ORIGINAL RESEARCH

In-vitro properties of calcium phosphate cement as a bone grafting material

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Abstract

In 1980's researchers discovered CPCs (calcium phosphate crystals) which are a bioactive and biodegradable bone grafting material. Phases form after mixing in different compositions with different end products which are mainly two types; Brushite, and a Apatite. Bioactive glass can undergo dissolution in physiological solutions and form a hydroxycarbonated apatite like phase (this includes Octacalcium or Flouroapatite). Novel material can be made by mixing bioglass and $Ca(H_2PO_4)_2$ and have cements set to form hydroxyapatite or brushite produce HAP, brushite and fluorapatite forming cements. The aims and objectives of this study were to investigate the influence of storage media on the Calcium Phosphate Cements combined with bioactive glass, with respect to properties and phase formed and strength of development. Would the outcomes of storing in a media enriched in calcium and phosphate (that more closely mimics the in vivo conditions, Simulated body fluid) and Tris buffer solution. To See histological and structural that do in-vivo implanted cements show the formation of more hydroxyapatite and higher mineral contents. To determine mechanical properties does it result in higher compressive strength. Functionally does it aid the conversion of Octacalcium phosphate to Hydroxyapatite. Calcium phosphate was measured 0.98 gms and mixed with bioactive glass 1.02 gms and placed in a 6 by 4 Cylinder, placed in aoven. Cements were immersed into both TRIS buffer and Simulated body fluid solution for 1hour, 1day, 7days and 28 days .The compressive strength was determined by a Instron machine. Characterization of phases was seen by analysis through FTIR(spectoscopy) and X-ray diffraction microtomography (determine quantitative measurements of mineral concentration in hard tissue). It was seen that the storage media does have a influence in properties and phases formed.

Keywords: Calcium phosphate; Brushite; Apatite; Bioactive glass; TRIS buffer; Simulated body fluid.

Introduction

One of the prerequisites of periodontal regeneration is the formation of bone. Bone grafting is possible because the bone tissue, unlike many other tissues, has the ability to regenerate completely if there is sufficient space to grow into. As bone grows, it will generally replace the graft material completely, and result in a totally integrated region of new bone. The biological mechanisms providing a rationale for bone grafting are the following osteoconduction, osteoinduction, osteopromotion and osteogenesis.[1] Osteoconduction phenomena occurred when the bone graft material served as a scaffold for new bone growth and therefore it was perpetuated by the native bone. Osteoblast from the margin of the defect being grafted utilized the bone graft material as a framework upon which to spread and to synthesize new bone.[1] Bone grafting is a surgical procedure that was performed to replace the missing bone with a material from a patient's own body, an artificial, synthetic, or a natural substitute. Bone grafting was possible when the bone tissue had the ability to regenerate completely if sufficient space was

provided into which the bone can grow. As natural bone grows, it can generally replace the graft material completely, and result in a fully integrated region of new bone. Classification of the bone grafts based on various material groups are[2] Allograft-based bone graft entails allograft bone, can be used alone or incombination with other materials (e.g., Grafton®, OrthoBlast®).

Factor-based bone graft are natural and recombinant growth factors, which are used alone or in either in merging with other materials, such as transforming growth factor-beta (TGF-beta), Platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and bone morphogenetic protein (BMP). Cell-based bone grafts use cells to generate new tissue alone or are added onto a support matrix, for example, haemapoetic stem cells. Ceramic-based bone graft substitutes which include material likes calcium phosphate, calcium sulphate, and Bioglass used alone or in combination; for example, OsteoGraf®, ProOsteon®, OsteoSet®. Polymer-based bone graft used degradable and nondegradable polymers alone or in combination with other materials, for example, open porosity polylactic acid polymers Flexible hydrogel-hydroxyapatite (HA) composite has a mineral to organic matrix ratio, which approximates that of the human bone. Artificial bone can be created from ceramics, like calcium phosphates (e.g., HA and tricalcium phosphate), bioglass, and calciumsulphate are biologically active depending on the solubility in a physiological environment [3]Alloplastic grafts can be manufactured from hydroxyapatite, and was a naturally occurring mineral (a main mineral component of bone), made from bioactive glass. Hydroxyapatite was a synthetic bone graft, which was commonly used now due to its properties e.g., osteoconduction, hardness, and acceptability by bone. Calcium orthophosphates have been studied as bone repair materials for the last 80 years. Calcium phosphates are part of a group of bioactive synthetic materials and the most frequently used are the hydroxyapatite and the tricalcium phosphate materials. They are commonly used due to their osteoconductivity, crystallographic structures, and chemical composition similar to the skeletal tissue. They are therefore classified according to their 'resorbability' which was that extent of degradation *in vivo*. Hydroxyapatite in turn hasbeen described as "non resorbable" and tricalcium phosphate has been described as "resorbable"[4,5]. Calcium phosphate materials demonstrate a positive interaction with living tissue that included also the differentiation of the immature cells towards bone cells[5,6]. Calcium Orthophosphate cements (CPC) have been reported to form two major end products: a precipitated poorly crystalline HA or CDHA and DCPD (also called ''brushite'') and apatite cements. The final setting product of the cements was of the paramount importance as this would determine the solubility and, therefore, the *in vivo* bioresorbability. The main difference between the two cement types was the solubility of the end-product: brushite was 1–2 orders of magnitude more soluble than apatite's at a physiological pH and therefore brushite CPCs normally resorb faster than that of apatite. It has numerous properties which include osteo-transductive, e.g., after implantation calcium orthophosphate cements may be replaced by new bone tissue and was osteo-conductive. Calcium phosphate [Ca(H2PO4)2] cements (CPC) have been used for the treatment of non-weight bearing bone fractures or defects.

Bioactive glass has also been used in dentistry as a bone substitute and a number of therapeutic agents may be incorporated into the glass structure, for example fluoride, strontium, chlorine etc. Bioactive glass (BG) has been reported to form hydroxycarbonate apatite (HCA). These materials also exhibit excellent Osseo-integration with bone and therefore were originally developed for applications in bone regeneration. Strontium has been reported *in vitro* and *in vivo* to enhance the replication of pre-osteoblastic cells and decreases the activity and the number of osteoclasts. The intake of these strontium containing drugs lead to a greater deposition of calcium in bone and DNA and bone collagen synthesis are enhanced[7].

Novel materials may also be formulated by mixing a bioglass composition with CPC in order to improve the physical properties. The ideal outcome of the new product was to utilize the bioactivity and resorbability of a bioactive glass with the added clinical advantages of *in situ* setting and extrudability of CPCs. Strontium can be added to bioglass to some samples in 25% ratio as that enhances bone formation.

The aim of the present study was to investigate the influence of a storage media (Tris buffer solution and Simulated Body Fluid (SBF) on a modified CPC combined with a Bioactive glass composition, with respect to both its properties (e.g., compressive strength) and the phase formed (e.g., conversion of Octacalcium phosphate [OCP] to Hydroxyapatite [HA]) and compressive strength

Materials and Methods

To investigate the influence of storage media on the Calcium Phosphate Cements, with respect to *properties and phase formed andstrength of development).What would be the outcomes of storing in a media enriched in calcium and phosphate (that more closely mimics the in vivo conditions, e.g., Simulated body fluid)* **Structural**; Do in-vivo implanted cements show the formation of more

hydroxyapatite and higher mineral contents. **Mechanical**; does it result in a higher compressive strength of the new material calcium phosphate and bioactive glass.**Functional**; does it aid the conversion of Octacalcium phosphate to Hydroxyapatite.

The CPC/Bioglass composition was formulated by measuring 0.98 gm of calcium phosphate and mixing with 1.02 gm. of bioactive glass. The cement paste was mixed and packed into 6 by 4 cylindrical steel moulds and placed in an incubator at 37^0c for 120 minutes. The cylinders were removed from the moulds and immersed in 50 ml of either TRIS buffer solution or SBF at 37^0c for 1hour, 24 hours, 7 days and 28 days The testing of the compressive strength (Mpa) of the samples (n=8) was by an Instron universal testing machine type 5567 and characterization of the different phases of the samples was by FTIR spectrum and X-rayDiffraction in order to determine the quantitative measurements of the mineral concentration in hard tissue.

Results

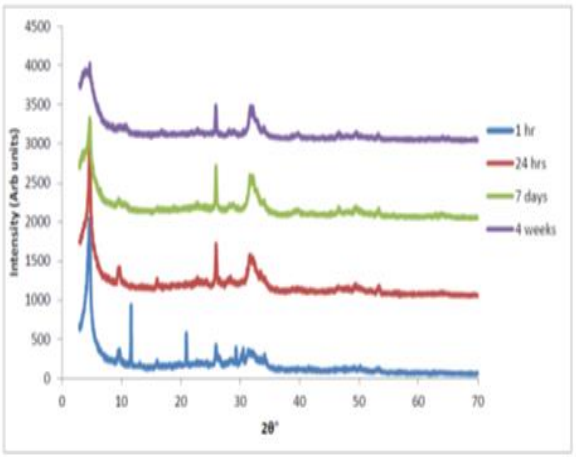

Fig 1: X-Ray Diffraction(XRD) of the 0% Sr cement after immersion in SBF : shows as a function of time had a XRD pattern that matched that of hydroxyapatite but in addition it also had a sharp diffraction peak at 4.7 degrees two theta which corresponded to the water layer in octacalcium phosphate (Ca8(PO4)6 H2.5H2O (OCP) which was thought to be a precursor to hydroxyapatite(HA) formation in the biomineralisation of tooth and bone [8] .The characteristic diffraction lines for apatite increased for longer immersion times and the diffraction line at 4.7 degree two theta characteristic of OCP decreased with immersion time.

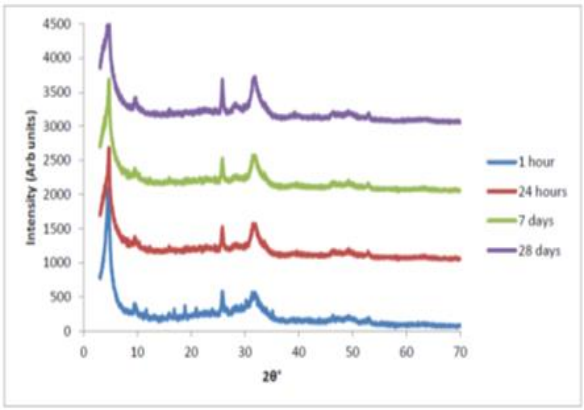

Fig 2: XRD of the 25% Sr cement after immersion in SBF Very similar behaviour was observed as for the 0% Sr glass. However the OCP peak at 4.7o two theta had now completely disappeared after four weeks of immersion and the diffraction lines for apatite were slightly sharper. This indicated that the 25% Sr cement was converting from OCP to hydroxyapatite more rapidly.

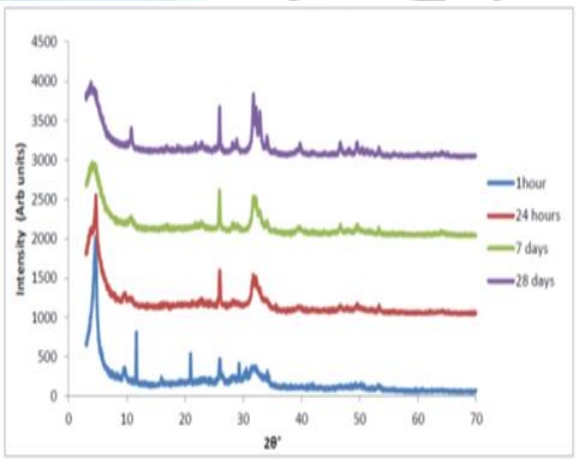

Fig 3: XRD of the 0% Sr cement after immersion in Tris The phase development and phases present in the 25%Sr cement immersed in Tris buffer were similar to the phases observed on immersion in SBF.

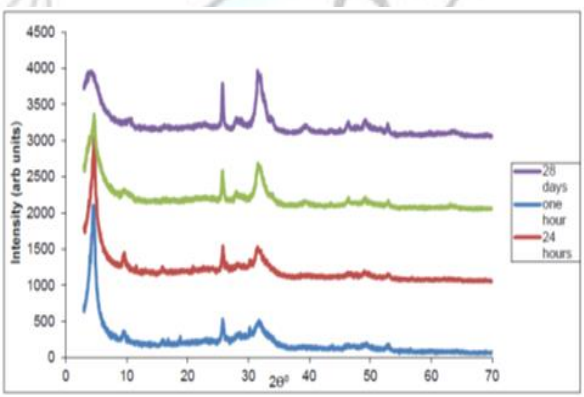

Fig 4: XRD of the 25% Sr cement after immersion in Tris: The phase development and phases present in the 25%Sr cement immersed in Tris buffer were similar to the phases observed on immersion in SBF.

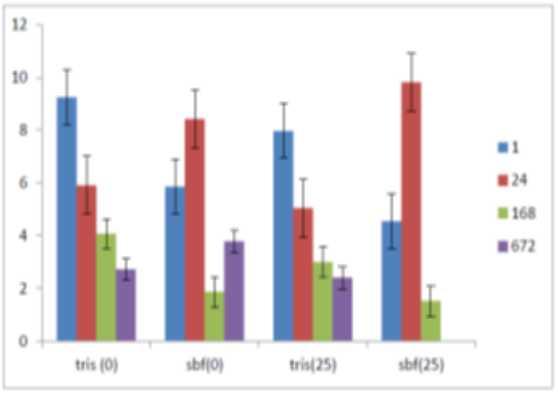

Fig 5: Shows the compressive strength for the cements after immersion in SBF. The cements exhibit an increasing compressive strength on immersion in SBF from 1 hour to 24 hours but then a marked decrease from 24 hours to 168 hours. This contrasted markedly with the behaviour found on immersion in Tris buffer. The reduction in the compressive strength has been associated with the conversion of OCP to HA for conventional; calcium phosphate cements.

The 4.7º two theta line of OCP was elucidating and the results would suggest that immersion in Tris buffer compared to immersion in SBF favoured the conversion of OCP to hydroxyapatite. It was also observed that the presence of calcium in SBF does not accelerate the conversion process as expected and in fact the conversion was slower in SBF compared to Tris buffer. In the compressive strength data cements exhibited an increasing compressive strength on immersion in SBF from 1 hour to 24 hours but then a marked decrease from 24 hours to 168 hours. This contrasted markedly with the behaviour observed on immersion in Tris buffer. The reduction in the compressive strength has been associated with the conversion of OCP to HA for conventional calcium phosphate cements.

Table 1: Presence of OCP as a function of time

	Time			
cements	1	24	168	672
Sr0Tris	√	√	x	x
Sr0SBF	√	√	x	√
Sr25Tris	√	√	√	x
Sr25SBF	√	√	√	√

Discussion

There are limited data with regard to the novel materials used in the present study although a recent study by Sadiasa*et al.*[9] in which the investigators used injectable bone substitutes modified by placing bioactive glass powders (synthesized via a ultrasonic energy-assisted hydrothermal method) to the calcium phosphate-based bone cement in order to improve its biocompatibility. The present study did not use this particular methodology (e.g., using the ultrasonic energy assisted hydrothermal method) and therefore it would be interesting to speculate on the differences between the methods. For example in the Sadiasa*et al.* [8] study the injectable bone substitutes were initially composed of a powder component (tetracalcium phosphate, dicalcium phosphate dihydrate and calcium sulfate dehydrate) and a liquid component (citric acid, chitosan and hydroxyl-propyl-methyl-cellulose) to which was added various concentrations of bioactive glass: 0%, 10%, 20% and 30%. By way of comparison in the present study the liquid and powder ratio was different and the bioactive glass content was with strontium 0% and Strontium 25%. Furthermore in the Sadiasa*et al.* [9]study the setting time and compressive strength of the injectable bone substitutes was evaluated and it was reported that the bone substitute improved (in terms of compressive strength) with the increased bioactive glass content. Another difference between the two studies was that the surface morphologies of the material was not evaluated by scanning electron microscope (SEM) before and after placing the samples into simulated body fluid in the present study.

There was however agreement between the two studies with regard to the observation that there was an increase in the apatite formation as shown by x-ray diffraction. The *in vitro* biocompatibility of the injectable bone substitutes would therefore appear to improve with the placement of bioactive glass as the proliferation/adhesion behaviour of cells on the material increased as reported by Sadiasa*et al*[9]. Another element to the Sadiasa*et al*[9]study was that human gene markers were expressed by real time-polymerase chain reaction and the samples were reported to promote cell viability which appeared to demonstrate an improved biocompatible as the concentration of bioactive glass was increased. This aspect was not explored in the present study. In addition the In vivo biocompatibility of the various samples containing 0% and 30% bioactive glass was also evaluated using a Micro-CT and histological staining after 3 months of implantation in male rabbits' femurs. An interesting observation from the that there was no inflammatory reaction and significant bone .Another study of interest by Yu *et al*[10] also evaluated a novel injectable bioactive cement in order to determine its composition, microstructure, setting time, injectability, compressive strength and to observe the behaviour of the material in simulated body fluid an aspect which was similar to the present study. The *in vitro* cellular

responses of the osteoblasts and the *in vivo* tissue responses following the implantation of calcium phosphate cement and bioglass in the femoral condyle defects of rabbits was also investigated by Yu *et al.*[10] As mentioned previously the present study did not undertake any similar procedures in an animal model to determine whether the product would be suitable in terms of biocompatible which in retrospect would have been an important component to the investigation. In the Yu *et al.* study CPC-BG was observed to have a retarded setting time and also an improved injectability and mechanical properties than CPC alone. It was also observed that a new Ca-deficient apatite layer was deposited on the composite surface after it was placed in SBF for 7 days. It was also observed that the CPC-BG samples demonstrated a significantly improved degradability and bioactivity compared to CPC in the simulated body fluid (SBF). The improvement in cell attachment, proliferation and differentiation on CPC-BG were superior to cells observed on CPC. Macroscopic evaluation, histological evaluation, and micro-computed tomography (micro-CT) analysis observations also demonstrated that CPC-BG enhanced the efficiency of new bone formation in comparison with CPC alone. No histological evaluation or proliferation studies were undertaken in the present study. The Yu *et al.*[10]study concluded that a novel.HydroSet represented the next generation in bone substitute technology and was reported to be an excellent bone substitute solution for a number of clinical applications and surgical specialties. HydroSet was a self-setting calcium phosphate cement and contained apatite which converted to hydroxyapatite (the principal mineral component of bone).

The crystalline structure and porosity of hydroSet indicated that it was an effective osteoconductive and osteointegrative material, with good biocompatibility An Ovine Implant study in Britain was undertaken by Hill *et al.*[11] on bioactive glass (with three kinds of glasses) plus Calcium phosphate and Hydroset. The research group implanted the material into femur sites both right and left sides distal and proximal. The implantation was placed in one animal for six weeks and in six animals for twelve weeks. Scattered SEMs demonstrated that for the 6 weeks ovine implanted there was relatively little resorbtion of the cement for all cements including Hydroset. No thermal emissions (isothermic) were observed during the hardening phase at 6 weeks and three months. Analysis was done using XMT, Histology, Peripheral quantitative computed tomography (pQCTBack). It was observed that there was excellent osseointegration with bioglass cements and that HydroSet was more radio opaque due to higher density at 6 weeks. New bone growth surrounded all thecements and interdigitation of cements with the host bone The novel cements were observed to set in-vivo and 'wash out' of the cement was not witnessed and excellent osseointegration of all cement compositions was evident. New bone formation surrounding implanted cement high level of resorption and remodeling at twelve weeks octacalcium phosphate & hydroxyapatite forming cements brushite Cements was observed.

Conclusion

The results from the present study demonstrated that the media influenced how compressive strength changes and storage in SBF resulted in an increase in the compressive strength initially compared to a reduction in Tris buffer. The presence of strontium inhibited the formation of brushite probably because the Sr2+ cation cannot replace Ca2+ ions in the Brushite crystal lattice. It would therefore appear according to the results obtained that storing the combined CPC/Bioglass composition in Tris buffer solution and Simulated Body Fluid had an influence on both the compressive strength and the phase formed over the media used to store the cements influenced the phases formed and in particular the conversion.

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