoteb (France)</td>
<td>granular</td>
</tr>
<tr>
<td>OsteoGen® (HA Resorb)</td>
<td>Impladent</td>
<td>granular</td>
</tr>
<tr>
<td>OsteoGraf ®/LD</td>
<td>CeraMed Dental</td>
<td>granular</td>
</tr>
</tbody>
</table>

2.4.2.4) Calcium Sulfate

This material otherwise known as plaster of Paris has been known to orthopaedic medicine for over 100 years and used to assist in bone fracture healing (Peltier et al, 1957). Much of the appeal of calcium sulfate stems from its favorable physical characteristics which include moldability, relative ease of handling, ability to acquire a hardened form, provision of a source of free Ca²⁺ ions, and capability of being combined with other materials as a binder or filler. Calcium sulfate is produced by heating gypsum (CaSO₄·2H₂O) so that it loses 75% of its water and thereby becoming the hemihydrate of calcium sulfate (Peltier, 1961; Beeson, 1981). When it is mixed with water, the resultant paste rapidly solidifies with a slight but clinically insignificant exothermic reaction (Peltier, 1961). It is utilized in dentistry for both tissue exclusion purposes (i.e. as a barrier on top of or mixed with particulate graft materials) and soft tissue support in regenerative procedures but is generally not used as a stand alone graft material. The calcium sulfate slurry binds to graft particles to improve their handling and
reduce their migration (i.e. loss of graft material) from the defect site. Repositioned mucogingival flaps are sutured over the hardening barrier to achieve an effective seal and protect the calcium sulfate from early dissolution. The material is porous and will allow fluid exchange to maintain vitality of overlying flaps while preventing epithelial migration into the grafted defect (Sottosanti, 1995). The material, however, cannot be left exposed to the oral cavity as this will encourage bacterial ingrowth and compromise the grafting procedure.

Available as CAPSET® from Lifecore Biomedical, (Chaska, MN) calcium sulfate will resorb within 4 to 6 weeks of placement. Sottosanti (1992) describes an initial learning curve in manipulating this material due to its fast-setting nature and suggests presuturing the overlying flap. Excessive bleeding at the site also will make for difficult placement and containment as the additional fluid may cause some wash-out prior to flap closure.

Lifecore Biomedical also provides HAPSET®, which is a porous, non-resorbable hydroxyapatite premixed with the powder component of the calcium sulfate. As with the Capset this material is hydrated and placed into the osseous defect without the need for an additional barrier.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Calcium Sulfate available as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand name</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>Capset®</td>
<td>Lifecore Biomedical</td>
</tr>
<tr>
<td>Hapset®</td>
<td>Lifecore Biomedical</td>
</tr>
</tbody>
</table>
2.4.2.2) Biologically Active Glass

The bioactive glass used in dentistry is known generically as 45S5 Bioglass. It is commercially available as: ERMI® from US Biomaterials Corporation (USB), PerioGlas® also from USB distributed by Block Drug, and BioGran® from FBFC International, (Dressel, Belgium)(Table 5). These graft materials have an amorphous structure with a composition of 45% silicon dioxide (SiO$_2$), 24.5% sodium oxide (Na$_2$O), 24.5% calcium oxide (CaO) and 6% phosphorous oxide (P$_2$O$_5$). The differences appear in the fact that PerioGlas® has a granule size in a large range of 90 to 710µm while BioGran® has a smaller size range of 300 to 355µm. The ERMI® or Endosseous Ridge Maintenance Implant is marketed as a solid cone-shaped bioactive glass insert, which is intended for placement into fresh extraction sockets in an attempt to prevent alveolar ridge collapse (Kirsch et al, 1994).

The developers and manufacturers of these materials stress that the rapid bonding of 45S5 bioactive glass to both hard and soft tissues is the prime characteristic conveying its ‘osteoproductive’ capabilities. The makers point out Bioglass® is not osteoinductive nor is it totally dependent upon osteoconduction for bone regeneration. US Biomaterials coined the phrase ‘osteoproduction’ to describe the ability of the bioglass to cause osteogenic cells that contact its surface to proliferate quickly and form bone on a glass substrate. This is in contrast to the typical mesenchymal cell ingrowth followed by slower development of bone seen with other CaP materials.

The composition of bioactive glass, which was formulated by Hench in 1969, results in a surface layer that interacts with the surrounding tissue fluids rapidly forming an apatite layer (Gross et al, 1982; Hench et al, 1982). Once implanted, bioactive glass reacts with bone by
rapid ion leaching, loss of soluble silica to the solution and subsequent formation of a silica gel on the surface of the glass particle. This silica gel layer which develops within minutes after exposure to body fluids has been shown by Damen and TenCate (1989) to accelerate the precipitation of an amorphous calcium phosphate phase on the surface and within the pores of the silica gel. Although initially amorphous, the CaP-rich layer crystallizes into mixed hydroxyl-carbonate apatite agglomerates within 7 to 10 days. When the apatite phase crystallizes in the presence of forming collagen fibers, the collagen becomes structurally integrated within the apatite agglomerates as an amorphous cementing zone on the CaP-rich layer. Further steps in bone development and bonding are controlled by osteoblasts at the site (Hench & Clark, 1982). Osteoblasts also have been shown to provide the collagen and ground substance for the primary "bond".

Altering the percentage of the components of a bioactive glass will result in slightly different products which can be categorized into four types, Class A through to Class D. Class A or bioglass 45S5, which includes the materials mentioned above, bonds quickly and efficiently to bone and soft tissue (i.e. has a high bioactivity index). This material does not possess the flexural and tensile strength necessary for load-bearing applications. Its relatively low fracture toughness prevents its use in monolithic form and restricts its general application to low load situations such as a particulate graft in periodontal applications and for middle ear prostheses (Hench et al, 1993).

Class B bioglass has a higher component of silicon dioxide (SiO₂), (≥ 54% as compared to 45% in Class A bioglass). As a result, this material requires two to four weeks to form a bonded bone interface and does not form a bond with soft tissues (lower bioactive index).
(Wilson et al, 1990). However, Class B bioglass has a higher fracture toughness, higher flexural strength as well as a higher elastic modulus than Class A and therefore approaches the necessary mechanical properties required of a load-bearing prosthesis (Hench, 1995). It has not found applications in dental and maxillofacial surgery. Examples of this material include Ceravital® or Cerabone®, which are used for spinal and iliac crest repair (Hench & Wilson, 1995). Class C and D materials have >60% silicon dioxide content, do not bond to tissue and are nearly bioinert materials.

In a comparative study using monkeys with surgically created periodontal defects, the investigators (Fetner et al, 1994) tested Class A 45S5 bioglass, a Class B particulate hydroxyapatite (HA), and tricalcium phosphate (TCP). Results revealed that the Class A bioglass led to a more rapid and complete repair of the defects including significantly more new attachment and little or no epithelial downgrowth.

In a human clinical trial in 20 patients having 44 periodontal intrabony defects, Zomet et al (1997) used PerioGlas® for half the surgical sites and compared these to debridement alone. At one year, standardized radiographs revealed significantly improved bone density for defects treated with the PerioGlas® when compared to the surgical debridement alone. Clinical examination revealed both treatment modalities resulted in significant improvement in probing depth and attachment levels which were not statistically significantly different. No histology was performed to examine whether new attachment was achieved with the PerioGlas® or whether the improved density was merely due to retained PerioGlas®. A similar human study again compared bioactive glass with open flap debridement in treating periodontal defects (Froum et al, 1998). A 12 month re-evaluation involving surgical re-entry but no histology
revealed significant improvement in clinical parameters including statistically significant reductions in probing depth, less recession and higher clinical attachment level gain in comparison to the flap debridement alone.

Studies with Biogran® investigated the potential advantage of smaller and consistent grain size for bioglass A. Schepers and Ducheyne (1997) used Biogran® in the partially edentulous jaws of beagle dogs to study bone growth around bioactive glass of various grain sizes and to compare it to various hydroxyapatite particles. After a minimum of three months and a maximum of 24 months of implantation the 300-350µm grain size demonstrated internal erosion with new bone formation within these protected pouches. By assessing the amount of new bone and osteoid the authors concluded that this particle size range showed better osteoconductive properties of the particles, better differentiation of osteoprogenitor cells to osteoblasts subsequent to excavation of the particles and enhanced bone ingrowth compared to the same material of grain sizes 212-300µm, 425-800µm, and 100-710µm. Other materials tested such as HA from Interpore 200® and Calcitek® did not show this excavation and pouch effect nor the same degree of osteoconduction as the 300-355µm bioglass.

Bioactive glass has been successfully used for the grafting of surgically created cystic defects, alveolar bone defects as well as for augmenting extraction sites. Schepers et al (1993) reported on a clinical study involving 106 of such bony defects in 87 patients. No histology was performed, but clinical examination revealed satisfactory to good ridge augmentation and preservation with minimal complications, none of which were related to the bioglass.

Another human clinical study involving ridge preservation with bioactive glass cones was performed on 16 adults (Yilmaz et al, 1998). Three different conditions were treated
including short span edentulous ridges that had undergone resorption due to a previous
eextraction (treated by splitting the ridge and inserting bioactive glass cones and particles) using
the Endosseous Ridge Maintenance Implant (ERMI), fresh extraction sockets treated
immediately with bioactive glass cones (ERMI), and fresh extraction sockets left empty. At 12
months post-surgery alveolar ridge dimensions were measured and compared to the pre-surgical
models. Both experimental conditions resulted in statistically greater alveolar bulk both
vertically and horizontally compared to the non-grafted sockets. However, at one year post-
operatively, the bone-splitting approach did reveal some loss of alveolar ridge dimension of
approximately 0.6mm while the fresh extraction socket revealed a loss of only 0.1mm. The
control site lost on average 0.75mm of horizontal bone and 1.35mm vertical height.

Sinus elevation procedures by the lateral wall approach were carried out on 25 patients
with the use of BioGran® as the bone graft material (Furusawa et al, 1997). Biopsies were
taken at 7 months post-surgically to assess hardness and bone ingrowth. The microhardness of
the newly formed bone was determined to be close to that of existing bone. Histological
sections revealed bone ingrowth enveloping the granules as well as into the excavated cores
within the granules. The authors noted that BioGran possessed this unique dual process of bone
formation distinguishing it from other synthetic materials.

Bioactive glass also has been investigated as a bone substitute material used in
conjunction with bony defects around dental implants (Hall et al, 1999). In a animal model
involving dogs three wall defects (3mm x 5mm x 5mm) were created on the mesial aspects of
previously integrated dental implants (TPS Calcitek 3.25x10.0mm). Thirty-two implants had
either PerioGlas®, BioGran®, canine demineralized freeze-dried bone (cDFDBA) or nothing
placed within the defect. At four months, the histological assessment revealed that the most bone-to-implant contact in the defect area was observed with the canine demineralized freeze-dried bone. The bioactive glasses produced results similar to the control sites and had significantly less bone ingrowth. Similar inferior results for the synthetic materials were reported for percent bone height fill and total area of new bone.

Other studies question the real advantage in using bioactive glass over other available materials. Schmitt et al (1997) compared bioactive glass 45S5 (PerioGlas®) to bovine bone mineral (Bio-Oss®) in a critical size defect created in rabbit radii. The amount of new bone was significantly greater at both 4 and 8 weeks in the Bio-Oss-treated group.

To date, there is no human histology that confirms significant new periodontium formation following the use of 45S5 bioactive glass in intrabony defects.

Table 6 Commercially-Available Bioactive glasses:

<table>
<thead>
<tr>
<th>Brand</th>
<th>Manufacturer</th>
<th>Available As</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERMI®</td>
<td>US Biomaterials</td>
<td>cone-shaped solid</td>
</tr>
<tr>
<td>PerioGlas®</td>
<td>US Biomaterials</td>
<td>particulate 90-710μm</td>
</tr>
<tr>
<td>BioGran®</td>
<td>Orthovita</td>
<td>particulate 300-355μm</td>
</tr>
</tbody>
</table>

2.4.2.3) Organic & Inorganic Composites

A non-resorbable polymer material has been promoted as an alternate to calcium phosphate materials for dentoalveolar reconstruction. HTR™ (Hard Tissue Replacement) polymer is manufactured by U.S. Surgical Corp. for Bioplant (New York, NY) and is
distributed by Septodont. It is a porous calcified plastic derivative with a negative surface charge (-8 to -15mV), the latter meant to assist in attracting cells and repelling microorganisms (Ouhayoun, 1996). This synthetic alloplast is a bioinert composite that is the result of combining polymethyl-methacrylate (PMMA) and polyhydroxyl-ethylmethacrylate (PHEMA). A calcium hydroxide coating is applied to the exterior and this forms a calcium carbonate apatite when hydrated with blood or tissue fluids. The patented process results in individual spherical particles each with a hollow, open-ended, egg shell-like structure. An external pore opening of 300 μm permits access to a 600μm diameter internal chamber. The effect when placed into a defect is to create an interconnecting porosity of 300-350μm diameter between the spherical bodies permitting tissue invasion. With its thin and hollow architecture, and approximately 90% porosity, HTR has been assessed as having a compressive strength of 50,000 psi (Boyne, 1995). The rationale behind this material is that the calcium hydroxide surface layer will interface with bone cells encouraging direct bone apposition, and subsequent bony ingrowth will occur into the inter-connected spaces amongst the spheres as well as into the open-ended globes of the extremely slowly resorbing material. (Epply et al, 1990; Ashman, 1991). The manufacturers and clinicians who employ this material claim that it does not require a membrane to protect it during healing as it has an inherent occlusive effect for non-bone cells (Ashman, 1985).

Septodont also distributes this polymer alloplast as HTR24®, a macro-mesh with a pore-size of 750μm, for larger defects such as ridge augmentation or ridge maintenance at time of extraction, or as HTR40®, a micro-mesh of pore size of 350 - 500μm for periodontal defects or around exposed implant surfaces (Haris et al, 1998). The material has gained in
popularity due to its ease of handling, radiopaque character and demonstrable therapeutic value as described in the following investigations. Boyne (1995) described its use in extraction sockets in monkeys. His investigation revealed no infection or soft tissue flap dehiscence and, histologically, for 10 month specimens, continuous remodeling with new bone formation at the alveolar crest.

Stahl et al (1990) reported on the use of HTR in 11 periodontal patients with intrabony lesions at post-op periods varying from 4 to 26 weeks. The clinical observations included reduction of pocket depth, which consisted of both gingival recession and clinical attachment gain. Histological samples revealed graft particles at the surgical site which were encapsulated by connective tissue and minimal bone formation at the periphery of the particles. Four of the 11 block sections did show evidence of new attachment in relation to previously placed notches in the root surfaces.

In a split-mouth design study Shamiri et al (1988) treated 15 individuals with bilateral intrabony defects of 5 to 9mm depth. HTR was placed in the defects on one side of the jaw while debridement alone was carried out on the contralateral defects. At three months clinical assessment revealed that pocket reduction was similar while clinical attachment gain was slightly better with the HTR at 3.0mm as opposed to 2.1mm with the control treatment.

Other clinical studies of 1 to 5 years duration (Ashman, 1991; Ashman & Bruins, 1985; Yukna, 1994; Biber, 1992) suggested acceptable bone preservation, osteopromotive and biocompatible properties. Well-controlled studies including split-mouth design and histological sampling of sites (Amler and LeGeros, 1990; Szabo et al, 1997; Haris et al, 1998) were used to investigate the use of this material in large osseous defects such as those
associated with tumor resections or sinus lift procedures to later accommodate dental implant placement. Histological assessment showed bone formation within the original defects but soft tissue encapsulation and no new bone where the HTR material had extended beyond the defects. The sinus lift procedures resulted in sufficient bone formation to stabilize subsequently placed implants. Although these studies revealed favorable results with the use of HTR, the small number of cases preclude the general acceptance of this material.

Some concern over the use of the monomer component of the graft material has encouraged the biopsy evaluation of longer healing intervals in previously treated HTR sites. These limited histological investigations of treated patients revealed one potential problem in that a chronic inflammatory cell infiltrate was seen where unresorbed HTR particulate material remained at 7 and 10 months. (Kwan et al, 1990). Clinically the authors described the overlying soft tissue as appearing healthy and without any indication of the underlying histologic chronic inflammation.

Although HTR® has been used as a bone substitute in ridge augmentation and preservation, sinus elevation procedures, and periodontal defects many clinicians are hesitant to incorporate it into their practices because of the unpredictable resorption pattern and unknown long-term effects of degradation byproducts.

**Table 7**  Polymer material available as:

<table>
<thead>
<tr>
<th>Brand</th>
<th>Manufacturer</th>
<th>available as</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTR® 24 &amp; 40</td>
<td>U.S. Surgical Corp. for Bioplant</td>
<td>hollow spheres of 500-750 μm</td>
</tr>
</tbody>
</table>
2.4.2.5) Biological Materials: Inductive Factors

One of the main limitations of synthetic calcium phosphate graft materials is that they lack osteoinductive capabilities and therefore there is impetus to use them in conjunction with osteoinductive substances added to induce new bone growth. Much research is being directed towards identifying and reproducing biological mediators that may promote wound healing and formation of bone. The importance of compounds such as bone morphogenetic proteins (BMPs) (Urist, 1965), platelet-derived growth factor (PDGF), and insulin-like growth factors (IGF-I) have become increasingly apparent in bone regeneration (Bowers et al, 1991; Sigurdsson et al, 1995). In an attempt to harness the great potential of these substances, biotechnological companies have developed genetically-engineered human proteins to mimic the naturally occurring proteins.

Very near to becoming commercially available, recombinant human bone morphogenetic protein-2 (rhBMP-2) (Genetic Institute Inc.) has shown very promising results in both animal models and limited human studies. Nevins et al (1996) implanted a collagen sponge impregnated with rhBMP-2 into the maxillary sinus of goats. This treatment induced rapid and extensive bone generation in comparison to no growth at the control site, which received the carrier collagen sponge only. A similar human clinical study by Boyne in 1997, revealed an overall mean height response of 8.51mm for the maxillary sinus floor in the 12 individuals participating. Schwartz et al (1998) implanted what was predetermined to be ‘inactive FDDBM’ in combination with rhBMP-2 and compared this to ‘active FDDBM’ and ‘inactive FDDBM’ alone in an intra-muscular rat model. The rhBMP-2 in combination with
the inactive FDDBM induced significant bone formation which was dose-dependent. Sites treated with the highest concentration of rhBMP-2 resulted in a 9-fold increase in amounts of new bone compared to that produced by active FDDBM. In marked contrast inactive FDDBM failed to produce any evidence of bone formation.

In a previously mentioned investigation, a newly developed calcium phosphate cement was combined with a polymeric cellulose carrier and injected into fresh extraction sockets in dogs (Gauthier et al, 1999). The purpose was to observe the potential for this graft to support new bone formation in fresh extraction sites. Although the control had similar amounts of bone fill, the novel graft material demonstrated osteoconductive and biocompatible properties which with the injectable property could potentially lend itself to being a carrier for inductive proteins as well.

Future development of such substances will require further human histology and a longer period of post-operative observation including the response of the regenerated bone to functional loading such as that applied by dental implants inserted into the grafted sites.

2.5) CONCLUSION

The difficulty in achieving acceptable and predictable levels of new bone and periodontal tissue regeneration has resulted in an ongoing quest for the ideal synthetic graft material. Biocompatible ceramics such as tricalcium phosphate, hydroxyapatite, bioactive glasses, polymers and natural coral all have been evaluated in human intrabony defects and elsewhere with inconsistent but generally positive and partial clinical improvements. The porous nature of many of these substances in particulate form potentially provides mechanical
support for bone ingrowth but no inductive influence for bone regeneration. (Table 8 for list of materials)

Considerable variation exists within the limited human clinical studies conducted using these alloplastic materials with respect to controls, assessment techniques, re-entry procedures and histological data. Consequently it is difficult to form definitive conclusions as to the relative efficacy of alloplastic implant materials in the management of periodontal defects and guided bone regenerative procedures. Reviewing published clinical and laboratory data, general comments and observations can be made concerning the use of these materials including; 1) the fact that although all these materials appear to be biocompatible, non-antigenic and non-inflammatory, autogenous bone remains the gold standard in treating osseous defects; 2) although clinical closure and defect fill is generally similar with most types of graft materials, regeneration of periodontal tissues is least likely to occur with alloplastic materials; 3) although new bone formation may be associated with some alloplastic materials, there is no evidence that these materials are osteoinductive or capable of stimulating new periodontal connective tissue attachment; 4) no current alloplastic implant material is superior to any other in treating periodontal defects, their use generally resulting in defect fill, stabilization of the remaining osseous structure, clinical attachment gain and decreased probing depth; 5) healing usually includes one of or a combination of the following, fibrous tissue encapsulation of the granules, bony ingrowth into the material, slow resorption of the material, and formation of a long junctional epithelium and/or connective tissue adhesion without the regeneration of a true periodontium.
Bovine-derived bone mineral or Bio-Oss in the very limited studies done in humans to date has revealed some potential in regenerating periodontal tissue (Camelo et al, 1998). Bio-Oss has also been shown to be superior to current alloplastic materials in regenerating bone in guided bone regenerative procedures such as ridge augmentation and immediate implant placement. The current data suggest no reason why any alloplastic material would be chosen over a bovine-derived bone powder for dental bone grafting procedures. Moreover, Bio-Oss produces significantly better clinical results in terms of bone volume and compressive strength than allograft material in situations of ridge augmentation (Fugazzato, 1998; Valentini et al, 1998).

The advent of molecular biological and in particular recombinant DNA techniques has substantially increased our understanding of molecules needed to induce bone formation. These methodologies have permitted the production of large quantities of growth proteins and this approach shows significant promise in regenerating bone tissue in particular.

Unquestionably, genetically-engineered substances such as recombinant human bone morphogenetic proteins will become a mainstay in the regenerative environment. Research however also continues to find the ideal carrier for these inductive molecules (Sigurdsson et al, 1996) and, synthetic calcium phosphates may in fact be useful in this regard. However, these graft materials will need to be designed so as to show more predictable resorption patterns than presently available. Moreover, the carrier must maintain effectively the defect space while allowing slow release of the inductive factor(s) at the recipient site and not impeding new tissue ingrowth and formation (Nevins et al, 1996; Marden et al, 1994). It was with this background that the present study was undertaken.
Table 8  Properties of Commercially Available Synthetic Bone Substitute Materials

<table>
<thead>
<tr>
<th>Product</th>
<th>Material</th>
<th>Resorbability</th>
<th>Method of breakdown</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>PerioGlas</td>
<td>Bioactive glass</td>
<td>small particles resorb</td>
<td>leaching, dissolution</td>
<td>90-710μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>large decrease in size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioGran</td>
<td>Bioactive glass</td>
<td>within 6 months under</td>
<td>osteoclast</td>
<td>300-355μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioplant HTR</td>
<td>Calcified co-polymer</td>
<td>surface only</td>
<td>solution mediated</td>
<td>500 and 700μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca²⁺ dissolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OsteoGraft/LD</td>
<td>low heat HA</td>
<td>yes</td>
<td>solution mediated</td>
<td>250-420μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OsteoGraft/N-300</td>
<td>Bovine-derived HA</td>
<td>yes</td>
<td>osteoclast</td>
<td>250-420μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO-OSS</td>
<td>Bovine-derived HA</td>
<td>yes</td>
<td>osteoclasts</td>
<td>250-1000μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and 1.0-2.0μm</td>
<td></td>
</tr>
<tr>
<td>Capset</td>
<td>Calcium Sulfate barrier</td>
<td>yes 4-6 weeks</td>
<td>hydrolysis</td>
<td>40μm</td>
</tr>
<tr>
<td>Hapset</td>
<td>HA with Calcium Sulfate</td>
<td>not HA</td>
<td>hydrolysis</td>
<td>250μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitite 2040</td>
<td>HA</td>
<td>no</td>
<td>N/A</td>
<td>420-840μm</td>
</tr>
<tr>
<td>Calcitite 4060</td>
<td>HA</td>
<td>no</td>
<td>N/A</td>
<td>250-420μm</td>
</tr>
<tr>
<td>PermaRidge</td>
<td>HA</td>
<td>no</td>
<td>N/A</td>
<td>block form</td>
</tr>
<tr>
<td>Orthomatrix</td>
<td>HA</td>
<td>no</td>
<td>N/A</td>
<td>1000μm or 420-840μm</td>
</tr>
</tbody>
</table>

(from Dentistry Today, August 1997)

Comparative Dissolution in Tris Buffer after one hour:

Biocoral®>PerioGlas®>BioOss®>OsteoGen®>Biphasic CaP>OsteoGraft®>Interpore®>Calcitite®
(LeGeros, R.Z. et al., 1996)
3.0) OTHER STUDIES RELEVANT TO THE PRESENT INVESTIGATION OF PARTICULATE CALCIUM POLYPHOSPHATE

As presented, there are a number of synthetic graft materials presently available to assist in regenerating bone after traumatic injury, disease or age-related deterioration. Currently available materials however lack predictable resorptive patterns and this may have negative impact upon bony ingrowth and tissue regeneration. Work at the Center for Biomaterials at the University of Toronto has been directed towards developing calcium phosphate materials with lower Ca:P ratios than the majority of existing ones. Most work with calcium phosphate materials to date has been with materials that have Ca:P ratios in the higher range of 1.5 and greater. It is thought that if the ratio of calcium to phosphorous is decreased during the preparation of CaP ceramics, the basic structure of the material may become more polymeric in form. (Wells et al, 1996; Wells, 1997). An example of a polymeric phosphate is a condensed phosphate in which phosphorous atoms share common oxygen atoms and are not exclusive to salts involving calcium. The structure can occur as a linear polyphosphate, ring structure metaphosphate or as a three dimensional ultraphosphate (Corbridge, 1995). Due to the repeating structure of the linear polyphosphate and its potential effect on resorption or degradation of the material, calcium polyphosphate (CPP) is of particular interest as a synthetic bone substitute material.
Figure 1. Schematic structural representation of calcium polyphosphate depicting its polymeric structure. The covalently bound PO$_3$ units are structurally linked through P----O----P bonds to form long polymeric chains. These chains are ionically bound to Ca$^{2+}$ between chains.

[Ca(PO$_3$)$_2$]$_n$. Calcium Polyphosphate

The structure of CPP can be seen in figure 1. The value of $n$ for calcium polyphosphates can be variable but usually reaches values of up to $n = 10^4$ (Corbridge, 1995). In vitro studies suggest that CPP will undergo hydrolytic degradation via chain scission to form calcium orthophosphate, a non-toxic salt which may be a potential building block for physiologic hydroxyapatite (Wells, 1997). Due to the added control during its manufacturing compared to other synthetic calcium phosphate materials, CPP can be custom-manufactured with different specifications regarding pore diameter, block shape and size, Ca/P ratio, crystallinity and material strength (Nelson et al, 1993).
Initial research and development of calcium polyphosphate involved its use as a biodegradable replacement for asbestos in insulation (Griffith, 1992). As such, the material was developed in fiber form and was incorporated as a reinforcing agent in degradable orthopaedic fixation devices (Andriano et al, 1992). Consequently there are limited references to calcium polyphosphate as a bone graft material in the published literature.

A number of investigators are actively investigating CPP as a synthetic biomaterial. Lagow (1995) in conjunction with dental, orthopaedic, and chemical investigators, has been able to produce a highly pure CPP in porous block form without the need for high temperature processing. Preventing the high temperature (>1200° C) processing avoids weaknesses associated with increased porosity, variable pore size and potential impurities (Jarcho, 1986; Frame, 1987). In an animal study, Lagow and his group evaluated the ability of porous calcium polyphosphate to function as a bone graft substitute (Nelson et al, 1993). Six weeks after the extraction of the mandibular first and second molars, alveolar ostectomies were performed bilaterally on five dogs. Ridge anatomy was restored using the CPP implant block material on one side and autogenous bone obtained from the contralateral ostectomy site on the control side. Specimens were retrieved after 4 months and examined with light microscopy and backscatter electron imaging. Histological assessment revealed that the CPP site showed extensive vascularization and cellularity within an ‘invading’ loose connective tissue matrix. The sites with autogenous grafts revealed loose connective tissue throughout with hypovascularity and hypocellularity. Neither site showed evidence of an inflammatory response to the implanted materials. The BSEI histometry revealed significantly greater bone formation in the experimental site than on the autogenous grafted control sites. Although not quantified, the
authors made note of the significant remodeling that had occurred with the CPP as opposed to the autogenous grafts. Only the one healing interval (four months) was examined and therefore this study provided no indication of the eventual outcome with the CPP block graft.

Other research activities by both Pilliar (Filiaggi et al, 1998; Wells et al, 1996) and Davies (Baksh et al, 1998) have involved in vitro and in vivo studies to assess CPP as a bone substitute material. Initial studies examined CPP rods formed from a condensation reaction of calcium and phosphate salts followed by sintering at just below melting temperature (Wells et al, 1996). The material was evaluated after each processing stage by x-ray diffraction to ensure appropriate characteristics of the initial calcined powders, glass powders and the final sintered rods. The rods were implanted into rabbit femurs for preliminary investigation of bone ingrowth at periods of 2, 6 and 12 weeks. The preliminary observations from this implantation study revealed that the rods were biocompatible and that sintering was capable of producing consistent interconnected channels of 100μm diameter. Similar CPP blocks were implanted in rabbit femurs and left for up to one year (Filiaggi et al, 1998). For this study rods of CPP with a porous crystalline form and controlled pore size and density were used. The intermediary glass powder and the final sintered rods were examined by x-ray diffraction to confirm the crystalline structure. A calcium to phosphate ratio of 0.5 was confirmed by neutron activation analysis. Histological assessment of sections of retrieved specimens demonstrated bone ingrowth into the pores, the amount of which corresponded with the amount of time the implant was in place. The amount of bone ingrowth was significantly greater at the longest time period than at two or six weeks which varied as well with different pore sizes where more growth was evident with the finer particle size. Morphometric assessments of the specimens were
quantified including the percent of CPP particles remaining within the porous implant in order to determine the amount of degradation that had occurred over the specified period for the different particle sizes. The results confirmed the ability of the material to support bone ingrowth and that degradation rates were inversely proportional to particle and therefore, pore size with the smallest particles of 45-106 µm having the highest degradation rate followed by 106-150µm and finally by 150-250 µm sized particles.

In vitro assessment of this material employed porous CPP disks seeded with chondrocytes derived from the deep zone of bovine articular cartilage. At two weeks it was evident that cartilaginous tissue had formed as a continuous layer over the entire disk implying that chondrocytes can attach to CPP and express their phenotype.

Other work with CPP (Baksh, Davies, Kim, 1998) was directed to the investigation of a novel scaffolding system which incorporates calcium polyphosphate onto a macroporous polymer substructure. Amorphous CPP particulate material was heat-treated in the presence of macroporous polyurethane scaffolds. The polyurethane was then burnt out and the resultant inorganic scaffold sintered at 900°C for 1 hour to produce a three-dimensional CPP porous template. The goal of this approach was to improve on the standard sintering process which usually results in sinter-necks or bonding zones between particles giving rise to the interconnecting pores of 100µm. The preferred scaffold created with this method was one with larger pores of 0.3-1mm. An in vivo component of this investigation involved the implantation of the scaffolds into rat femora while the in vitro portion included seeding templates with rat bone marrow cells. Results indicated that at two weeks the templates seeded with rat bone marrow-derived cells had become populated on their entire surface forming a continuous sheet
of cells. The scaffolds retrieved from the rat femora demonstrated that bone had grown throughout the porous structure. The authors concluded that the newly developed macroporous CPP scaffold was able to support bone growth both in vitro and in vivo (Baksh et al, 1998).

To date there is no known published work investigating calcium polyphosphate in amorphous particulate form. This form is often the desirable one for any bone graft or substitute material meant to be used in periodontal or peri-implant regeneration or in well-enclosed bony defects that are of irregular shape. The ability to be able to pack or fill such a defect with loose or moldable graft material ensures superior adaptation of substance to the existing bony walls, tooth root and/or endosseous dental implant. Such sites are not weight-bearing and as such, the material will not be placed under immediate function or load. As well, graft particles of an amorphous form can be made without the need for processing at high temperatures thus avoiding concerns over different phases which may form inconsistencies with porosity and long degradation periods generally associated with crystallized material.
3.1) OBJECTIVES

There have been numerous attempts to develop the ideal synthetic bone substitute material, i.e. one that could predictably and consistently support bone regeneration. The properties of such a material would include, 1) promotion of neovascularization, 2) the ability to encourage and conduct bone cells across its surface and bone matrix production upon it, 3) suitability to act as a carrier for osteoinductive mediators, 4) the ability to maintain structural integrity without particle migration or micromovement during initial healing, 5) resorption within a defined period of time; and 6) having minimal to no toxicity, either locally or systemically.

Calcium polyphosphate (CPP), considered a member of the calcium phosphate ceramic family, has some unique characteristics offering potential as a biodegradable synthetic bone substitute material. Its ability to act as a reinforcing component and as a biodegradable material have made it useful as a reinforcing component in load-bearing composite implants, such as fracture fixation plates (Kelley et al, 1987; Andriano et al, 1992). As well, recent work (Baksh et al, 1998; Filiaggi et al, 1998) has shown that CPP is applicable as a synthetic bone graft in monolithic or block form. There have been no attempts to date however to test CPP in particulate graft form.

The current study was meant to be a preliminary assessment of the usefulness of biodegradable calcium polyphosphate in amorphous particulate form in bone regeneration using the rat calvarial bone wounding model.
3.2) HYPOTHESIS:

Calcium polyphosphate in amorphous particulate form of 150 - 250μm particle size will act effectively in the rat calvarial model as an osteoconductive bone substitute graft material especially when combined with an overlying barrier membrane providing occlusive properties.

4) METHODS AND MATERIALS:

4.1) Manufacturing Amorphous Calcium Polyphosphate Particulate

The calcium polyphosphate (CPP) for our investigation was procured from the Center for Biomaterials, University of Toronto. The raw material used in the fabrication of the amorphous product was calcium phosphate monobasic monohydrate, Ca(H₂PO₄)_2·H₂O (J.T.Baker, Phillipsburg). As obtained from the manufacturer, this powder has the desired molar calcium to phosphate ratio of 0.5. This precluded the need to achieve this by combining exact amounts of calcium and phosphate salts, avoiding the potential formation of other unwanted calcium phosphate phases that may lead to a variable osteoconductive and degradative response.

The initial phase in the formation of CPP involves the calcining or heating of the powder to 500°C for 10 hours (Thermolyne Type 1500 furnace). This calcining process drives off most of the H₂O loosely bound to the salts. Any initial impurities in the precursor powder would result in increased amounts of OH⁻ remaining attached to the salts and prevent the desired long chain polymerization characteristic of CPP. This initial condensation process however does not liberate all of the attached water groups, necessitating the subsequent higher temperature processing. The calcined powder is therefore placed in a tube furnace where it is
melted at 1100°C, 100°C over its melting point for 2 hours. Although this time is arbitrarily selected, it is believed that two hours in molten state allows sufficient time to induce chain lengthening (personal communication Filiaggi 1999). Overheating of the molten mass would result in phosphorus formation and possibly disrupt polymerization. To avoid crystallization, the molten CPP undergoes a quick cool-down quench as it is poured into distilled water to form an amorphous frit.

The final amorphous particulate is produced by ball milling of the frit using a stainless steel mortar and balls. The frit is pulverized for ten seconds and the resultant powder sifted through selective screens to capture particulate in the range of 150-250μm (-60/+100 mesh).

All the powder used in this investigation was derived from a single batch of particulate. The particulate was pre-measured in amounts of 0.125g to 0.15g and securely packaged into Baxter Dual Peel Tubing. The material was sterilized with ɣ-irradiation at 2.5 Mrads prior to utilization.

4.2) Animal Model

The rat calvarial model was used for the following reasons; minimal expense in procuring and housing, ease at running a larger sample size, and minimal quantities of experimental material. In addition, calvarial bone allows extrapolation to facial bones including that of the jaw because of similar patterns of bone formation (membranous ossification) and embryonic origin (Frame, 1980; Bosch et al, 1998; Schmitz et al, 1986).

In testing the ability of a CPP material to enhance bone regeneration in this model, the experimental defects needed to be of a so-called “critical-size”; that is, any defect used in
testing the efficacy of a substance or a procedure to stimulate new bone regeneration should be one which does not heal spontaneously with bone during the period of the experiment (Schmitz, et al, 1986; Bosch et al, 1998).

Reviewing previous studies employing the rat calvarial model to evaluate osteopromotive capabilities revealed confusion in regard to the size of what is considered to be a critical size defect. Freeman and Turnbull (1973) utilized 2.0mm diameter defects in 500g rats, and reported that none of the defects healed by spontaneous bone regeneration. Other studies used 4.0mm and 5.0mm diameter defects as critical sized (Glowacki et al, 1981; Deporter et al, 1985; Bosch et al, 1998), and confirmed the required non-union of the control sites. Other investigators (Hollinger and Kleinschmidt, 1990; Takagi and Urist, 1982) proposed that an 8.0mm diameter critical size defect be used. These investigators used animals that were sacrificed at 12 to 13 months, and the results showed non-unions containing fibrous connective tissue in the control sites. The obvious disadvantage however with an 8.0mm defect is that the size of the rat calvarium precludes using a paired design experiment as only one defect per animal is possible. To ensure a credible difference between the experimental and control sites, an 8.0mm defect as described by Hollinger was selected for the present investigation.
4.3) Surgical Procedure

Young adult male rats of 250 to 275g from Charles River Breeding Laboratories were anesthetized with 3% halothane for induction and maintained at 1.5% in 600ml N₂O and 300ml O₂ via nose cone. Presurgical medication included 7ml of warm lactated ringer intraperitoneally (I.P.) to accommodate for blood loss during the procedure. The animals also received 0.05 ml of dexamethazone I.P. as an anti-inflammatory. Post-surgical analgesia was addressed with Buprenorphine HCl 0.025 ml subcutaneously (S.C.) to comply with regulations of the Canadian Council on Animal Care and the University of Toronto Animal Care Policies and Guidelines.

Using aseptic technique, the dorsal part of the cranium was shaved and washed with iodine solution. A trapdoor-like dermal flap was prepared with two longitudinal incisions running along the outer limits of the cranial vault and a horizontal incision joining these longitudinal ones just distal to the orbits. The flap was reflected posteriorly and a circumferential area of 12.0 to 13.0 mm of bone at the mid-parietal suture was exposed by displacing the remaining periosteum laterally with a periosteal elevator. Due to the location of the 8.0mm defect, there was an anticipated high risk of encroaching upon the dura and more importantly the sagittal sinus just below the mid-sagittal suture that would result in extensive bleeding. Previous literature did not make mention of the potential high mortality of this size defect at this particular site (Schmitz et al, 1986; Tagaki et al, 1982; Hollinger et al, 1990).

The 8.0 mm trephine (courtesy of J.O. Hollinger) was initially engaged with a slow-speed electric driven bench-top handpiece (Kavo Model EWL 3) at approximately 3,000 to 4,000 rpm. This initial engagement of the calvarium under irrigation at a relatively high speed allowed the creation of a trough without the skipping of the trephine over the bone which
frequently occurs at lower speeds. Once the trough was created at a depth of approximately 0.3 to 0.4 mm within bone, the trephine was placed into a slower speed handpiece, (bench-top electric belt-driven handpiece) to continue the cut preparation to completion at speeds of 300 to 400 rpm. In employing the slower handpiece, the operator (F.M.) had more tactile sense and could monitor the resistance of the remaining bone at the site. The bone disc of 0.75 to 0.9 mm in thickness created with the trephine was teased from the underlying dura by sliding a thin flat periosteal elevator below the cut edge and working around the perimeter of the disc in a similar fashion as in opening a can of paint. In doing so, the disc was elevated and removed with dental pliers (Figure 2).

Due to the elliptical shape of the calvarium, the trephine did not penetrate uniformly. Thus the mid-crest section of the cranium was penetrated before the lateral margins of the defect. Just below this mid-crest region is located the sagittal sinus blood plexus preventing any deeper cutting with the trephine. Therefore in order not to encroach upon and damage this vessel, the lateral aspects of the defect had to be completed using the trephine in a free-hand manner angled first towards one side and then towards the other. Unfortunately, attempts to remove these lateral bony margins were not always successful and in ~25% of the animals resulted in one of two complications; 1) the breaching of the blood vessel plexus, massive hemorrhaging and the death of the animal or 2) persistently falling short of cutting through all the bony attachment on the periphery and relying on prying the bony disc off with pliers. This prying action also resulted in trauma to the blood vessel and death. The overall mortality rate after three sessions of surgery was 32% and this high death rate necessitated a request to the
Animal Care Committee for additional rats from the mid-way through the investigation to ensure sufficient animals for statistical purposes.

Once the site was successfully prepared, each animal was placed into one of the following four groups:

1) Control (n=3): Control animals received no material in the created defect site, and the flap was simply repositioned and sutured with interrupted 4-0 vicryl sutures. No attempt was made at repositioning the periosteum, but care was taken to bring the skin wound edges together without overlapping or bunching. Only three animals were included in the control to confirm the literature that the 8.0mm defect could not spontaneously heal.

2) Membrane only (n=8): In this group, a commercially available dense polytetrafluoroethylene membrane (PTFE), Cytoplast™ Regentex GBR®(Innova Corp. Toronto, Canada), was placed over the entire defect which as with the control received no CPP (Figure 4). The membrane was stabilized over the defect with two drops of Tissee™ (Immuno AG, Vienna, Austria), a two-component fibrin sealant hemostatic agent, at the membrane edges, taking care that none of this tissue glue seeped underneath the membrane. The flap was sutured as before.

3) Particulate only (n=8): In these animals the defects were packed with CPP particulate using a no. 9 periosteal elevator. The CPP particulate was dispensed into a glass dish and wetted with 35µl of sterile saline solution. The paste-like consistency of the material enhanced handling and ease of placement. The defects were slightly over-filled with material to compensate for the slight swelling of the brain into defect (Figure 3). Approximately 0.1g of
material was utilized in each defect and the flaps were repositioned and sutured as previously described.

4) Particulate and membrane (n=9): The last group involved the placement of CPP particles into the defect and the same barrier procedure as used in group 2 (Figure 4).

All animals were given post-analgesic medication consisting of 0.025 ml S.C. of Buprenorphine HCl and initially caged in single accommodations to prevent any mutilations of the surgical area by a cohabitant. After 48 hours of observation and full recovery, the rats were reinstated in twin accommodations.

A total of 41 rats received surgery in 5 sessions over a period of 21 days, 29 animals survived and 13 died, giving a mortality rate of 32%. Three rats died without apparent cause within 24 hours of the surgery, while ten died due to excessive hemorrhaging at the time of surgery. One animal of the 29 surviving rats which had particulate and membrane placed (group 4) developed an exposed membrane on day 12, and was withdrawn from the study.

The remaining 28 rats healed uneventfully with normal weight gain and no macroscopic evidence of infection of the surgical site. There were no signs of paralysis, convulsions, respiratory distress, or persistent pain. The animals were sacrificed at 4 weeks by CO₂ asphyxiation. The calvaria were dissected with a wide margin of normal bone and the overlying soft tissue as well as the underlying brain remaining attached to the skull.

4.4) Histological Preparation

For proper quantitative assessment of newly formed mineralized tissue, specimen preparation included non-decalcified sections and selective staining. This protocol allowed both light microscopy and backscattered electron image analysis (BSEI) (Schenk et al, 1984).
At the time of collection, the specimens were fixed in 10% buffered formalin for two weeks. Dehydration of the specimens was achieved by upgrading the alcohols from 70% absolute, to 95% and finally 100% over a period of three days. Xylene was used as the clearing agent to ensure total removal of water, lipids and fats so that there would be good infiltration and uniform hardening during polymerization.

Embedding of the non-decalcified specimens was done in methyl methacrylate (Osteobed®, Polysciences Inc. Warrington, USA), which ensures minimal shrinkage and increased stability in high vacuum and under the electron beam for electron microscopic assessment. During sectioning, the initial cut was made with a 0.006 mm diamond wafering blade (Norton Co. Worcester, USA) in an Isomet Low Speed Saw (Buehler Ltd., USA) through the specimen block, just off center of the sagittal midline of the circumferential defect (Deporter et al, 1986). This cut surface was polished (Planopol-2 polishing machine, Struers, Denmark; silicon carbide paper from Struers, FEPA 320, 800, 4000) and then the entire block was surface-stained with Toluidine blue and Van Gieson (Maniatopoulos et al, 1986). The cut surface of the block was than glued to a glass slide (LePage 5 minute Epoxy, Brampton, Ontario; thinner: toluene) and another sagittal cut of 150 μm in thickness was executed creating a stained specimen slide. The exposed specimen surface was polished and a cover glass was glued over this. This unstained surface was used for BSEI.

The initial thickness of the cut specimen attached to each slide was approximately 150μm, and during polishing was reduced to 50-60 μm. Each animal provided 3 sagittal sections, one taken as close as possible to the center of the 8 mm defect (as described above),
and the other two, 2 to 3 mm anterior and posterior to the initial midline cut. Avoiding sections from the edges ensured conformity in size of all sections of the defects sampled.

4.5) Quantification Method

Histomorphometric assessment of the three sections per animal was accomplished with Backscattered Electron Imaging (BSEI-SEM) with a Scanning Electron Microscope (Hitachi Model S-2500, voltage 15KV; with a Robinson Detector, Sidney, Australia) at a magnification of 25X. The images were captured on computer (Dell Optiplex GX1 Pentium II 320-MHz computer) employing the SigmaScan Image Measurement Software, Version 4.0 (SPSS® Inc. Chicago, USA). Having 8 mm defects magnified 25X, resulted in the need for three overlapping images to record properly both margins of the defect and the area between them.

The software allowed splicing of the overlapping structures to produce an accurate representation of the SEM image of the entire defect. The spliced image (Figure 5) was then cropped to include a rectangular area within which could be measured any new bone formation from the periphery or within the defect including that amongst residual particles of graft material. This rectangular area was defined laterally by the bony margins of the initial trephine cut, superiorly by the highest point above the overlying defect where there was evidence of new bone or residual material and inferiorly by the superior surface of the dura mater (Diagram 1, Figure 5).

Using the light microscope (Leitz, Wetzlar) at 10X magnification and the highly delineated images produced with the SEM, it was possible to clearly outline newly-formed immature bone, existing bony margins of the calvarial wound (old bone), CPP graft material and the dense teflon membrane. Employing the SigmaScan software, through colour
differentiation and edge detection, the bone and particles were identified and quantified. The measurements were recorded in mm-square units for new bone, residual graft material (where applicable), and total defect space occupied in the cropped image in each animal as described above. The average of new bone and residual material of the three sections from each animal was computed. A mean for each of the four groups representing mean new bone and residual material was then determined (Table 9).

The SigmaScan software produces these values by allowing the operator to select a part or portions within the image and assign these items a specific colour, thereby differentiating the new bone, particles and unfilled area in the defect. By pre-calibrating in the desired units, (i.e. mm$^2$), the program will be able to determine an accurate value of the different coloured areas selected in the image. (See Figures 7,9,11,13)

4.6) Qualitative Assessment

Microscopy with transmitted light (Leitz at 10X) was utilized to assess any inflammatory response at the defect sites. Magnified images were also captured from the microscope using a Sony CCD colour camera (model DXC15, Japan). Although these images were not used for histomorphometric analysis, they provided good hard copies (Tektronix Laser Printer, Phaser 740) of representative views of the different treatments and the tissue response at the termination of the investigation. (Figures 6,8,10,12)
4.7) Statistical Analysis

Statistical comparisons were carried out by using one-way analysis of variance ANOVA followed by Duncan’s multiple range test for all 4 conditions. The mean amount of particulate material in group 3 and 4 was also tested to determine if there was any significant difference. $P$ values $< 0.05$ were considered statistically significant.

5.0) RESULTS

5.1) Clinical Results

Intraoperatively, the handling of the CPP particulate in paste form after being hydrated with small amounts of saline solution proved to be convenient. The material was easily stabilized with little migration or spilling of particles out of the defect. Once the particulate was in place it appeared to behave in a hemostatic manner, reducing or totally arresting the small amount of bleeding from the wound. The placement of the membrane, although slightly more tedious, was facilitated by the use of the fibrin glue. The glue worked remarkably well at maintaining the membrane in place as the flap was re-approximated and sutured.

At the end of the twenty-eight days, 28 of 29 animals recovered fully from the surgery. The skin wounds had healed completely with no sign of infection or untoward reaction to the particulate or membrane employed. One of the 29 rats did have an exposure of the membrane at day 12 of the investigation. It was not certain as to why this complication arose except to speculate that the sutures may have been dislodged by another animal, exposing the site and resulting in subsequent infection. This animal was initially treated with topical antibiotic (polymyxin) but the unresolved infection resulted in the animal’s early exit from the study.
At the time of dissection and removal of the calvaria, there were no indications of infection or inflammatory response to either of the implanted materials. Where membranes had been placed, it was evident that these were maintained in place by overlying soft tissue.

5.2) Histological Results

5.2.1) Light Microscopic Evaluation

One month after the surgical procedure, the healing response varied considerably depending on the conditions employed at that site. The stains used (Toluidine blue and Van Gieson) affected bone in various shades of red depending on its stage of development (Maniatopoulos et al, 1986). Osteoid stained light green, mature bone a paler red and immature bone an intense red. The mineralizing front between old and new bone was clearly delineated. Soft tissue including that of the brain and the nuclei of cells including any inflammatory cells appeared a distinctive blue while fibrous connective tissue stained with a bluish-green appearance. The particulate did not pick up any stain and appeared as angular particles in shades of gray.

Group 1: Control

The predominant feature of the three control specimens (no graft material placed) was the amount of new bone bridging the 8mm defects. Although thinner than the existing old bone, the ingrowth from both sides of the wound edges approximated 50 to 70 % of the original defects. There appeared to be a small amount of fibrous tissue over the healing defect but no detectable inflammatory cells (Figure 6 and 7).
Group 2: Membrane Only

Similar to the control sites, the membrane-only specimens resulted in significant bony ingrowth from both wound edges. Again the 8mm gap experienced closures of 40 to 70%, albeit in a thinner growth pattern. Organized fibrous tissue was evident over the top surface of the membrane, with a more loosely organized fibrous tissue below the membrane and above the developing bone. No inflammatory component was apparent in these sections (Figure 8 and 9).

Group 3: Particulate Only

In striking contrast, these specimens clearly depicted minimal if any bony ingrowth or regeneration from the perimeter or within the particulate mass. The particulate material appeared to have been adequately maintained at the site with only a small number of particles having migrated into the overlying connective tissue. Some loosely organized fibrous tissue had infiltrated the spaces amongst the particles. Also within this fibrous tissue was evidence of on average 6 to 8 multinucleated giant cells, representing a mild inflammatory response with no other untoward reaction to this material. None of these multinucleated cells were actually on the surface of the CPP material but rather within the fibrous tissue. No material migrated below the dura or into the brain tissue. There was minimal vasculature within the fibrous tissue (Figure 10 and 11).

Group 4: Particulate and Membrane

Similar to the particulate-only group, minute amounts of new bone were detected in these specimens. The membrane appeared to have remained stable over the defect and effectively maintained the particulate in the defect as well. As with the particulate only, a mild inflammatory response was present with multinucleated giant cells detected within the fibrous
connective tissue. It appeared that the residual particulate material was denser in this group of defects than it was for group 3 (i.e. no membrane placed). The fibrous ingrowth appeared to lack vasculature (figure 12 and 13).

5.2.2) Scanning Electron Microscopy Evaluation

As described in the methods section, the quantitative component of this study was accomplished with BSEI recorded and then transferred and analyzed in a computer using SigmaScan® software. The mean average new bone per mm² in descending order was highest for control at 1.17mm², followed by membrane only (1.04mm²), by particulate and membrane (0.12mm²), and finally with the least amount of bone, the particulate only (0.08mm²) group (table 10). The mean for each animal and the standard deviations are shown in table 9.

Using the one way ANOVA followed by the Duncan multiple page test, a significant difference was detected between the two groups without the material and the two that had the CPP material placed within the defects (graph 1a). Significantly (p<0.0001) more bone was measured in groups 1 and 2 than in groups 3 and 4. (Appendix 5)

An assessment of the amount of residual material remaining with the membrane (mean 0.52 mm² ± 0.17mm²) and without the membrane (mean 0.87mm² ± 0.41mm²) revealed no statistically significant difference, p>0.05 (Graph 1b).
Figure 2. Prepared defect with mid-sagittal sinus venous system (SV) exposed but not damaged.

Figure 3. Particulate CPP (P) placed into the defect with some slight overfilling.

Figure 4. Dense Teflon membrane (M) placed over graft and defect extending 2mm beyond margin of defect.
**Figure 5a.** Image generated from BSEI at 25x. Boxed area defines area being measured.

**Figure 5b.** Cropped image from previous specimen. New bone coloured in red.
Figure 6a  Control specimen (13x). New bone formation involves more than 70% of the defect. Difference in new bone (NB) and mature cortical bone (OB) evident. Bone formation below cortical bone from endosteum not included as new bone in the defect.

Figure 6b.  Higher magnification (32x) reveals the apposition line of the newly forming bone (NB). Old bone (OB) with distinct laminar pattern with new bone (NB) formation extending beyond wound edge (W).
Figure 7a-c. Computer image of a control specimen (4x) with BSEI cropped image (10x) and analyzed for total bone ingrowth. Red represents new bone.
**Figure 8a.** Example from Group 2 (8x) with membrane (M) only revealing the new bone growth (NB) extending into the defect in a thin-like manner from the wound edge (W).

**Figure 8b.** Higher magnification (13x) of an example of membrane only depicts the close adaptation of the membrane (M) to the new bone (NB) forming below it. Distinct line evident of wound edge (W) between old bone (OB) and new bone (NB).
Figure 9a-c. Computer image of a membrane only (9a) specimen (4x) with the BSEI cropped image (9b)(10x). Sigma Scan analysis (9c) for quantification of new bone in red with gray edges representing margin of old bone. Remaining white areas represent BSEI artifact.
Figure 10a. Example from group 3 (13x), particulate only (P), with no new bone evident in the defect.

Figure 10b. Higher magnification (32x) of particulate only. While old bone (OB) can be seen at the wound margin (W) there is no evidence of new bone in the defect but there is some new bone (NB) apposition along the endosteum not related to the graft material. The wound remains filled with particulate graft material (P) and encapsulating fibrous tissue (F).
Figure 11a-c. Computer image of an example of a particulate (11a) specimen (4x) with the BSEI cropped (11b) image (10x) and analyzed image (11c) with new bone in red, gray periphery represents wound margin and green represents residual particulate material.
**Figure 12a.** Example from group 4, particulate and membrane (M)(13x). All elements of defect clearly distinct with no evidence of new bone. Membrane (M) overlying the defect and extending beyond the wound margins.

**Figure 12b.** Higher magnification (32x) of figure 12a., with old bone (OB) and wound margin not having any new bone growth. The defect remains filled with particulate material (P) which is encapsulated with fibrous tissue (F).
Figure 13a-c. Computer image of an example of a particulate with membrane (13a) specimen (4x) with the BSEI cropped (13b) image (10x) and analyzed image (13c) with new bone in red, gray periphery represents wound margin and green represents residual particulate material.
Table 9) **New Bone Growth:** Individual Means (of the three sections per animal)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN BONE (MM$^2$)</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.680</td>
<td>0.232</td>
</tr>
<tr>
<td>C</td>
<td>0.473</td>
<td>0.170</td>
</tr>
<tr>
<td>C</td>
<td>1.373</td>
<td>0.127</td>
</tr>
<tr>
<td>M</td>
<td>0.380</td>
<td>0.234</td>
</tr>
<tr>
<td>M</td>
<td>0.830</td>
<td>0.177</td>
</tr>
<tr>
<td>M</td>
<td>1.673</td>
<td>0.685</td>
</tr>
<tr>
<td>M</td>
<td>1.013</td>
<td>0.208</td>
</tr>
<tr>
<td>M</td>
<td>0.840</td>
<td>0.087</td>
</tr>
<tr>
<td>M</td>
<td>0.840</td>
<td>0.363</td>
</tr>
<tr>
<td>M</td>
<td>1.647</td>
<td>0.440</td>
</tr>
<tr>
<td>M</td>
<td>1.687</td>
<td>0.361</td>
</tr>
<tr>
<td>P</td>
<td>0.093</td>
<td>0.078</td>
</tr>
<tr>
<td>P</td>
<td>0.090</td>
<td>0.057</td>
</tr>
<tr>
<td>P</td>
<td>0.057</td>
<td>0.040</td>
</tr>
<tr>
<td>P</td>
<td>0.133</td>
<td>0.075</td>
</tr>
<tr>
<td>P</td>
<td>0.043</td>
<td>0.032</td>
</tr>
<tr>
<td>P</td>
<td>0.117</td>
<td>0.055</td>
</tr>
<tr>
<td>P</td>
<td>0.093</td>
<td>0.035</td>
</tr>
<tr>
<td>P</td>
<td>0.070</td>
<td>0.010</td>
</tr>
<tr>
<td>PM</td>
<td>0.200</td>
<td>0.050</td>
</tr>
<tr>
<td>PM</td>
<td>0.023</td>
<td>0.015</td>
</tr>
<tr>
<td>PM</td>
<td>0.140</td>
<td>0.106</td>
</tr>
<tr>
<td>PM</td>
<td>0.077</td>
<td>0.046</td>
</tr>
<tr>
<td>PM</td>
<td>0.080</td>
<td>0.026</td>
</tr>
<tr>
<td>PM</td>
<td>0.220</td>
<td>0.010</td>
</tr>
<tr>
<td>PM</td>
<td>0.290</td>
<td>0.151</td>
</tr>
<tr>
<td>PM</td>
<td>0.060</td>
<td>0.017</td>
</tr>
<tr>
<td>PM</td>
<td>0.173</td>
<td>0.065</td>
</tr>
</tbody>
</table>

C- CONTROL
M- MEMBRANE ONLY
P- PARTICULATE ONLY
PM- PARTICULATE & MEMBRANE
Table 10  Histomorphometric Measurements of Each Treatment Group at 28 Days

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>New Bone (mm$^2$) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>3</td>
<td>1.165 ± .697</td>
</tr>
<tr>
<td>2</td>
<td>membrane only</td>
<td>8</td>
<td>1.043 ± .467</td>
</tr>
<tr>
<td>3</td>
<td>particulate only</td>
<td>8</td>
<td>0.080* ± .037</td>
</tr>
<tr>
<td>4</td>
<td>membrane and particulate</td>
<td>9</td>
<td>0.115* ± .071</td>
</tr>
</tbody>
</table>

*Denotes significance at P< .0001 as compared to the control and membrane only group.
### Dependent Variable: AVNEWB

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>6.41935268</td>
<td>2.13978423</td>
<td>20.13</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>2.55136875</td>
<td>0.10630703</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>27</td>
<td>8.97072143</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**R-Square** 0.715589  
**C.V.** 67.52465  
**Root MSE** 0.32604759  
**AVNEWB Mean** 0.48285714

### Source

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>3</td>
<td>6.41935268</td>
<td>2.13978423</td>
<td>20.13</td>
</tr>
</tbody>
</table>

### Duncan's Multiple Range Test for variable: AVNEWB

**NOTE:** This test controls the type I comparisonwise error rate, not the experimentwise error rate

**Alpha= 0.05 df= 24 MSE= 0.106307**  
**WARNING:** Cell sizes are not equal.  
**Harmonic Mean of cell sizes= 5.76**

**Number of Means:** 2 3 4  
**Critical Range:** .3965 .4165 .4293

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Duncan Grouping</th>
<th>Mean</th>
<th>N</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.1650</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>1.0431</td>
<td>8</td>
<td>M</td>
</tr>
<tr>
<td>B</td>
<td>0.1150</td>
<td>9</td>
<td>P+M</td>
</tr>
<tr>
<td>B</td>
<td>0.0806</td>
<td>8</td>
<td>P</td>
</tr>
</tbody>
</table>

Appendix 2
Mean New Bone $\text{mm}^2$

- significant difference between control/membrane and particle/particle+membrane ($p<0.0001$)

Residual Particulate material $\text{mm}^2$

- no statistical difference with membrane over particulate material, $p>0.05$
TERMS:  

OSTEOGENESIS: All the steps and the processes leading to bone formation. An osteogenic graft material possesses vital osteoblastic cells that result in bone formation is described as osteogenic.

OSTEOINDUCTION: A material is said to be osteoinductive if when implanted in a non-osseous environment such as the rodent muscle pouch, will result in bone formation. Growth factors such as BMP’s present in such graft are capable of recruiting pre-osteogenic cells or directing undifferentiated mesenchymal cells towards osteochondrogenic differentiation. Such a graft does not require viable cells.

OSTEOCONDUCTIVE: A material is said to be osteoconductive when it facilitates bone cell migration and new bone formation from existing bone.

OSTEOPRODUCTION: This is defined as a process whereby a bioactive surface is colonized by osteogenic stem cells free in the defect environment as a result of surgical intervention. (Hench & Wilson 1993)

CERAMIC MATERIAL: A product made by baking or firing of a non metallic mineral and formed by combination of one or more metallic elements with one or more non-metallic elements to form partially ionic, partially covalent compounds.

BIOACTIVE MATERIAL: defined as a material that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material. (Hench & Wilson 1993)
6) DISCUSSION:

This would appear to be the first investigation of the use of particulate calcium polyphosphate (CPP) as a bone graft substitute material in an in vivo model. The rat calvarial model as used here traditionally has been employed in preliminary in vivo assessments of novel bone replacement materials (Hollinger et al, 1990) as it has the potential to offer insights into the physiology of bone grafting in craniofacial reconstruction (Murray et al, 1979).

Conventional grafting of bony defects with osteoconductive materials is based upon “creeping substitution”, that is the graft is gradually vascularized and resorbed as new bone is synthesized. The purpose of the graft is to provide support to the overlying muscle and skin tissue, to retain space for clot formation and to act as a scaffold for bony ingrowth (Klawitter et al, 1971; Osborn et al, 1980; Jarcho, 1981). In these previous investigations using 8 mm defects in rats, total closure of the defects occurred only in animals over 350 g body weight at the commencement of the procedure and during healing periods of more than 3 months. The studies resulting in the greatest defect closure employed rats of 475 to 525 g and the use of double membranes and/or autogenous graft material combined with bovine bone BMP’s (Dahlin et al, 1991; Katsumasa et al, 1982). Other investigators were not able to report total healing of the defects in the control or experimental conditions (Naaman et al, 1998; Isaksson et al, 1993).

The results of the present investigation indicate that not only did the bony ingrowth into the particulate material fail to occur but that there was actually a near total inhibition of bone ingrowth by the particulate material. The control sites (N = 3) and those which received an occlusive teflon membrane had between 40% and 70% of the defect width filled with new
bone. These results are in agreement with other similar studies incorporating 8.0mm critically-sized defects to test procedures and materials. For example, Naaman et al (1998) used rat calvarial defects for the purpose of investigating bone formation using grafts composed of discs or particulate of natural coral with and without a polymer membrane for occlusive purposes. The authors incorporated Hollinger's definition of critical-sized defect (i.e. 8 mm diameter) so as to ensure that the wounds would not heal spontaneously in the time frame of the investigation. All thirty animals appear to have survived the surgery as no fatalities were reported. The results revealed that only the combination of coral granules with a polyglactin membrane had significantly more bone formation than the controls. The other groups including natural coral disc, natural coral granules alone and polyglactin membrane alone showed slightly more bone than the controls, but none of the differences were significant. The controls in their study produced slightly less bone fill (0.804 mm^2 ± 0.076) than in the present study (1.165mm^2 ± 0.690). This variation between the controls in the two studies is not likely attributed to age since younger rats (45 days vs 3 months) were used in Naaman's study and therefore should have provided more active osteoprogenitor cells for wound healing. Bone formation in their study was evident in close approximation to the coral, be it in block or granular form. The authors concluded that the use of the membrane enhanced retention of the granular form of the material at the site as the lack of a membrane resulted in less residual graft material at the defect with evidence of granule migration.

Dahlin et al (1991) reported being able to create two 8mm calvarial defects side by side in 72 adult rats weighing 475 to 525 g with only two deaths due to bleeding and 4 due to infection. This was certainly in contrast to the present investigation where much higher
mortality was experienced. Dahlin's study design included defects that were treated with no material (control), with PTFE-membrane (Gore-Tex) over an empty defect, with two membranes, one internally between the dura and the skull and the other over the defect, and using the same 3 conditions above but with addition of autogenous bone chips procured from the bone disk resulting from the defect creation. The least amount of bone was formed in the two control conditions where no material was placed. These empty defects revealed similar mean percentage bone fill (18.3%) as that achieved in the present study (21.5%). The most bone was regenerated within the defects packed with autogenous bone chips and isolated with the double membrane approach. These results were consistent at all time intervals used (3, 6 and 12 weeks). The addition of the second inner membrane and the autogenous bone chips resulted in a major improvement in bone repair from the rims with a 90% mean bone fill including a few defects that were totally filled with new bone. As well, the authors clearly reinforced the superiority of fresh autogenous bone as a bone graft particularly when used in combination with occlusive membranes.

There is no apparent explanation for the lack of significant bone ingrowth seen in the calvarial model with the novel particulate CPP material used here. This material had been used earlier in block form and proved to be an effective osteoconductive scaffold. As demonstrated by Filiaggi et al (1998) and Baksh et al (1998) when different forms of block CPP were used in animal models good osteoconductive properties were observed. Both investigations included the use of cylindrical block forms of CPP which were fitted into surgical sites in rabbit or rat femur. The surgical sites ensured that the graft material was in rigid contact with both cortical bone for stabilization of the material and with trabecular bone to provide an ample source of
osteogenic cells. Furthermore, CPP disks when seeded with chondrocytes or osteoblastic precursors in culture, resulted in cellular attachment and proliferation. These investigations gave no indication of cellular inactivation or inhibition (Filiaggi et al, 1998; Baksh et al, 1998; Nelson et al, 1993; Wells et al, 1996; Varrela et al, work in progress). Thus it was an unexpected finding that particulate CPP graft material as used here should actually appear to inhibit bony ingrowth. A number of suggestions might be considered to explain this outcome. Firstly the animal model utilized to test the material may be challenged. A number of authorities who promote the use of the rat calvarial model recommend an 8mm defect as the critical size needed to discourage spontaneous healing of control sites. Accordingly, any significant bony healing such as the total closure of the wound with new bone could only be attributed to the use of the material being tested (Hollinger et al, 1994; Takagi et al, 1981). Employing a defect of this size however, results in minimal support for the overlying soft tissue which may easily collapse into the defect area. As the cranium is elliptical in shape, the mid-sagittal region where the bulk of the particulate material was placed had minimal support for the overlying soft tissues once the flaps were repositioned. Nor did the defects have a hard floor onto which the material could be packed with positive pressure. Instead the floor of the defect consisted of a smooth, slightly pulsating soft dural surface which due to normal physiological pressure, tended to swell into the newly formed defect. Regardless of the use of an occlusive membrane, the particulate material could be vulnerable to some amount of motion, be it from the animal’s musculature during normal movement or from an overt manipulation by the animal or its caged partner. Such motion of the graft could have been sufficient to stimulate fibrous tissue rather than bony ingrowth. However, the empty control defects also had no space
maintenance material with likely collapse of the overlying soft tissue and interestingly this situation did not arrest bone growth but merely created a thinning effect near the center of the defect where the collapse was most pronounced (Figures 8a & 8b).

Using smaller defects, for example 5mm defects, in the rat calvarium may have been a better option. This experimental protocol would have allowed the use of two 5mm defects in each animal creating a paired-design and avoiding encroachment upon the sagittal suture. This defect size has been used and proven to be a critical size defect by Bosch et al (1998) who were able to demonstrate that at 12 months controls did not progress to complete bone healing. However, having an amount of bone growth as resulted in the control groups in the present investigation would suggest that in a 5mm defect spontaneous healing of the critical size defects would be a real possibility.

The phenomenon of diminished bone growth with motion of particulate or block materials has been reported elsewhere. For example, Snyders et al (1993) evaluated the use of negatively charged dextran beads with and without the use of calcium sulfate slurry as a binding agent in 5 mm diameter calvarial defects in rat. The combination of the two materials promoted better bony repair. The dextran beads used alone produced mostly fibrous tissue with less bone in the wound than even the controls. The authors attributed the success of the combined treatment to the binding effect of the calcium sulfate and its ability after setting both to maintain the material at the site without movement and to retain the space, preventing soft tissue collapse into the wound area. Lindholm et al (1994) have also provided evidence of poor osteoconductivity of granular or particulate graft material in the calvarial model. Hydroxyapatite (HA) granules were tested in rabbit calvaria with or without rabbit autogenous
bone. Eleven mm defects and four conditions were used including a control, HA alone, HA mixed with autogenous bone marrow and autogenous bone alone. At completion of the healing period (12 weeks) computerized analysis of histologic sections in combination with immunohistological staining revealed that the HA alone or in combination with the autogenous marrow produced the least amount of new bone and the greatest amount of fibrous connective tissue, while the control and autogenous marrow produced primarily bone. The authors hypothesized that migration of and mechanical irritation from the HA granules resulted in granulation tissue proliferation. The immunohistological staining revealed fibronectin and collagen type III at the wound site. Fibronectin is associated with fibrinogen, fibrin, denatured collagen and other inflammatory and degeneration products at wound sites. In conclusion, the authors speculated that migration and motion of HA granules initiated the formation of inflammatory granulation tissue while impeding new bone formation.

Other studies conducted by Pilliar and colleagues using a porous-surfaced endosseous dental implant with porosities similar to that which should have developed in conjunction with the CPP material used here may be of relevance. These studies were aimed at determining the amount of permissible micromotion at the porous-surface-to-bone interface which would not hinder bony ingrowth into this surface. It was determined using animal models that the extent of allowable movement was less than 50μm. Greater initial movement resulted in the development of a fibrous connective tissue layer at the bone-to-implant interface (Pilliar et al, 1981; Pilliar et al, 1986; Pilliar et al, 1993; Pilliar et al, 1995; Cameron et al, 1973). Although this work was directed at a metal porous surface, the findings may be germane to the concept of
the need for sufficient stability of implanted biomaterials during healing in order to allow for bone formation.

Lindhrom (1995) also included HA block material and HA granular material in his study using calvarial defects in rabbit. The results revealed that where the HA block was not in tight apposition to the bone edge, connective tissue developed. Furthermore, HA granules impeded bone formation and appeared to encourage fibrous tissue proliferation. Significantly more bone development was detected in the control than in any sites harboring the HA granules.

In another much referenced paper, Glowacki and colleagues (1981) examined the differences between demineralized and non-demineralized bone powder of variable size as well as HA when placed in 4mm rat calvarial defects. None of the empty control defects healed even after 6 months. Sites implanted with mineralized bone graft also showed very little if any new bony healing but did reveal resorption of the mineralized powder. By contrast, sites treated with demineralized bone powder revealed significant bony healing. Similar to the present study, sites treated with either mineralized material impeded bone growth into the wound. The lack of bone healing in the 4mm control defects is in contrast to the response seen in the present study of 8mm defects where significant bony ingrowth occurred. The discrepancy is an oddity as immature animals like the ones used by Glowacki, should heal at a faster rate than older animals like the ones used in the present study (Hollinger et al, 1990).

An anatomical feature of the calvarial model which might contribute to the minimal bone growth observed is that of vasculature or lack thereof. In contrast to many long bones that contain a primary nutrient artery supply, there is none in the calvarium (Schmitz et al, 1986). It
is known that bony ingrowth in the calvarial model occurs in a centripetal manner starting from
the perimeter of the wound, the wound edges being the source for neovascularization and
subsequent invasion of osteogenic cells. A 250 to 275 g rat has a calvarium of approximately
0.75 to 0.9 mm thickness and the degree of vessel ingrowth from such a thin-rim source of
cortical bone would likely be minimal under the best of circumstances and perhaps easily
discouraged by the placement of the CPP or HA particles.

A further possible explanation for the poor result seen with the CPP in the present study
may have been the size of the porosity achieved between the graft particles and the effect that
this may have had on any scaffolding property and osteoconduction. The literature is replete
with work conducted to determine the ideal pore size for bone cell ingrowth and a number of
different opinions prevail as to this ideal size and the particle size range required to achieve it.
Work during two decades has indicated that interconnecting porosity, be it in block graft form
or resulting from the packing of particulate material, should be in the range of 100 to 800 μm
(Klawitter et al, 1971; Ferraro, 1979; deGroot, 1980; Jarcho, 1981; Rawlings, 1993; Oonishi et
al, 1999). For the present study, it was hoped to achieve the same pore size with the CPP
particulate as with a block CPP graft used in rabbit femur, i.e. 150 to 250 μm (Filiaggi et al,
1998). Using an amorphous particulate material of particle size less than 100μm likely would
have resulted in too rapid a dissolution rate (Klawitter et al, 1971; Jarcho, 1981). Therefore a
particle size range of 150μm -250μm was used so that when packed into the defect the result
would be an effective interconnected pore size above 100μm and slightly below 250μm.
Although not measured with the SEM, the images derived from the investigation depicted the
desired scaffolding pattern and therefore it was believed that the size of the particles was appropriate and not the reason for the hindrance to bony ingrowth.

Consideration was also given to the possibility that the inhibition of bony healing might have been the result of excess calcium or phosphate ion release from the grafted CPP material into the local microenvironment. Knabe et al (1997) evaluated the cellular response to a number of rapidly resorbing calcium phosphate ceramics. The novel materials were formed by the addition of ions such as sodium, magnesium, magnesium silicate or potassium phosphate to the conventional hydroxyapatite formulation. In vitro investigation involved the exposure of these compounds to rat bone marrow cells for 14 days with the daily analysis of calcium and phosphate content in the media. The results revealed significant inhibition of cell growth with two compounds which developed a relatively higher phosphate-ion release, these being Ca₃PO₄ and CaNaPO₄ combined with Mg₂SiO₄.

Miyamoto et al (1997), in an attempt to decrease setting time for a novel calcium phosphate cement (FSCPC), added 0.2 mol/L neutral phosphate to the liquid phase of the conventional calcium phosphate cement (CPC). This cement then was placed in surgical defects created in rat tibia and compared to the conventional cement at intervals including 0.5, 1, 3, 6, 12, 24 days and 2, 4, 8 weeks. At the completion of this study, the tissue response to the FSCPC with its elevated level of phosphate was similar to that of regular CPC, i.e. minimal tissue irritation or inflammation throughout the entire period. The higher phosphate concentration did not hinder nor did it increase the amount of new bone ingrowth into the cement-treated sites but did decrease the setting time. The authors concluded that it is safe to
incorporate higher levels of phosphate to achieve faster setting time without compromising the osteoconductive properties of the material.

Although calcium and phosphate dissolution was not measured in the present investigation, histological images revealed residual particulate at the site with some of the original angular configurations created in the manufacturing process still intact. While some dissolution of the material had occurred, which was evident from the rounded edges of some particles, the bulk of the residual graft material remained at the site perhaps suggesting that there was not likely sufficient dissolution of material to achieve cytotoxic levels. Observations in this study did indicate minimal inflammatory response to the CPP biomaterial. The few multinucleated giant cells observed were not in close proximity to the material but more so in the fibrous connective tissue. This might indicate minimal cellular degradation of the CPP particulate and resorption primarily by dissolution. This observation and the assumption that material loss is by dissolution would be supported by the results of an in vitro study by LeGeros R.Z. et al (1995) in which physical and chemical properties of various synthetic materials were compared. The amounts of dissolution in a physiological tris buffer were assessed as the calcium ions released into solution after one hour exposure to the various materials (table 8). Results indicated that the amorphous particulate material, Perioglas®, and the naturally occurring, less organized crystal structure of Biocoral® had the greatest amount of calcium release when compared to crystalline materials such as Calcitite® and Interpore®. Although these results do not preclude possible adjunctive osteoclastic resorption of the material when placed in vivo, they do suggest that there is sufficient dissolution of the CPP in this model to account for its eventual loss.
The examples cited above concerning the use of high levels of phosphate ion in calcium phosphate cements in rat tibia, or dissolution in vitro of synthetic graft material in tris buffer are meant to mimic human physiologic conditions with good vascularization. The rat calvarium which is mostly dense cortical bone has a poor blood supply and a relative deficiency of bone marrow (Prolo et al, 1982). Not having the same level of vascularization or arterial supply as long bones may result in an inability to clear local ions and or inhibit the neovascularization critical for bony growth. Furthermore, although CPP in block form was shown to be an effective graft in the rabbit femur and rat tibia, the calvarial defect with its poor vascularization potential may be a poor site in which to test the material in particulate or block form.

The hypothesis for the present study was that calcium polyphosphate (CPP) in amorphous particulate form of particle size 150 - 250μm would act effectively as an osteoconductive bone substitute material in the rat calvarial model and provide clear evidence of superior bone ingrowth into the grafted site when compared to control non-grafted sites. The results failed to support this hypothesis and demonstrated that the CPP material appeared to impede bone formation and/or may have encouraged the proliferation of fibrous connective tissue. However, the author believes that the study may have been flawed in the choice of model. What the model may have been doing is indicating the need for total stability of a particulate graft material like CPP because of minimal tolerance of host bone cells to any minimal motion of graft particles. This finding has clinical significance when considering the employment of any form of bone graft material other than vital autogenous bone. Even in areas that are non-weight-bearing, such as extraction sockets or intrabony periodontal defects, the importance of graft stabilization and proper adaptation and contact with the recipient bed
cannot be over-emphasized (Lindholm et al, 1994; Lindholm, 1995). This concept of particle stabilization is clearly supported by differences previously mentioned with materials that undergo bone-bonding such as 45S5 Bioglass as opposed to more bioinert materials such as non-resorbable HA particles, the latter generally becoming encapsulated with fibrous tissue.

While performance of the particulate CPP graft material used was disappointing, since the same material in block form when implanted in other animal models performed admirably in promoting osteoconduction, there may be some value in assessing further particulate calcium polyphosphate under different test conditions. A more appropriate model might be one like the femoral bone or mandible having a more definitive recipient bed after defect creation so as to better stabilize the material during the healing period. Furthermore, Oonishi et al (1999) described the use of bilateral 6 mm holes drilled into the femoral condyles of mature Japanese White rabbits to test the comparative bone growth behavior achieved with granules of bioceramic materials of various sizes. The investigators reported no difficulties with maintaining material at the site. Such alternative investigative approaches would need to provide a more appropriate source for vascular ingrowth by having more than just dense cortical bone contributing to the healing process.
7) CONCLUSIONS

From the results reported here it may be concluded that:

1) at 4 weeks of healing, none of the 8mm defects created in rat calvaria exhibited total closure with bone confirming that the defect size chosen was of "critical size";

2) amorphous particulate calcium polyphosphate with or without the use of a dense teflon membrane appeared to inhibit new bone ingrowth in the rat calvarial model;

3) calcium polyphosphate appeared to be a biocompatible material resulting in minimal histological inflammation at the implanted sites;

4) the 8mm defect size used in the rat calvarial model may not be suitable for evaluating the osteoconductive properties of synthetic particulate graft materials;

5) the use of a dense, non-resorbable teflon membrane may not prevent micromotion of particulate graft materials when the latter are used in 8mm defects in the rat calvarium;

6) dense teflon membranes used alone do not significantly impair bone formation in calvarial defects.
8) REFERENCES


Boyne, P.J., Use of HTR in tooth extraction sockets to maintain alveolar ridge height and increase concentration alveolar matrix. Gen. Dent. 45:470-473, 1995.


Pilliar, R.M., personal communication 1999.


