Bioactive Composite Materials for Bone Augmentation

K.E. Tanner

Departments of Mechanical and of Civil Engineering, James Watt South Building,

University of Glasgow, Glasgow, G12 8QQ, UK

Corresponding Author:
Professor K.E. Tanner FREng
Departments of Mechanical and of Civil Engineering,
James Watt South Building,
University of Glasgow,
Glasgow,
G12 8QQ,
UK
Phone: +44-141-330-3733
Fax: +44-141-330-4343
e-mail: e.tanner@eng.gla.ac.uk

ABSTRACT
Biomaterials have been used to repair the human body for millennia, but it is only since the 1970s that man made composites have been used. Hydroxyapatite reinforced polyethylene is the first of the "Second Generation" biomaterials that have been developed to be bioactive. The mechanical properties have been characterised, using quasistatic, fatigue, creep and fracture toughness testing and these studies have allowed optimisation of the production method. The *in vitro* and *in vivo* biological properties have been investigated with a range of filler content and have shown that the presence of sufficient bioactive fillers leads to a bioactive composite. Finally the material has been applied clinically initially in the orbital floor and then in the middle ear. From this initial combination of hydroxyapatite in polyethylene other bioactive ceramic polymer composites have been developed.

Keywords: Bioactive composites, hydroxyapatite, polymers, bone replacement, biomechanical properties, biological properties

INTRODUCTION

Biomaterials have been used since prehistoric times, such as the use of nacre (mother of pearl) by the Mayans to integrate dental implants into bone (Westbroek & Marin, 1998). While in the 19th Century silver coated steel fracture fixation plates used to repair patella fractures in the USA (Parkhill, 1897). However, in the 1970s the early hip and knee replacements started to fail in significant numbers. Prior to this time, joint replacements had been confined to an elderly and very low activity patient cohort and the implants were able to outlive their recipients. The implants were loosening with large areas of osteolytic (bone) resorption, so much so that at revision surgery the implants could be removed with minimal effort. The rationale for these failures was considered in detail and the concepts of "cement disease" and "stress shielding" were developed (Engh *et aI*., 1987; Harrigan *et al*., 1992).

"Cement disease" was considered a biological response to the long term application of bone cement (Charnley, 1975; Jones and Hungerford 1987). Fatigue of bone cement leads to the production of cement particles and the release of the zirconia or barium sulphate particles used as radiographic opacifiers. These opacifier particles then become entrapped in the articulating surfaces of the prosthesis and accelerate the production of wear particles of the polyethylene and metal components of the joint replacement. Cement, cement opacfier, polyethylene and metal particles have all been found in the osteolytic lesions surrounding loose joint replacements. A dose dependant response has been seen with over 10^{10} polymer particles per gram of wet tissue being found in osteolytic lesions (Kadoya *et al*., 1997, Kobayashi *et al*., 1997). Other studies have shown that these wear particles can cause macrophages to initiate bone resorption (Quinn *et al*., 1992; Sabokbar, *et al*., 1996).

Stress shielding is due to the mis-match between the stiffness of bone which has a Young's modulus of 7-25GPa (for example, Bonfield & Grynpas, 1977; Currey, 1998) and 210GPa for stainless steel implant stems. Various methods of solving this problem were considered including changing the size and shape of the stem to reduce the differences in the structural stiffness of the implant and the surrounding bone and changing the implant material from steel to commercially pure titanium or Ti-

6%Al-4%V alloy (Sarmiento *et al*., 1979).

At this time Katz and Lakes (Lakes et al 1977; Lakes & Katz 1977a&b), Currey (1969 & 1999) and Bonfield (Bonfield & Li, 1966 & 1967; Bonfield & Grynpas 1977) were all investigating the mechanical properties of bone, its structure and anisotropy. Katz presented the idea that bone itself is "a composite of a composite of a composite" working from the macroscale to the nanoscale (Katz *et al*., 2007). Thus Bonfield and colleagues at Queen Mary College, University of London developed the concept of manufacturing an analogue of bone itself of polyethylene reinforced with hydroxyapatite (Bonfield *et al*., 1980, 1981; Bonfield, 1988). All the biocompatible metals and bioceramics available at that time are stiffer than bone, while the polymers are more flexible, both cases leading to stress concentrations at the implant bone interface. Bonfield and colleagues also realised that if they used polyethylene (PE) and hydroxyapatite (HA) the material could potentially be bioactive due to the presence of the HA and that approval for clinical use should be simplified as the individual materials were already approved for clinical applications. The composite with 40vol% HA was given the trade name HAPEX™, thus in this paper HA/PE is used to describe materials with different filler contents and HAPEX™ the 40vol% HA composite used clinically.

Bonfield (1988) described bioinert materials such as stainless steel and PE as the "First Generation" biomaterials and the materials aiming to interact beneficially with the body, such as HAPEX™and Bioglass® as "Second Generation" biomaterials while Hench and Polak (2002) have described resorbable and bioactive materials as "Third Generation" biomaterials, as they "help the body heal itself".

MATERIAL PRODUCTION

In composites the mechanical properties are strongly controlled by the production method. In collaboration with Brunel University twin screw compounding extrusion was used to add HA into high density PE (HDPE). Through all the studies HDPE was used as it can be processed by compounding and extrusion unlike ultra high molecular weight PE, which can only be compression moulded from powder. The initial studies used calcined bovine bone ash with a mean particle size of 10μm (Bonfield *et al*., 1980, 1981). The bone ash was replaced with synthetically produced hydroxyapatite with a smaller mean particle size of 4μm, which did, to some extent, improve the mechanical properties, but also allowed more reproducible and controllable starting HAs to be used. Twin screw compounding extrusion generates high shear forces ensuring that upto 50vol% HA could be compounded into the polyethylene. Tensile testing showed that the increase in HA content lead to increases in the Young's modulus from 1.3±0.2GPa for the unfilled polyethylene through to 7.7±1.3GPa for 50vol%, however at 45vol% the tensile strength started to drop and the failure mode changed from ductile to brittle, as instead of the material consisting of HA particles totally surrounded by PE which acted as matrix, HA to HA particle contact occurred (Table 1). Thus from this time the all studies were confined to no higher than 40vol% HA. In comparison the volume content of bone mineral of human bone is between 45 and 50vol% and the bone mineral particles are nanometre size with various estimates for particle size

including 5×5×20 and 5×10×40 nm (Currey 1998).

Table 1 Mechanical properties and bioactivity of hydroxyapatite reinforced polyethylene composites (from Bonfield, 1988)

Hydroxyapatite volume fraction	Young's Modulus /GPa	Strain to Fracture /%	Fracture mode	Bioactivity (bone apposition around implant)
0	1.3 ± 0.2	>90	Ductile	×
10	1.4 ± 0.2	79 ± 10	Ductile	??
20	2.0 ± 0.1	50 ± 4	Ductile	??
25	2.5 ± 0.2	43 ± 3	Ductile	??
30	3.0 ± 0.2	34 ± 5	Ductile	??
35	3.7 ± 0.4	32 ± 8	Ductile	??
40	4.4 ± 0.7	29 ± 5	Ductile	√
45	5.9 ± 0.5	7 ± 3	Brittle[a]	??
50	7.7 ± 1.3	3 ± 1	Brittle[b]	√

[a] $K_{IC} = 2.9 \pm 0.3$ MN $m^{-3/2}$
[b] $K_{IC} = 2.4 \pm 0.2$ MN $m^{-3/2}$

Various studies have investigated the effect of altering particle size and particle morphology. Wang *et al*. (1994 & 1998b) compared two grades of spray dried HA: one with a mean particle size of 4.14μm and a specific surface area of 8.27m^2g^{-1} and the other with a mean particle size of 7.32μm and a specific surface area of 7.61m^2g^{-1}, but both produced by spray drying leading to each particle being an agglomerate of nanocrystallites. Minor differences in the tensile strength were seen for the different filler particles and the filler contents investigated, however the tensile and torsional stiffnesses were higher with the smaller filler particles. Joseph *et al*. (2002a) investigated the effect of HA particle shape and used a spray dried and a sintered grade of HA powder with similar particle size distributions and mean particle sizes of 4.02 and 3.58μm respectively, but a factor of over 10 difference in the specific surface areas. They compound extruded the composite and then characterised the rheological properties. The HA/PE manufactured with the spray dried HA had substantially higher viscosity than that manufactured using the sintered HA (Figure 1). They suggested that the difference was produced by the amount of PE involved in coating the HA particles and thus how much was free to act as matrix between the coated particles (Figure 2). To improve the impact properties of the composites Zhang and Tanner (2008) produced composites using sintered particles of HA for comparison to the spray dried HA filled composites. These two HAs were again chosen to have similar particle size and particle size distribution with mean values of 3.80μm and 4.46μm, but different specific surface areas of 13.536 and 0.965m^2g^{-1} for the spray dried and sintered HA, respectively. Composites with 20, 30 and 40vol% filler were manufactured and subjected to impact testing, using an instrumented falling weight impact tester. For all volume fractions both the initiation and propagation energies were higher with the sintered HA with the lower surface area. SEM of the fracture surfaces

showed that the smoother particles allowed more polyethylene drawing during the fracture process (Figure 3) in agreement with the concept of Joseph *et al.* (2002a) of the effect of specific surface area.

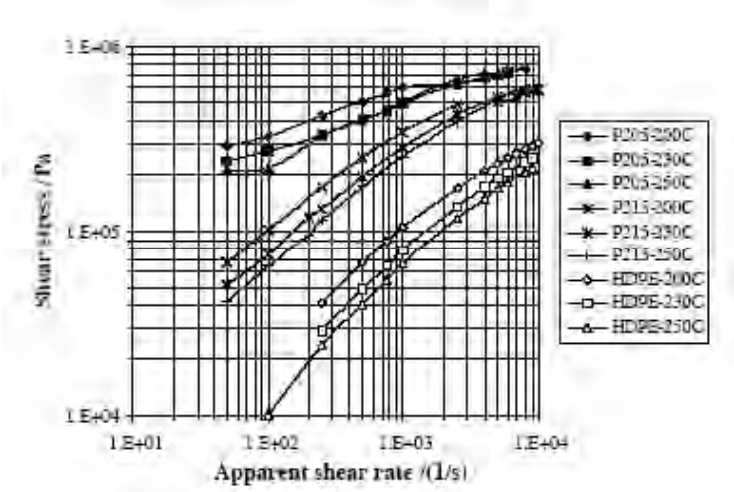


Figure 1 Apparent shear viscosity of 48,650 Mw PE reinforced with 40vol% spray dried HA (P205) and sintered HA particles (P215) at temperatures between 200 and 250˚C. (From Joseph *et al*., 2002a).

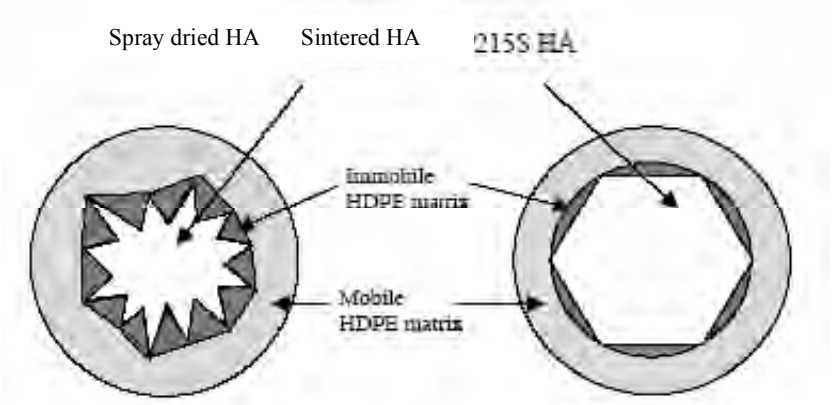


Figure 2 Schematic showing the different amounts of matrix used to coat high specific surface area spray dried HA (P205) versus sintered low specific surface HA (P215S) (From Joseph *et al*., 2002a)

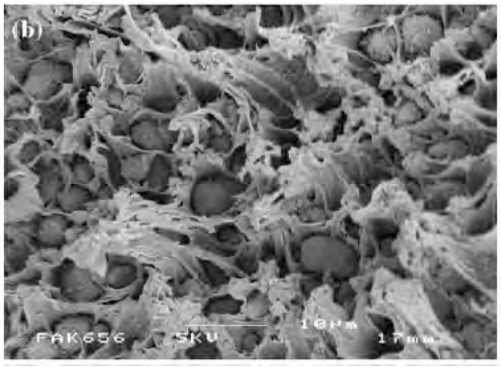
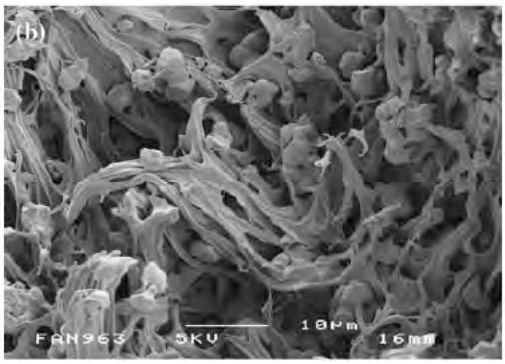
a)  b) 

Figure 3 Impact facture surfaces of 30vol% a) spray dried HA and b) sintered HA in polyethylene, showing the longer draw fibrils with the lower surface area filler particles. (From Zhang & Tanner, 2008)

The effect of HDPE molecular weight was investigated by both Joseph *et al*. (2002b) and Zhang and Tanner (2003). Joseph *et al*. (2002b) were interested in producing an injection moulding grade of HA/PE, thus they used three grades of HDPE with melt flow indices between 7.6 and 26 g $(10min)^{-1}$, that is molecular weights between 72,350 and 48,650 respectively and filled these PEs with 0, 20 and 40vol% spray dried HA. The effect of the increasing filler content or the molecular weight was to increase the shear stress at any shear rate during injection moulding. Zhang and Tanner (2003) used two different HDPEs with melt flow indices of 6 and 26 g $(10min)^{-1}$ that is molecular weights of 249,233 and 48,650 respectively. Under drop weight impact testing the effect of molecular weight was substantial with the energy absorbed being 5.1J for the non-filled lower molecular weight HDPE compared with 93.6J for the non-filled high molecular weight HDPE. The impact energy absorbed decreased to 7.6J for the high molecular weight HDPE and to less than 2J for the lower molecular weight HDPE when these were filled with 40vol% HA. As with the studies changing the HA filler morphology the tougher material showed longer drawn polymer fibrils on the fracture surfaces.

MATERIAL MODIFICATIONS

Failure of such composites is initiated by debonding of the filler matrix interface (Friedrich and Karsch, 1983; Bonfield *et al*., 1998). Chemically coupling the HA and the PE phases to increase the mechanical properties was investigated by Deb and colleagues (Deb *et al*., 1996; Wang *et al*., 2000; Wang & Bonfield, 2001) who silanated the HA phase and acrylic acid coupled the PE. Silane coupling without acrylic acid grafting reduced the Young's modulus and did not affect the strength however, the Young's modulus and tensile strength were increased when both silane coupling and grafting were combined. However, the most substantial difference was the increase in the strain to failure at both 20 and 40vol% filler by silane coupling with or without acrylic acid grafting (Table 2).

Table 2 Mechanical properties of HA/PE composites at 20 and 40vol% HA showing the effect of silane coupling and acrylic acid grafting showing significant differences at * $p<0.05$ and ** $p<0.01$ when compared to the non-coupled, non-grafted material of the same volume fraction (from Deb *et al.*, 1969).

Filler volume content	Silane coupling	Acrylic acid grafting	Tensile Strength /MPa	Young's Modulus /GPa	Strain to Fracture /%
20	No	No	17.77 ± 0.09	1.60 ± 0.02	34.0 ± 9.5
20	Yes	No	17.01 ± 0.19**	1.54 ± 0.02*	>100
20	Yes	Yes	19.97 ± 0.07**	1.81 ± 0.05**	39.7 ± 1.5
30	No	No	22.67 ± 0.17	4.29 ± 0.17	2.6 ± 0.4
30	Yes	No	22.08 ± 0.05	3.66 ± 0.20**	7.8 ± 0.6**
30	Yes	Yes	23.16 ± 0.40*	3.87 ± 0.21*	6.8 ± 0.6**

A different approach to increase the mechanical properties was used by Ladizesky *et al*. (1997), McGregor *et al*. (2000), (Wang *et al*., 2000) and Bonner *et al.* (2002). They hydrostatically extruded HAPEX™ to orientate the polymer chains and thus produce an anisotropic material. Extrusion ratios of up to 11:1 were used to increase the stiffness and flexural strength. While the stiffness continued to increase with increases in the extrusion ratio the flexural strength and ductility peaked at an extrusion ratio of 8:1 (Figure 4). All the materials were ductile and at the highest extrusion ratios the failure was due to fibrillation parallel with the extrusion direction, rather than actual fracture of the material.

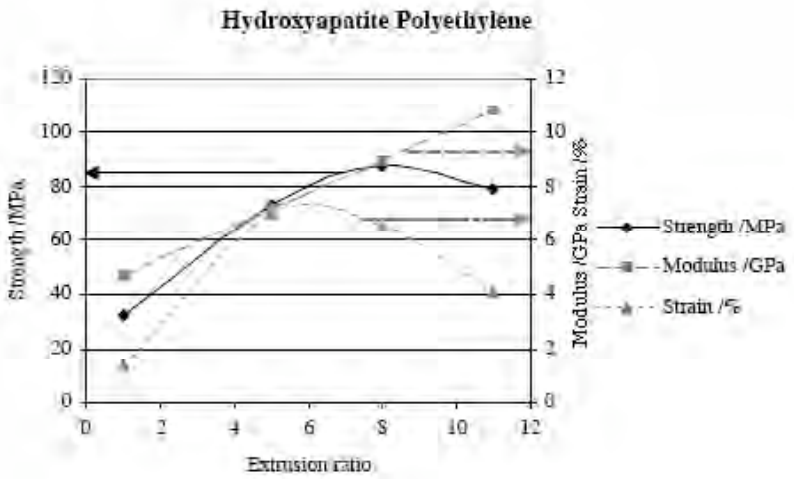


Figure 4 Effect of extrusion ratio on the flexural strength (♦), flexural modulus (■) and strain to failure (▲) of hydrostatically extruded 40vol % HA/PE (from Bonner *et al*., 2000)

More recently selective laser sintering (SLS) was used to manufacture porous scaffolds of HA/PE and HA/polyamide (HA/PA) composites (Savalini *et al*., 2006 & 2007; Hao *et al*., 2006a, 2006b, 2007, 2009; Zhang *et al*., 2008). Twin screw extrusion was used to process the composites which were then ground down into particles of the order of 40 to 300μm across. These composite particles were then sieved into two groups, those with particle

size greater or less than 165μm and these were selectively laser sintered. A purpose built SLS system was manufactured to allow infrared heating of the powder to just below the polymer melt temperature and then low laser power (<10W) could be used to perform the sintering. By optimising the particle size and laser power scaffolds of 20vol% HA/PE and 30vol% HA/PA could be sintered successfully. Infiltration of the samples with resin and subsequent sectioning and polishing for microscopy showed that the material was all open celled which is essential for tissue ingrowth (Figure 5). Optimisation studies showed that 20vol%HA in PE and 30vol% HA in PA produced the greatest consolidation. Dynamic mechanical analysis was used to measure the mechanical properties as this allowed much smaller test samples to be used (Zhang *et al*., 2008). The stiffness was lower than of the fully dense material, but still had moduli of 0.7GPa at 37˚C compared to 2GPa for the fully dense compression moulded HA/PE composite.

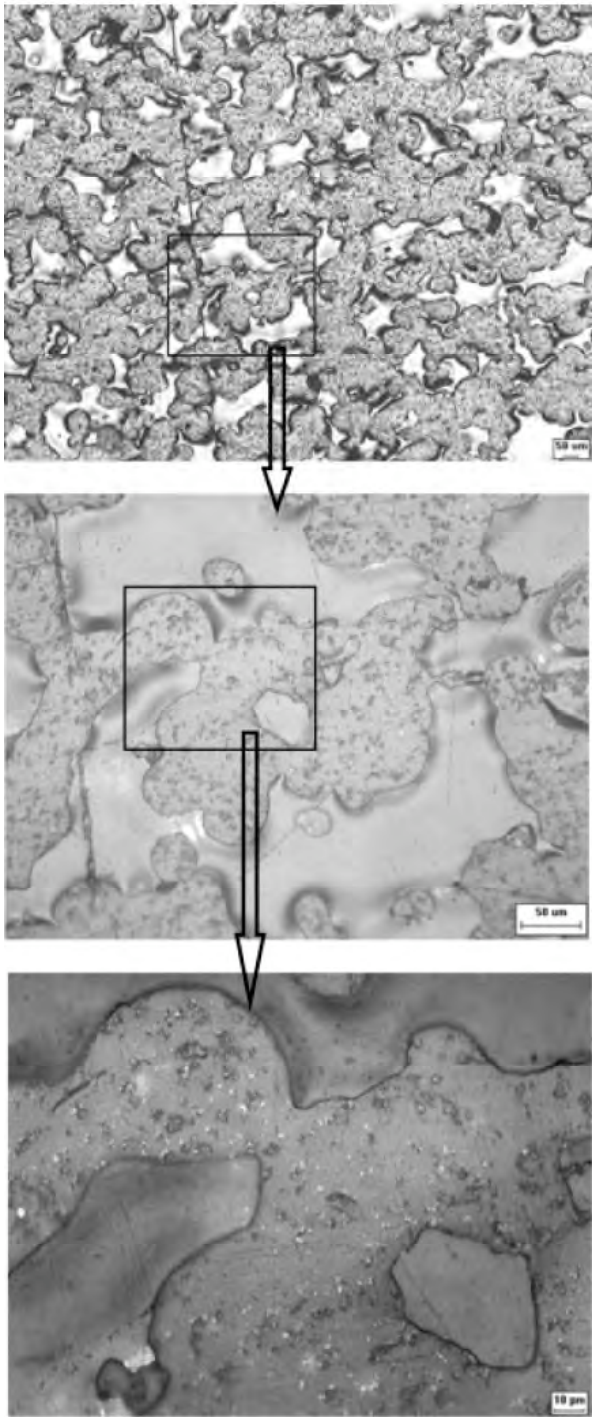
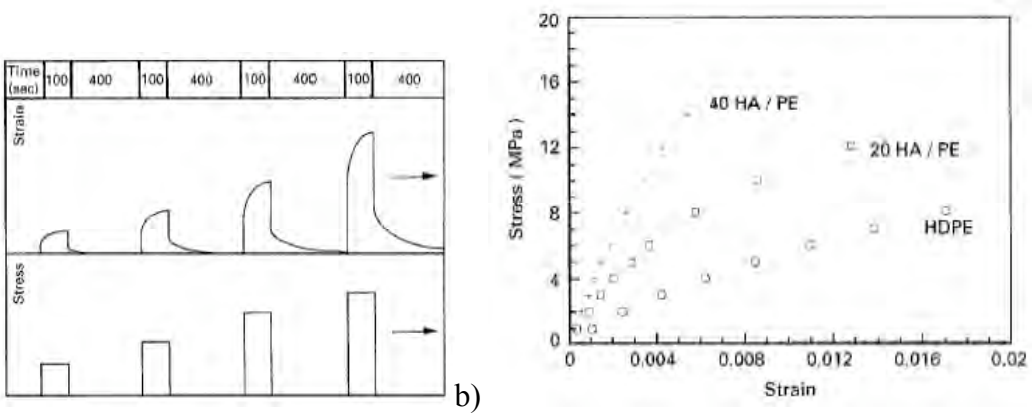


Figure 5 Optical micrographs of selectively laser sinterered 20vol% HA in PE showing the infiltration of the resin and thus that it is and open celled material with each particle composed of HA/PE composite. Scale bars are 50µm, 50µm and 10µm respectively (From Zhang *et al*., 2008).

MECHANICAL TESTING

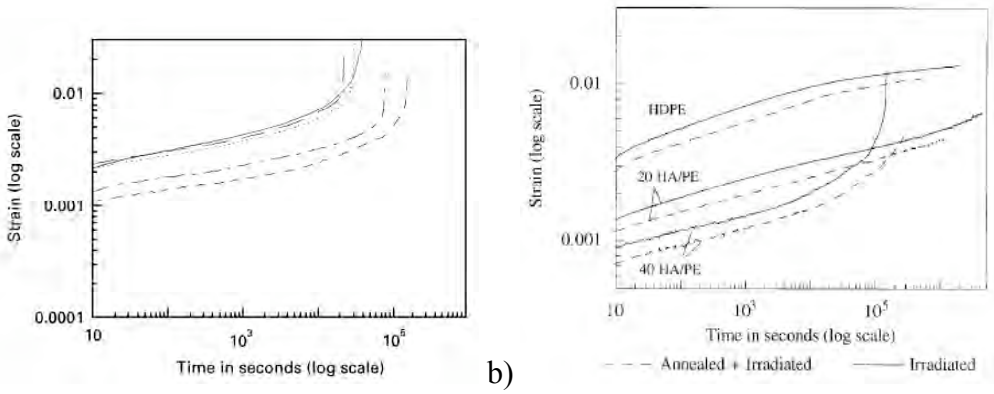
The initial mechanical testing concentrated on quasi-static testing to optimise the production methods using compression moulded samples although some both injection moulded samples were tested. Implants are subjected to both creep and fatigue loading *in vivo*. Suwanprateeb *et al.* (1995) investigated the creep behaviour at 37˚C in saline and started with isochronous testing, where a

small stress is applied for 100s and then measuring the strain at the end of the loading time, followed by unloading for 400s and then applying a higher strain for 100s and continuing with gradually increasing stress levels to produce isochorous stress-strain curves (Figure 6). These isochronous tests allowed basic comparison of the creep behaviour of the difference volume fracture fillers and the optimisation of load levels for conventional long term creep testing. They found that increasing the filler content from 0 to 20vol% or 20 to 40vol% halved the creep strain for a given applied load, however these increases in filler content also decreased the time to creep rupture. In subsequent studies (Suwanprateeb *et al*., 1997, 1998) they showed that pre-soaking the composite in saline at 37˚C increased the strain deformation due to the plasticising effect of the absorbed liquid, but that gamma irradiation decreased the creep due to the cross-linking of the polymer and finally that thermal annealing also decreased the creep and increased gthe time to creep rupture (Figure 7).



a) b)

Figure 6 Isochronous creep testing of HAPEX showing a) the loading regime and b) the results obtained for high density polyethylene (HDPE) and reinforced with 20 (20 HA/PE) and 40vol% HA (40 HA/PE) (from Suwanprateeb *et al*., 1995).

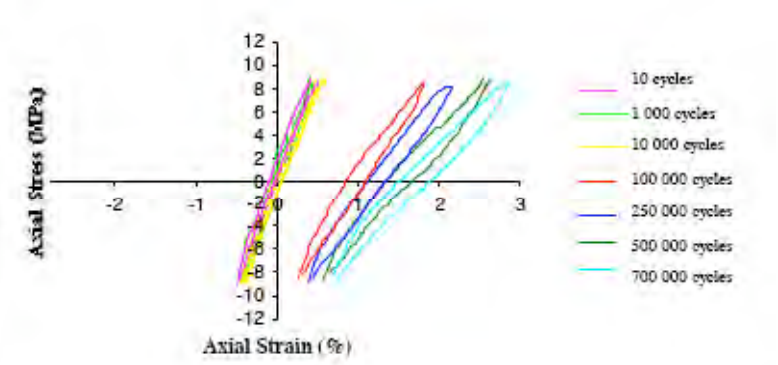


a) b)

Figure 7 a) Influence of immersion upon the creep behaviour of 2.5Mrad γ-irradiated 40 HA/PE at 6 MPa applied stress at 37˚C in Ringer's solution: ---- non-immersed, - · - 1 day, —— 7 days, ····· 90 days, ——150 days (from Suwanprateeb *et al.,* 1997) and b) effect of thermal annealing on the creep behaviour (from Suwanprateeb *et al*., 1998).

Ton That *et al*. (2000 a & b) studied the fatigue behaviour of HAPEX™ under both uniaxial tension-compression and when combined with fully reversed torsional loading. As to be expected for a filled polymer the

tensile phase of the loading caused more damage to the material than the compressive phase as the interface between the polymer and the HA opened up. This behaviour lead to non-symmetrical stress strain loops with dynamic creep and increased energy absorbed per load cycle. However, fully reversed torsional loading produced symmetrical loops which reduced in secant modulus and increased in energy absorbed as the material failed (Figure 8). Failure was seen first as a reduction in the secant modulus followed by an increase in the energy absorbed per load cycle. Increasing the phase angle between the axial and torsional loading increased the fatigue life and as with the quasistatic and creep testing, failure was by debonding of the HA from PE and drawing of polyethylene fibrils between the filler particles. Joseph & Tanner (2005) performed uniaxial tensile only fatigue tests, in saline at 37˚C, with the maximum stress being 75% of the ultimate tensile strength, on 40vol% HA/PE manufactured using both spray dried and sintered HA. They found that the energy absorbed per load cycle was higher and the secant modulus was lower with the sintered filler particles. These differences lead to earlier initiation of failure with the sintered filler particle composite.



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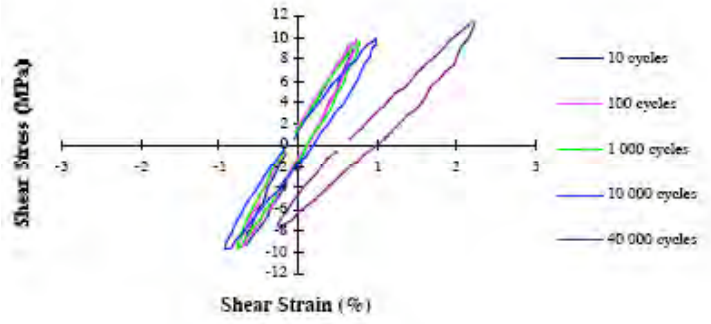


b)

Figure 8 Stress strain loops for fully reversed a) tension compression at ±50% of ultimate tensile strength and b) torsion at ±50% of ultimate shear strength (from Ton That *et al.*, 2000a)

Nazhat *et al.* (2000) used dynamic mechanical analysis (DMA) at 1HZ and using a temperature scan between 20 and 100˚C, of different volume fraction HA/PE using the same spray dried HAs with different particle sizes and the same PE as used by Wang *et al.* (1998b). Similar to other studies, they found that increasing the filler content increased the stiffness and that the smaller particle size lead to higher modulus for a given volume fraction.

The use of DMA allowed the damping to be measured as tanδ, the ratio of the loss modulus (E") to the storage modulus (E') and this was found to increase with increasing temperature, but to be reduced by higher filler content with minimal differences between the two types of filler particles.

Eniwumide *et al*. (2004) used compact tension testing to measure the fracture toughness of composites manufactured with two different molecular weight PE and both sintered and sprayed dried HA. They showed that testing the material at 37˚C rather than room temperature increased the fracture toughness from 1.30±0.04 MN $m^{-3/2}$ to 1.34±0.07 MN $m^{-3/2}$ for 40vol% HA/PE manufactured with 240,000 molecular weight PE. When the molecular weight was reduced to 50,000 the fracture toughness decreased to 0.5 MN $m^{-3/2}$ while decreasing the specific surface area of the filler particles increased the fracture toughness.

To elucidate the failure mechanisms Guild and Bonfield (1993 & 1998) used microscale FEA modelling of spherical filler particles in a polymeric matrix. The tensile modulus estimated from the FEA analysis is slightly lower than that measured experimentally at intermediate filler contents. They showed that the highest stresses occurred at the poles of the filler particles thus the failure started by debonding of the filler from the polymer and thus that the filler particles are seen between drawn fibrils as has been seen from mechanical testing. They also showed the increase in stress concentration at about 40vol% filler in agreement with the experimental results presented in Bonfield (1988) (Figure 9).

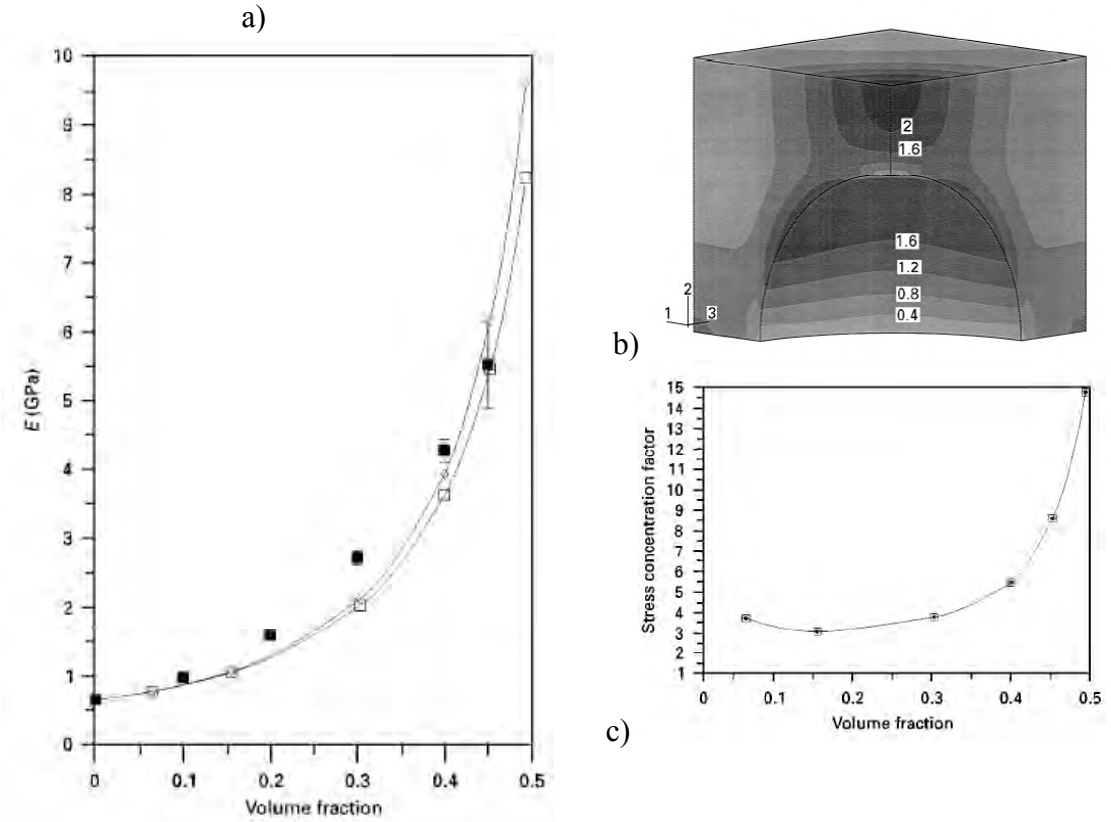
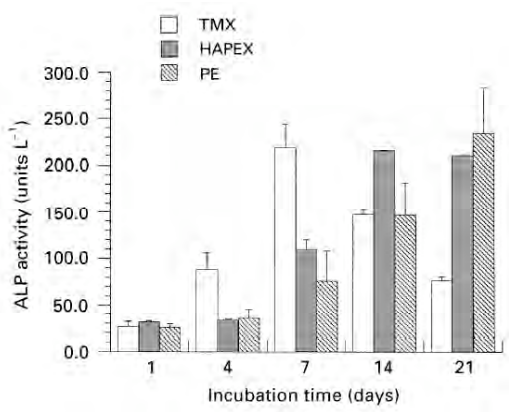


Figure 9 a) Comparison of the Young's modulus of HA in PE measured experimentally (■) and predicted using FEA modelling with the PE bulk modulus at 5GPa (◊) and 10GPa (□), b) contours of stress concentration of the von Mises stress in the polyethylene matrix viewed from the centre of the HA sphere, with the sphere removed and c) effect of volume fraction of HA spheres on the stress concentration factor (from Guild & Bonfield, 1998).

IN VITRO TESTING OF BIOACTIVITY

When HAPEX™ was initially developed cell culture on biomaterials was insufficiently developed to be used to assess the biological response to materials. Thus the initial biological assessment was performed *in vivo* (Doyle *et al*., 1990). As cell culture studies on biomaterials developed *in vitro* studies of HAPEX™ were performed. Di Silvio and colleagues (Huang *et al*. 1997 b & c; Di Silvio *et al*. 1998; 2002 a & b; Dalby *et al*., 1999, 2000, 2002) cultured osteoblasts obtained at elective surgery from human femoral heads on various HA/PE composites and showed that the material was biocompatible. Huang *et al*. (1997b) and Di Silvio *et al*. (1998) also showed that the osteoblasts proliferated and matured faster on HAPEX™than on plain PE although the response was slower on both the composites than on Thermanox (polyethylene terephthalate) coverslips used as a control (Figure 10a). The most interesting finding was that the osteoblasts cell processes attached down onto the HA particles in obvious preference to the surrounding PE (Figure 10b).



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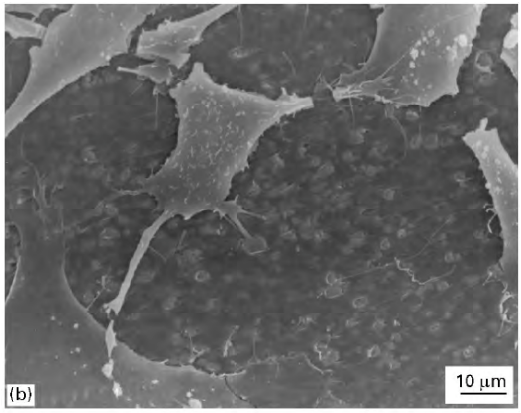


b)

Figure 10 a) Alkaline phosphatase activity on samples of HAPEX™ and PE with Thermanox (TMX) as a control surface and b) osteoblasts attaching down on to the HA particles in HAPEX™ (from Huang *et al.*, 1997b).

In a later study (Di Silvio *et al*., 2002a) 20 and 40vol% HA/PE were compared with Thermanox controls. Cell proliferation was measured using tritiated thymidine incorporation and cell differentiation using alkaline phosphatase activity. The cells proliferated faster on the 40vol% HA/PE than on the 20vol% HA/PE and then differentiated more, the difference peaking at day 7 of culture. Scanning electron microscopy showed the osteoblasts attaching down on to the HA particles and showed cells in the process of division. Confocal laser microscopy showed actin stress fibres in the cells and with vinculin staining adhesion plaques could be seen, although the number of adhesion plaques and actin fibres were higer and more organised on the 40vol% HA/PE than the 20vol% HA/PE. Transmission electron microscopy of the cell culture showed more collagen was formed between the cells with the higher HA content.

Dalby *et al*. (2000) showed the importance of surface features by culturing osteoblasts on machined rather than polished surfaces of HAPEX™. The osteoblasts aligned themselves with the grooves on the surface. Rea *et al*. (2004c) also investigated the effect of surface texture and produced a series of different grooves pillars and pits on the surface of HAPEX™. They found that compared to a polished surface there was not much difference in the cell number on the textured surfaces with the possible exception of 0.3mm wide grooves (Figure 11). However, cell differentiation, as measured by alkaline phosphatase activity was increased on the textured surfaces.

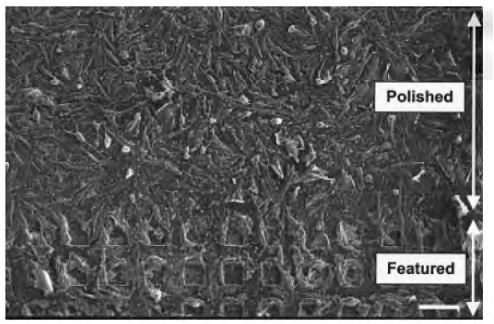
a)  b) 

Figure 11 Effect of surface texture on osteoblast attachment to a) grooved with grooves 50µm deep and 50µm wide and b) pitted with pits 50µm deep and 50µm wide and polished areas of HAPEX™ all marker bars =100µm (from Rea *et al.,* 2004c)

The importance of the HA morphology was shown by Zhang *et al*. (2007) who cultured osteoblasts on polished sections of 30vol% HA/PE manufactured using the sintered and spray dried HA particles investigated in Zhang & Tanner (2008). Three control materials were used, 100%PE and 100% HA in addition to Thermanox. When the composite surface was polished the spray dried particles were seen to be slightly porous and thus textured while the sintered particles were solid and thus smooth when polished (Figure 12 a & b). The osteoblasts were from both human femoral heads removed at hip replacement and from human calveria, again taken at elective surgery. The human calveria origin cells reacted slightly more slowly than those from the femoral heads although with a similar trend. Both origin cells attached with more, but finer, cell processes on to the spray dried HA containing composite than the sintered HA composite with higher cell proliferation followed by higher cell differentiation (Figure 12c). However these responses were higher and faster on the composite than either the plain polyethylene or the solid HA.

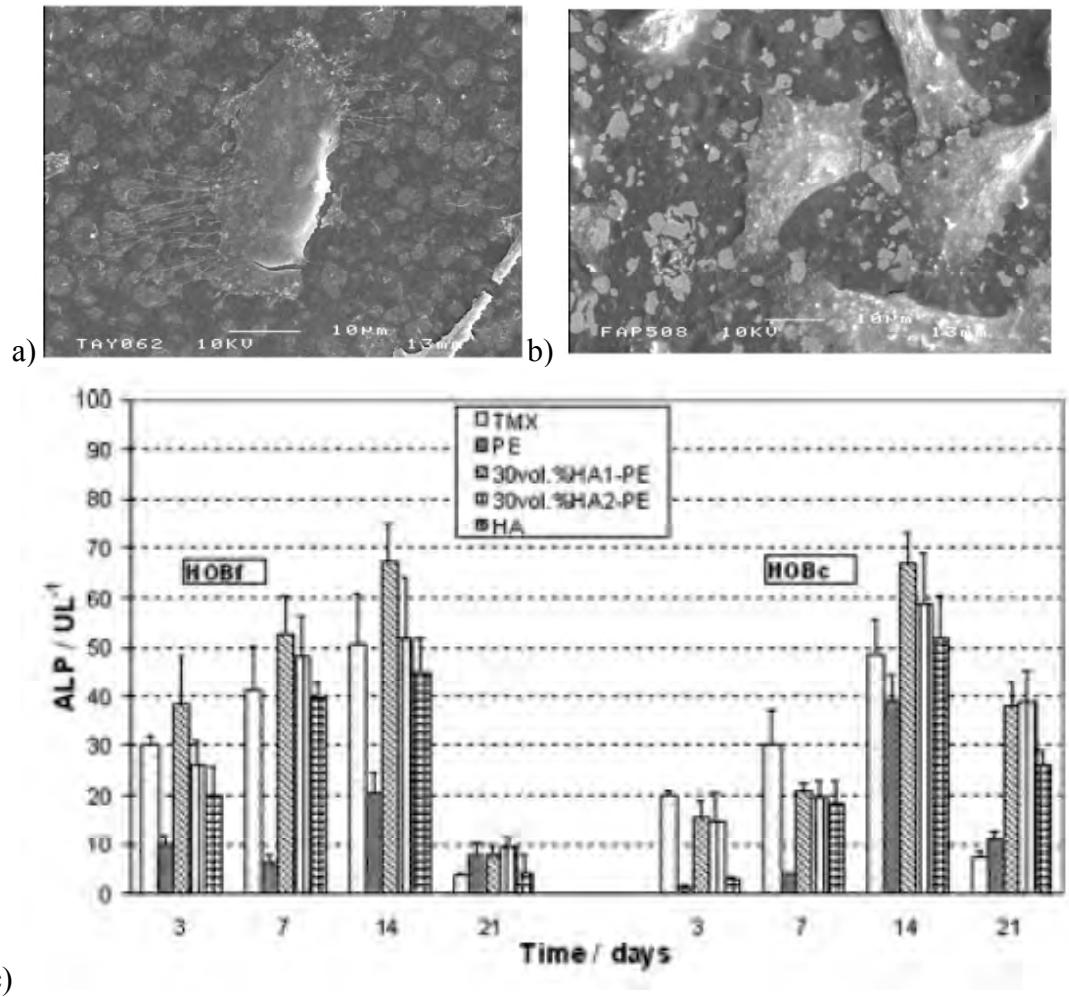


Figure 12 The interaction of femoral head origin osteoblasts with 30vol% HA/PE manufactured using a) spray dried HA and b) sintered HA particles and c) the alkaline phosphatase activity of osteoblasts from femoral heads (HOBf) and calvaris (HOBc) cultured on Thermanox controls (TMX), non-filled PE (PE), 30 vol% spray dried HA in PE (30ol%HA1-PE), 30 vol% sintered HA in PE (30vol%HA2-PE) and HA (HA) (from Zhang *et al*., 2007).

The cellular response to selectively laser sintered HA/PE and HA in polyamide (HA/PA) composites were investigated by Zhang *et al*. (2009). They used 20vol% HA and 30vol% HA in PE, as processing constraints had shown these to be the highest HA content possible in these two matrix materials (Savalini *et al*., 2006 & 2007; Hao *et al*., 2006a, 2006b, 2007, 2009; Zhang et al., 2008). In this study two controls were used, Thermanox which should lead to a beneficial cellular response and tin doped polyvinylchloride (SnPVC) a known toxic material. The cell proliferation and differentiation were higher on the 30vol%HA/PA than the 20vol%HA/PE, but the production of osteocalcin, a marker for bone mineralization, was higher on both composites than on the Thermanox (Figure 13). Cell culture was progressed through to 28 days and, without the addition of dexamethasone or any other additional factors to drive bone mineralization, on both the composites mineralised nodules were seen indicating the strong drive for the osteoblasts to progress through to bone

formation.

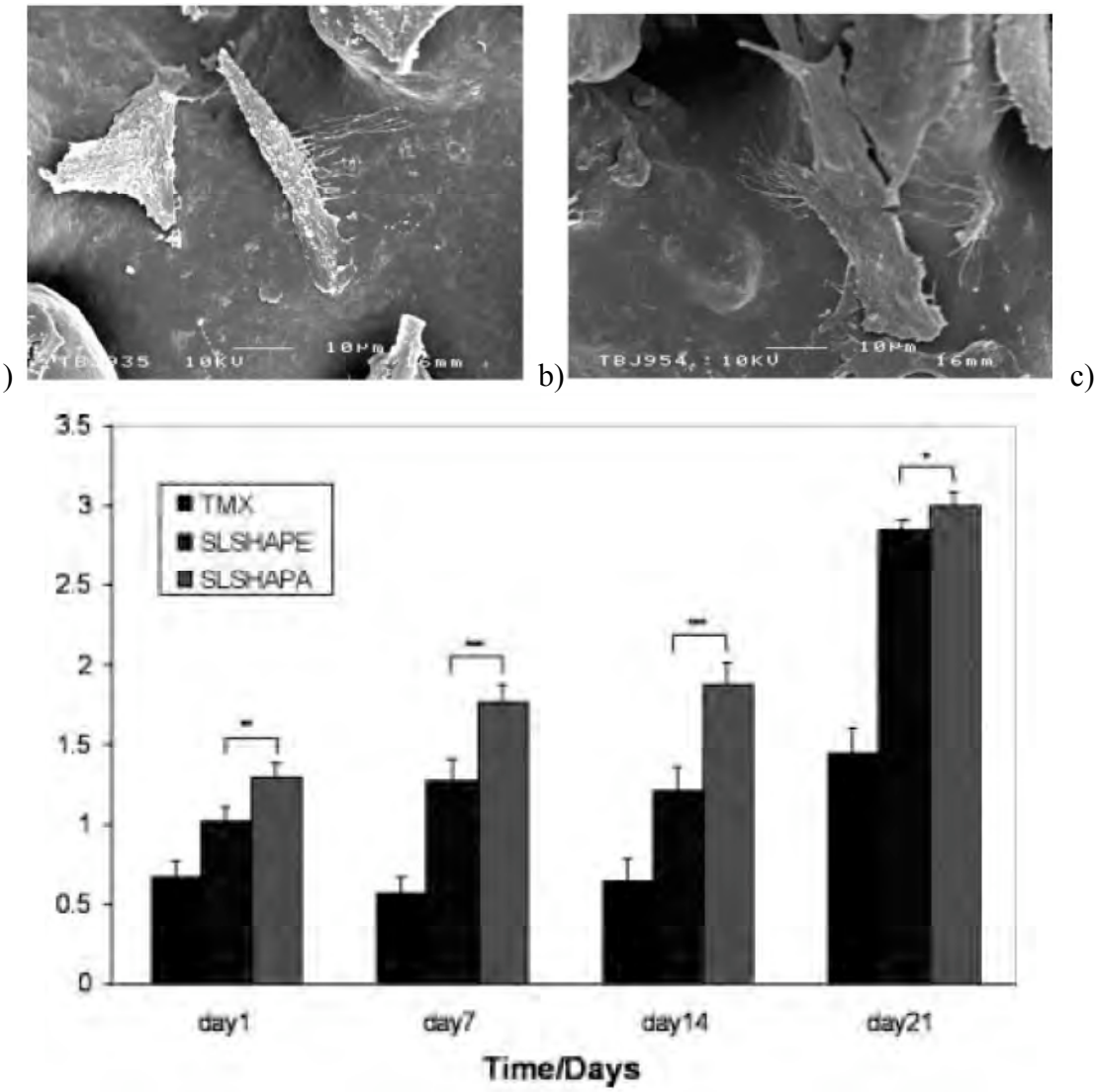


Figure 13 The interaction of human osteoblasts on selectively laser sintered a) 20vol% HA/PA and b) 30 vol% HA/PA and c) osteocalcin levels on Themanox control (TMX), electively laser sintered 20vol% HA/PA (SLSHAPE) and 30 vol% HA/PA(SLSHAPA) with differences by Student's t-test, * = $p < 0.05$, *** = $p < 0.001$ (From Zhang *et al*., 2009).

IN VIVO TESTING OF BIOACTIVITY

The *in vivo* biocompatibility and bioactivity testing was started before the *in vitro* as the development of this material pre-dated the use of *in vitro* cell culture studies for biocompatibility testing. In the first of these studies Bonfield *et al*. (1986) implanted rivet shaped implants of HAPEX™ with non-filled PE as a control into the femoral shaft of rabbits, passing through both cortical and cancellous bone. While the PE was surrounded with a fibrous layer direct bone contact onto the HAPEX™ was seen. In later studies high resolution TEM was used to investigate the crystallographic detail of this interface and showed that the mineral phase in the newly formed bone was aligned with that in the HA in the composite indicating that the

newly formed bone appeared to be using the HA on the composite surface to nucleate mineralisation (Doyle *et al*., 1990).

Tanner *et al*. (1990) performed push-out testing to measure then strength of the interface produced bone and between HAPEX™, apatite-wollastonite (A-W) glass ceramic provided by Professor Kokubo or non-filled PE. Even after 3 months implantation the non-filled PE-bone interface failed with a shear strength of than 0.6MPa, while the A-W glass ceramic-bone interface reached 20MPa and the HAPEX™-bone interfacial strength gradually increased up to 10MPa at 3 months.

Revell *et al*. (1997) implanted rods into the distal femora of rabbits, each rod had a slot cut into in 2mm deep and 2mm across. Two materials were used, HAPEX™ and titanium where the inside of the slot was plasma sprayed with HA (Ti+HA) to model plasma sprayed titanium used for the stems of non-cemented joint replacements. After 5 weeks the new bone formation was down to the bottom of the HAPEX™ slot and filled 80% of the available space unlike the Ti+HA where the new bone filled only 25% of the available space (Figure 14).

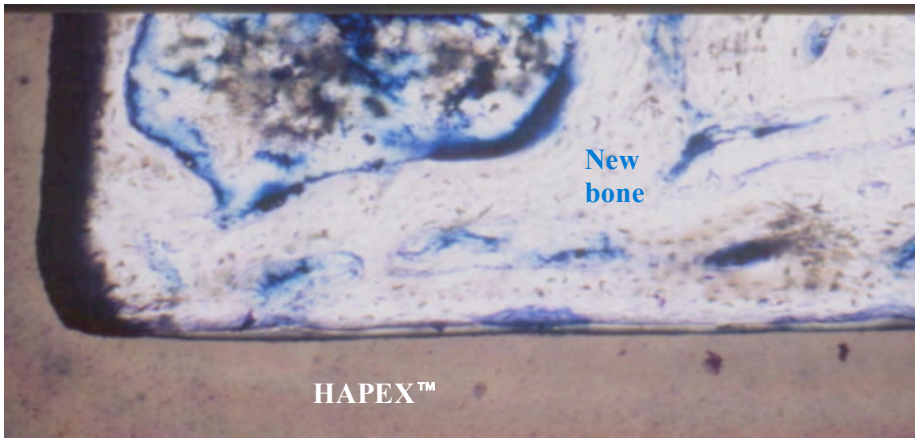


Figure 14 Section through the base of the slot machined into a HAPEX™ cylinder and implanted in a rabbit knee for 5 weeks (from Revell *et al*., 1977).

CLINICAL APPLICATIONS

HAPEX™ was the first of the bioactive composites implanted into patients and was used by Downes *et al*. (Downes *et al*. 1991; Tanner *et al*., 1994) in orbital floor applications. The two designs of implants either filled the space left after an eye was removed to allow the artificial eye to fit better or restored eye alignment in patients with orbital floor fractures. These were low load bearing applications, but showed that the material encouraged the supporting bone to bond to the implant material. Previous implants were manufactured of silicone and manual palpation had shown them lie loose in a fibrous capsule and some extruded. None of the HAPEX™ implants moved on palpation or were extruded. CT images (Figure 15) showed no gaps between the implant and the supporting bone.

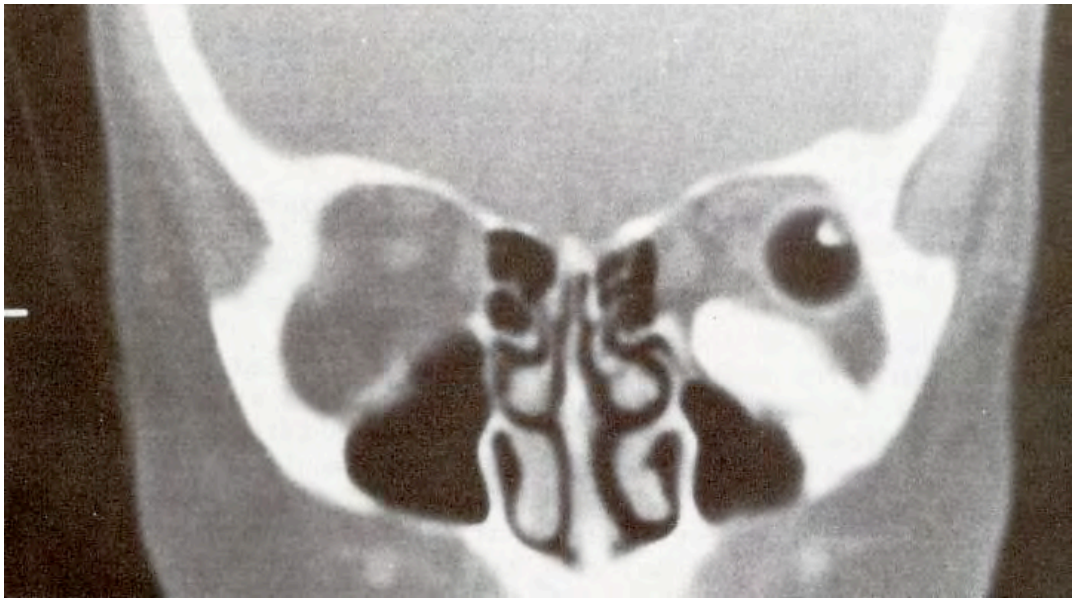


Figure 15 An HAPEX™ implant in the orbital floor of a patient who has lost an eye. The HAPEX™ implant is shown on the right of the image with the same radiographic density as the bone to which it is bonded while the spherical black object is a glass ball implanted in an earlier attempt to increase the volume of material in the orbital socket (from Downes *et al*., 1991)

This initial study had only 18 patients, but allowed the material to be used by Smith & Nephew ENT to manufacture the shaft of a range of middle ear implants including the Goldenberg (Goldenberg, 1994) and Dornhoffer (Dornhoffer, 1998) implants. These implants consist of a monolithic HA head on a shaft and are designed to replace the mechanical function of sound transmission through the three bones of the middle ear. The HA head is placed against the tympanic membrane (ear drum) and the surgeon cuts the shaft to fit the individual patient. The range of implants allows for patients where all three bones of the middle ear have been lost or damaged or where only two bones were affected and the stapes is still present. Originally, the shafts were manufactured of PE and the surgeon trimmed them to shape with a scapel blade, when the PE shafts were replaced with HAPEX™ shafts the ability of the surgeon to trim the implant to shape was retained and indeed improved (Figure 16). One study by Meijer *et al.* (2002) has reported the clinical application of these implants. They used Scanning Electron Microscopy to review the response to 11 HAPEX™ implants which had been removed due to re-occurrence of the original clinical problem, rather than for device failure. In most of the cases the implants were covered with fibrous tissue and in half of these there was a thin epithelial outer layer. The response to implantation was considered to be good even upt o 30 months implantation. Also in no cases were macrophages or other signs of inflammation seen, unlike similar retrieved implants manufactured of Proplast® or Plastipore® where macrophage activity was obvious and some inflammation was seen.

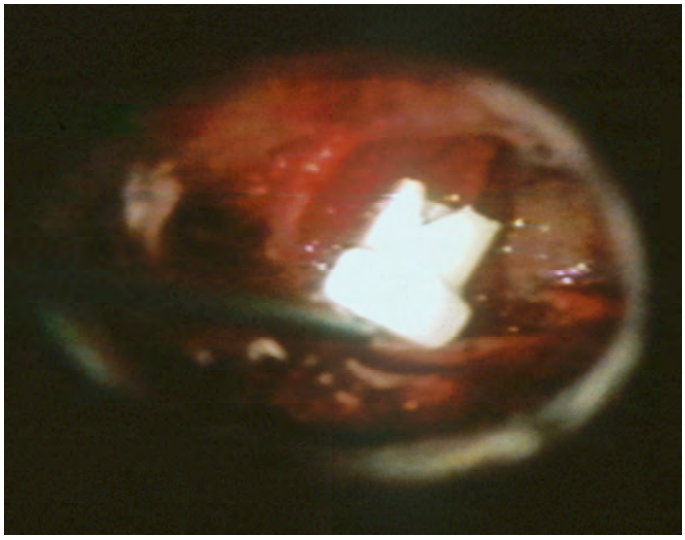


Figure 16 Middle ear implant *in situ* showing the notch cut in the HAPEX shaft to allow the implant to sit over the arch of the stapes (top) and the HA head resting on the tympanic membrane (bottom).

OTHER COMPOSITES

This work with hydroxyapatite reinforced polyethylene has lead to a range of other potentially bioactive composites to be investigated., both by Bonfield and his colleagues and by other groups. These composites can be degradable or non-degradable depending on the matrix polymer. For non-degradable materials PE remains the most popular matrix material, while a range of degradable polymers have been used as matrix materials in composites, including polylactic acid and polyhydroxybutyrate.

Harper (1998) reinforced PMMA bone cement with 17wt% (6vol%) HA in an attempt to confer bioactivity without reducing the mechanical properties. Dalby *et al*. (1999 & 2001) investigated the cellular response to this composite. The addition of the HA increased the cellular response to the PMMA with again the osteoblast processes attaching down onto the HA particles and higher actin organisation in the cells grown on the filled PMMA than the non-filled (Figure 17). Bonner *et al*. (2001) reported the manufacture and hydrostatic extrusion of polypropylene reinforced with 40vol% HA (HA/PP). Hydrostatic extrusion increased the modulus from 7.5 to 9.3GPa and the flexural strength from 25 to over 80MPa. Neither of these materials have progressed beyond the initial testing.

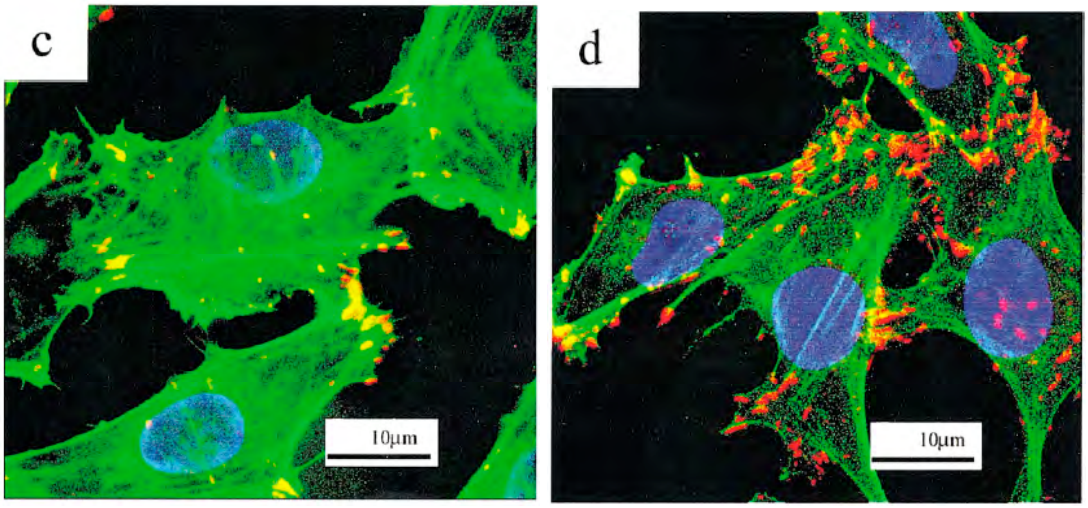


Figure 17 Actin cytoskeleton (green)/vinculin (red) interaction surrounding the cell nucleus (blue) on a) PMMA and b) PMMA reinforced with 6vol% HA (from Dalby *et al*., 2001).

In the 1980s, polyhydroxybutyrate (PHB) and its co-polymer polyhydroxyvalerate (PHV) were being developed as biodegradable polymers with similar mechanical properties as polypropylene. Doyle *et al*. (1991) manufactured HA reinforced PHB and characterised the mechanical properties and performed *in vivo* analysis of the material, including push out testing similar to that performed by Tanner *et al*. (1990). The non-filled PHB had a Young's modulus of 4GPa which was increased to 11GPa with the addition of 40vol% HA however the tensile strength dropped from 37MPa to 22MPa. The biological response to implantation of 40vol%HA/PHB was bone ongrowth (Figure 18), but in push out-testing failure occurred though the material rather than at the implant-bone interface as occurred with HA/PE composites. Luklinska and Bonfield (1997) using high resolution TEM showed that the bone had bonded across the interface. Knowles and Hastings (1993) manufactured composites by dry blending phosphate based glasses 20 to 40wt% into PHB with 7vol%PHV and then injection moulded samples. The presence of the glass accelerated the degradation. However, these studies stopped the cessation of production of medical grade PHB for commercial reasons. However more recently PHB has again been used in bioactive composites such as PHB reinforced with 5 and 15wt% HA short fibres produced by Coskun *et al*. (2005) and PHB12%PHV reinforced with up to 14vol% nanoHA produced by Chen *et al*. (2007). As a matrix material PHB has the advantages of higher modulus than most other degradable polymers, furthermore by including PHV the ductility can be increased although with reductions in the stiffness and strength.

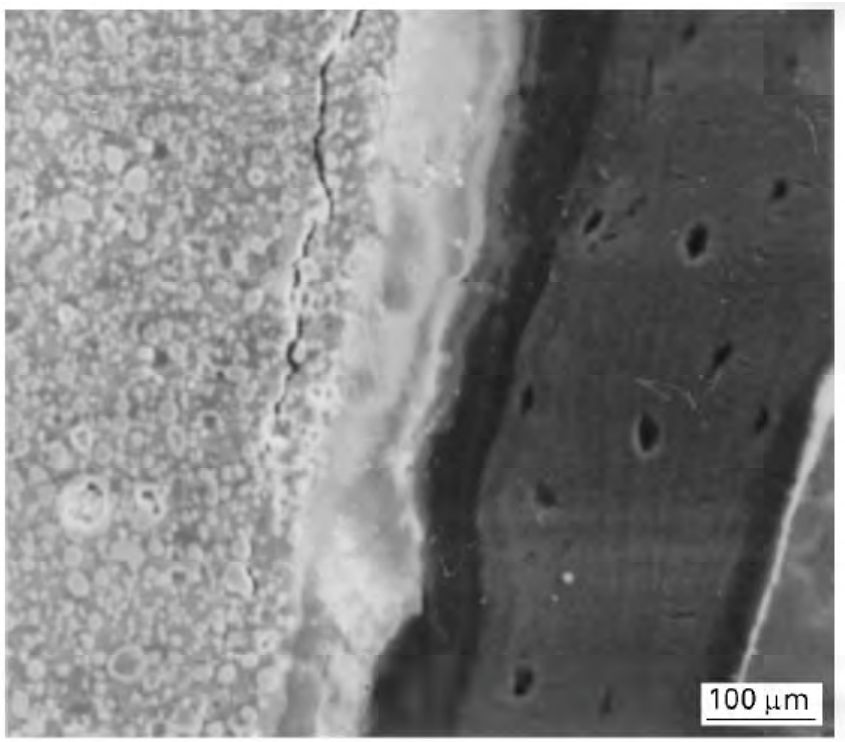


Figure 18 Interface developed between cortical bone and 40vol% HA/PHB after 1 month marjer bar = 100µm (from Luklinska and Bonfield, 1997).

In contrast to these composites with different polymers reinforced with HA, Huang *et al*. (1997 a & c; 1998) and Wang *et al*. (1998a) reinforced polyethylene with Bioglass®. Again increases in the modulus were seen with the addition of the Bioglass®, however, the sharp corners of the Bioglass® lead to stress concentrations and thus significant reductions in the strength (Figure 19). However, the response to soaking in simulated body fluid was excellent with dissolution of the Bioglass® acting to initiate the development of a hydrated carbonate layer (HCA) which started from the Bioglass® particles and then progressed across the composite surface (Figure 20). The material was seen to be biocompatible with increased cell viability when cultured in cell culture medium that had been exposed to the composite for 24 hours. This increase in cell viability was presumably due to the ions released during the dissolution of the Bioglass® in the cell culture medium.

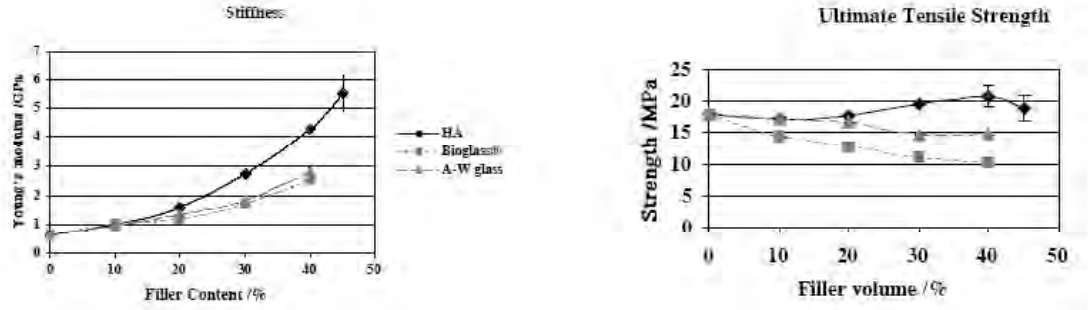
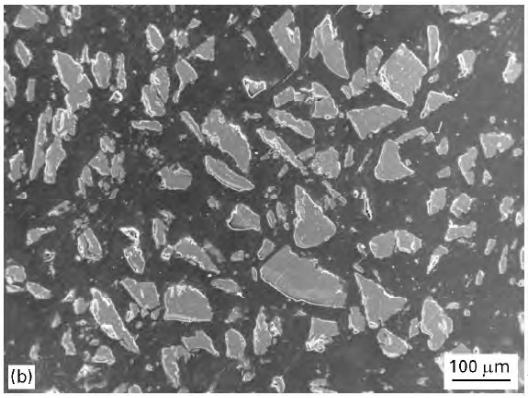


Figure 19 Comparison of the reinforcing effects of hydroxyapatite (HA) Bioglass® and A-W glass ceramic in polyethylene on the Young's modulus and tensile strength at a range of volume fractions.

a) 

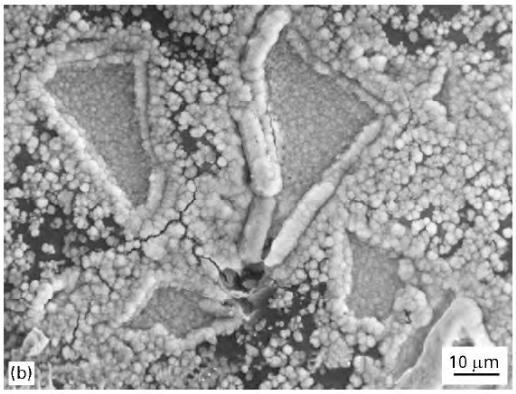
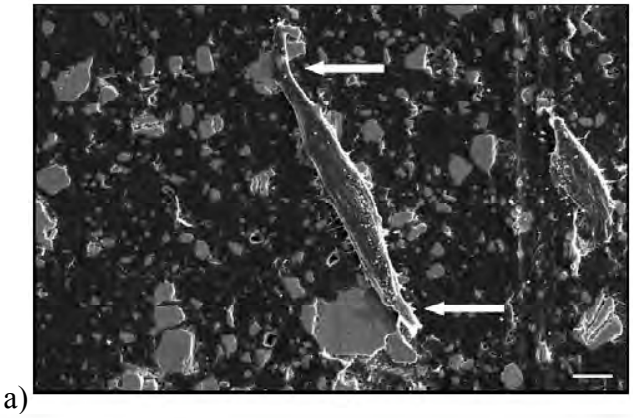
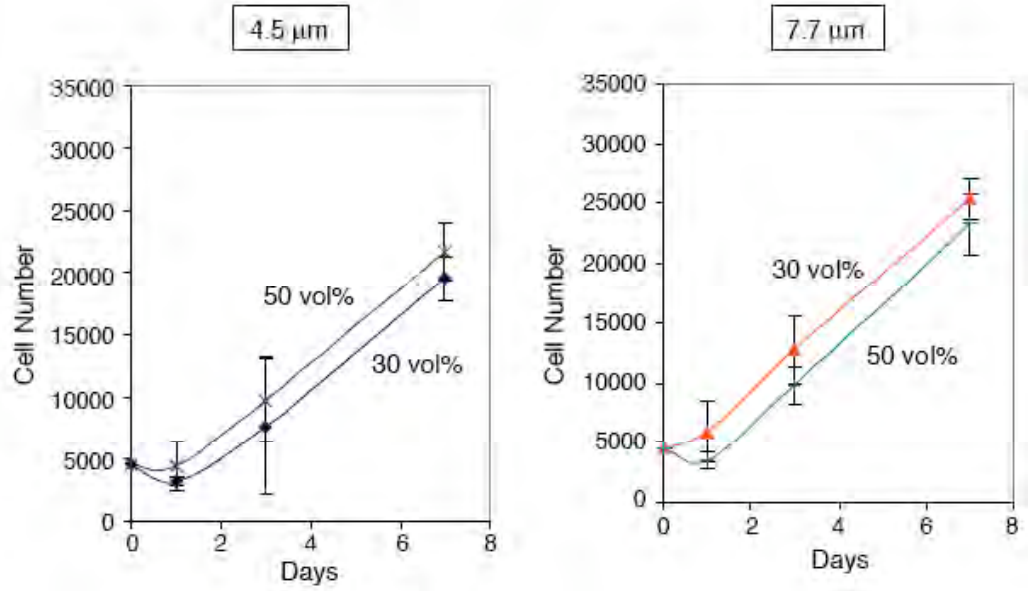
b) 

Figure 20 Polished sections of 40vol% Bioglass® reinforced PE a) polished section and b) after 7 days in simulated body fluid (from Huang et al 1997a)

Judhasz *et al*. (2003 & 2004) reinforced PE with A-W glass-ceramic and again found that the addition of the glass-ceramic increased the stiffness of the composite, but due to the shape of the A-W glass ceramic particles stress concentrations lead to low strength materials. However the bioactivity as measured be time to produce a surface layer after soaking in SBF was excellent. With even as little as 10vol% A-W glass-ceramic 100% of surface of samples was covered in 3 days and with 50vol% total coverage took less than 6 hours. Rea *et al*. (2004 a, b & c) compared the response of osteoblasts like cells on HAPEX™ and A-W glass ceramic-PE composites and showed that as with HAPEX™ when the cells were cultured on A-W glass ceramic-PE they preferentially attached down on the filler particles via filopodia. The response increased with increasing A-W glass ceramic content but for all surface textures, be they grooves, pits or pillars the response, in terms of cell proliferation and differentiation, was higher on HAPEX™ than on A-W glass ceramic-PE (Figure 21).



a)



b)

Figure 21 a) an osteoblast growing A-W glass ceramic in PE composite showing the cell attaching preferentially onto the A-W glass ceramic particles and b) the increases in cell number with time for 30 and 50vol% A-W glass ceramic in PE with mean particle sizes of 4.5µm and 7.7µm (from Rea *et al*., 2004b).

The idea of reinforcing a polymer with a bioactive filler was taken further by Törmälä *et al*. (1997) and Bleach *et al*. (2001, 2002) who reinforced polylactic acid (PLA) with HA or tricalcium phosphate (TCP) particles and then used this composite to act as the matrix around drawn PLA fibres thus creating a three phase fibre reinforced composite. The stiffness and strength were at the lower bounds of those for cortical bone at over 7GPa and 70MPa respectively. An interesting effect of adding HA or TCP was to chemically buffer the degradation of the PLA so that the start of loss of strength was delayed from 8 weeks to 12 weeks. DMA performed by Nazhat *et al*. (2001) at 1Hz test frequency and scanning from -50 to +100˚C showed the glass transition temperatures for both the PLAs used in the manufacture of the composite. The drawn fibres increased the storage modulus from 3.5 to over 7GPa and that the loss modulus was relatively unaffected by temperature upto the glass transition temperature.

Other composites containing polymer fibres have been developed. For example Waris *et al*. (1994)

manufactured plates of PLA reinforced PLA and used these in the repair of skull defects in growing children as the degradation of the plates allowed skull growth continue once the plate had disappeared. More recently some of the studies using fused deposition and electrostatic spraying have used composites of

CONCLUSIONS

The idea of adding HA to polymers has lead from the bioinert monolithic materials used in the 1970s and 1980s through to the bioactive biodegradable composites used now as modulus matched materials and more recently as the scaffolds for tissue engineering. The work has lead to the application of conventional composite manufacturing techniques in the field of biomaterials, with materials that have similar mechanical properties to bone. The addition of the bioactive phases has lead to materials that have been shown to be bioactive in both *in vitro* studies using soaking in simulated body fluid and *in vitro* cell culture where the interaction of the cells will the filler phases has indicated the manner of the biological and cellular response *in vivo*. Finally the materials have been used clinically leading to improvements in the quality of life of patients worldwide.

REFERENCES

Bleach, N. C., Nazhat, S. N., Tanner, K. E., Kellomäki, M. & Törmälä, P. 2002 Effect of filler content on mechanical and dynamic mechanical properties of particulate biphasic calcium phosphate polylactide composites. *Biomaterials*, **23**, 1579-1585.

Bleach, N.C., Tanner, K. E., Kellomäki M., & Törmälä, P. 2001 Effect of filler type on the mechanical properties of self-reinforced polylactide calcium phosphate composites. *J. Mater. Sci. Mater. Med.* **12**, 911-915. (doi:10.1023/A:1012884310027)

Bonfield, W. 1988 Hydroxyapatite reinforced polyethylene as an analogous material for bone replacement. *Ann. New York Acad. Sci*. **523**, 173-177.

Bonfield, W. & Grynpas, M.D. 1977 Anistropy of the Young's modulus of bone. Nature **270**, 453-454.

Bonfield, W. & Li C.H. 1966 Deformation and fracture of bone *J. Appl. Phys*. **37**, 869-

Bonfield, W. & Li C.H. 1967 Anisotropy of non-elastic flow in bone *J. Appl. Phys*. **38**, 2450-

Bonfield, W., Bowman, J. & Grynpas, M. D. Composite materials for use in orthopaedics, UK patent no. GB2085461B; 1980.

Bonfield, W., Doyle, C. & Tanner, K. E. 1986 *In vivo* evaluation of hydroxyapatite reinforced polyethylene composites. In "Biological and Biomechanical Performance of Biomaterials", Edited by P. Cristel, A. Meunier and A.J.C. Lee, Published by Elsevier Science Publishers, pp. 153-158.

Bonfield, W., Grynpas, M., Tully, A. E., Bowman, J., Abram, J. 1981 Hydroxyapatite reinforced polyethylene - A mechanically compatible implant materials for bone-replacement. *Biomaterials*, **2**, 185-186.

Bonfield, W., Wang, M. & Tanner, K. E. 1998 Interfaces in analogue biomaterials. *Acta Materialia* **46**, 2509-2518.

Bonner, M., Saunders, L. S., Ward, I. M., Davies, G. W., Wang, M., Tanner, K. E. & Bonfield, W. 2002 Anisotropic mechanical properties of orientated HAPEX™. *J. Mater. Sci.* **37**, 325-334. (doi: 10.1023/A:1013652312670)

Bonner, M., Ward, I.M., McGregor, W. J., Tanner, K. E. & Bonfield, W. 2001 Hydroxyapatite/polypropylene composite: A novel bone substitute material. *J. Mater. Sci. Letters*, **20**, 2049-2051.

Charnley, J. 1975 Histology of loosening between acrylic cement and bone. *J. Bone Jt. Surg*. **57-B**, 245-245.

Chen, D. Z., Tang, C. Y., Chan, K. C., Tsui, C. P., Yu, P. H. F., Leung, M. C. P. & Uskokovic, P. S. 2007 Dynamic mechanical properties and *in vitro* bioactivity of PHBHV/HA nanocomposite, *Comp. Sci. and Tech.*, **67**, 1617-1626.

Coskun, S., Korkusuz, F., Hasirci, V. 2005 Hydroxyapatite reinforced poly(3-

hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) based degradable composite bone plate, *J. Biomater. Sci. – Poly, Ed.*, **16**, 1485-1502.

Currey, J. D. 1998 Mechanical properties of vertebrate hard tissues. *Proc. Instn. Mech. Engrs.: Part H J. Eng. Med.* **212-H**, 399-413.

Currey, J.D. 1969 Mechanical consequences of variation in mineral content of bone. *J. Biomech.* **2**, 1-

Dalby, M. J., Di Silvio, L., Davies, G. W. & Bonfield, W. 2000 Surface topography and HA filler volume effect on primary human osteoblasts *in vitro*. *J. Mater. Sci. Mater. Med.* **11**, 805-810.

Dalby, M. J., Di Silvio, L., Harper, E. J. & Bonfield, W. 1999 *In vitro* evaluation of a new polymethylmethacrylate cement reinforced with hydroxyapatite. *J. Mater. Sci. Mater. Med.* **10**, 793-796.

Dalby, M. J., Di Silvio, L, Harper, E. J. & Bonfield, W. 2001 Initial interaction of osteoblasts with the surface of a hydroxyapatite-poly(methylmethacrylate) cement Biomaterials 22 (2001) 1739}1747.

Dalby, M. J., Kayser, M. V., Bonfield, W. & Di Silvio, L. 2002 Initial attachment of osteoblasts to an optimised HAPEX™topography. *Biomaterials* **23**, 681-690.

Deb, S., Wang, M., Tanner, K. E. & Bonfield, W. 1996 Hydroxyapatite-polyethylene composites: Effect of grafting and surface treatment of hydroxyapatite. *J. Mater. Sci. Mater. Med.*, **7**, 191-193. (doi:10.1007/BF00134322)

Di Silvio, L., Dalby, M. J. & Bonfield, W. 1998 *In vitro* response of osteoblasts to hydroxyapatite-reinforced polyethylene composites. *J. Mater. Sci. Mater. Med.* **9**, 845-848.

Di Silvio, L., Dalby, M. J. & Bonfield, W. 2002a Osteoblast behaviour on HA/PE composite surfaces with different HA volumes. *Biomaterials* **23**, 101-107.

Di Silvio, L., Dalby, M. J., Gurav, N., Annaz, B., Kayser, M.V. & Bonfield, W. 2002b Optimizing HAPEX™ topography influences osteoblast response. *Tissue Engineering* **8**, 453-467.

Dornhoffer, J. L. 1998 Hearing results with the Dornhoffer ossicular replacement prostheses. *Laryngoscope*, **108**, 531-536.

Downes, R. N., Vardy, S., Tanner, K. E. & Bonfield, W. 1991 Hydroxyapatite-polyethylene composite in ophthalmic surgery. In "Bioceramics 4 - Proceedings of the Fourth International Symposium on Ceramics in Medicine", Edited by W. Bonfield, G.W. Hastings and K.E. Tanner, Published by Butterworth-Heinnemann Ltd. pp. 239-246.

Doyle, C., Luklinska, Z. B., Tanner, K. E. & Bonfield, W. 1990 An ultra-structural study of the interface developed between hydroxyapatite/polymer composite and bone. In "Clinical Implant Materials", Edited by G. Heimke, U. Soltèsz and A.J.C. Lee, Published by Elsevier, pp. 339-344.

Doyle, C., Tanner, E. & Bonfield, W. 1991 *In vitro* and *in vivo* evaluation of polyhydroxybutyrate and of polyhydroxybutyrate reinforced with hydroxyapatite. *Biomaterials*, **12**, 841-847.

Engh, C. A., Bobyn, J. D. & Glassman, A. H. 1987 Porous-coated hip replacement – the factors governing bone ingrowth, stress shielding and clinical results. *J. Bone Jt. Surg.* **69-B**, 45-55.

Eniwumide, J. O., Joseph, R. & Tanner, K. E. 2004 Effect of particle morphology and polyethylene molecular weight on the fracture toughness of hydroxyapatite reinforced polyethylene composite. *J. Mater. Sci. Mater. Med.,* **15**, 1147-1152. doi:10.1023/B:JMSM.0000046398.17027.97

Friedrich, K. & Karsch, U. A. 1983 Failure processes in particulate filled polypropylene. *Fibre Science & Technology* **18**, 37-52.

Goldenberg, R. A. 1994 Ossiculoplasty with composite prostheses - PORP and TORP. *Otolaryngologic Clin. N. Am.* **27**, 727-745.

Guild, F. J. & Bonfield, W. 1993 Predictive modeling of hydroxyapatite polyethylene composite. *Biomaterials* **14**, 985-993.

Guild, F. J. & Bonfield, W. 1998 Predictive modelling of the mechanical properties and failure processes in hydroxyapatite-polyethylene (HAPEX™) composite. *J. Mater. Sci. Mater. Med.* **9**, 497-502.

Harper, E.J. 1998 Bioactive bone cements. . *Proc. Inst. Mech. Eng.: Part H J. Eng. Med.* **212-H**, 113-120.

Hao, L., Savalani, M. M., Zhang, Y., Tanner, K. E. & Harris, R. A. 2009 Rapid manufacturing of bioceramic/polymer composite implants by selective laser sintering. *Internat. J. Computer Appl. Tech.*, **36**, 97-103. (doi: 10.1504/IJCAT.2009.026667)

Hao, L., Savalani, M. M., Zhang, Y., Tanner, K. E. & Harris, R.A. 2006a Selective laser sintering of hydroxyapatite reinforced polyethylene scaffolds for bioactive implants and tissue scaffold development. *Proc. Inst. Mech. Eng.: Part H J. Eng.*

Med., **220-H**, 521-531. (doi:10.1243/09544119JEIM67)

Hao, L., Savalani, M. M., Zhang, Y., Tanner, K. E. & Harris, R.A. 2006b The effect of material and processing conditions on characteristics of hydroxyapatite and high density polyethylene bio-composite by selective laser sintering. *Proc. Inst. Mech. Eng.: Part L J. Mater. Design and Appl.*, **220-L**, 125-137. (doi:10.1243/14644207JMDA92)

Hao, L., Savalani, M. M., Zhang, Y., Tanner, K. E., Heath, R. J. & Harris, R. A. 2007 Characterisation of selective laser sintered hydroxyapatite based biocomposite structures for bone replacement. *Proc. R. Soc. A*, **463**, 1857-1869. doi:10.1098/rspa.2007.1854

Harrigan, T. P., Kareh, J. A., O'Connor, D. A., Burke, D. W. & Harris, W. H. 1992 A finite element study of the initiation of failure of fixation in cemented total hip components. *J. Orthop. Res.* **10**, 134-144.

Hench L. L., Polak J. M. 2002 Third-generation biomedical materials. *Science* 295 1014-1017. (doi: 10.1126/science.1067404)

Huang, J., Di Silvio, L., Wang, M., Rehman, I., Ohtsuki, C. & Bonfield, W. 1997a Evaluation of *in vitro* bioactivity and biocompatibility of Bioglass®-reinforced polyethylene composite *J. Mater. Sci. Mater. Med.* **8**, 809-813.

Huang, J., Di Silvio, L., Wang, M., Tanner, K. E. & Bonfield, W. 1997b *In vitro* mechanical and biological assessment of hydroxyapatite-reinforced polyethylene composite. *J. Mater. Sci. Mater. Med.* **8**, 775-779.

Huang, J., Di Silvio, L., Wang, M., Tanner, K. E. & Bonfield, W. 1997c *In vitro* assessment of hydroxyapatite- and Bioglass®- reinforced polyethylene. In "Bioceramics 10 - Proceedings of the Tenth International Symposium on Ceramics in Medicine", Edited by L. Sedel and C. Rey, Published by Elsevier Science Ltd, pp. 519-522.

Huang, J., Wang, M., Best, S.M., Tanner, K. E. & Bonfield, W. 1998 Evaluation of Bioglass®- and hydroxyapatite-reinforced polyethylene composites after aging in simulated body fluid. In "Bioceramics 11 - Proceedings of the Eleventh International Symposium on Ceramics in Medicine", Edited by R.Z. LeGeros and J.P. LeGeros, Published by World Scientific Press, pp. 165-168.

Jones, L. C. & Hungerford, D. S. 1987 Cement disease. *Clin. Orthop. Rel. Res*. **225**, 192-206.

Joseph, R. & Tanner, K. E. 2005 Effect of morphological features and surface area of hydroxyapatite on the fatigue behaviour of hydroxyapatite-polyethylene composites. *Biomacromolecules*, **6**, 1021-1026. (doi: 10.1021/bm0493180)

Joseph, R., Martyn, M. T., Tanner, K. E. & Coates, P.D. 2006 Interfacial stick-slip transition in hydroxyapatite filled high density polyethylene composite. *Bulletin Mater. Sci.*, **29**, 85-92.

Joseph, R., Martyn, M. T., Tanner, K. E., Coates, P. D. & Bonfield, W. 2001b Rheological characterisation of hydroxyapatite filled polyethylene composites Part 1: Shear and extensional behaviour. *Plastics, Rubbers Composites*, **30**, 197-204.

Joseph, R., Martyn, M. T., Tanner, K. E., Coates, P. D. & Bonfield, W. 2001c Rheological characterisation of hydroxyapatite filled polyethylene composites Part 2: Isothermal compressibility and wall slip. *Plastics, Rubbers Composites*, **30**, 205-212.

Joseph, R., McGregor, W. J., Martyn, M. T., Tanner, K. E. & Coates, P.D. 2002a Effect of hydroxyapatite morphology/surface area on the rheology and processability of hydroxyapatite filled polyethylene composites. *Biomaterials*, **23**, 4295-4302.

Joseph, R., McGregor, W. J., Martyn, M. T., Tanner, K. E., Coates, P. D & Bonfield, W. 2002b Effect of polymer matrix on the rheology of hydroxyapatite filled polyethylene composites. *Poly. Eng. Sci.*, **42**, 326-335.

Juhasz, J. A., Best, S. M., Bonfield, W., Kawashita, M., Miyata, N., Kokubo, T. & Nakamura, T. 2003 Apatite-forming ability of glass-ceramic apatite-wollastonite - polyethylene composites: Effect of filler content. *J. Mater. Sci. Mater. Med.* **14**, 489-495.

Juhasz, J. A., Best, S. M., Brooks, R., Kawashita, M., Miyata, N., Kokubo, T., Nakamura, T. & Bonfield, W. 2004 Mechanical properties of glass-ceramic A-W-polyethylene composites: Effect of filler content and particle size. *Biomaterials* **25**, 949-955.

Katz, J. L., Misra, A., Spencer, P., Wang, Y., Bumrerraj, S., Nomura, T., Eppell, S. J. & Tabib-Azar, M. 2007 Multiscale mechanics of hierarchical structure/property relationships in calcified tissues and tissue/material interfaces. *Mater. Sci. & Eng. C – Biomimetic and Supramolecular Systems* **27**, 450-468.

Kadoya, Y., Revell, P. A., Kobayashi, A., Al Saffar, N., Scott, G. & Freeman, M. A. R. 1997 Wear particulate species and bone loss in failed total joint arthroplasties. *Clin. Orthop. Rel. Res*. **340**, 118–129.

Knowles, J.C. & Hastings, G.W. 1993 *In vitro* and *in vivo* investigation of a range of phosphate glass-reinforced polyhydroxybutyrate-based degradable composites, *J. Mater. Sci. Mater. Med.* 4, 102-106.

Kobayashi, A., Freeman, M. A. R., Bonfield, W., Kadoya, Y., Yamac, T., Al Saffar, N., Scott, G. & Revell, P. A. 1997 Number of polyethylene particles and osteolysis in total joint replacements - A quantitative study using a tissue-digestion method. *J. Bone Jt. Surg*. **79B**, 844-848.

Ladizesky, N. H., Pirhonen, E. M., Appleyard, D. B., Ward, I. M. & Bonfield, W. 1998 Fibre reinforcement of ceramic/polymer composites for a major load-bearing bone substitute material. *Composites Science and Technology* **58**, 419-434.

Ladizesky, N. H., Ward, I. M. & Bonfield, W. 1997 Hydrostatic extrusion of polyethylene filled with hydroxyapatite. *Polymers for Advanced Technologies* **8**, 496-504.

Lakes, R.S., Katz, J.L. & Sternstein, S.S. 1979 Viscoelastic properties of wet cortical bone 1. Torsional and biaxial studies. *J. Biomech.* **12**, 657- 678.

Lakes, R.S. & Katz, J.L. 1979a Viscoelastic properties of wet cortical bone 2. Relaxation mechanisms. *J. Biomech.* **12**, 679-687.

Lakes, R.S. & Katz, J.L. 1979b Viscoelastic properties of wet cortical bone 3. Nonlinear constitutive equation. *J. Biomech*. **12**, 689-698.

Luklinska, Z. B. & Bonfield, W. 1997 Morphology and ultrastructure of the interface between hydroxyapatite-polyhydroxybutyrate composite implant and bone. *J. Mater. Sci. Mater. Med.* **8**, 379-383.

McGregor, W. J., Tanner, K. E., Bonfield, W., Bonner, M.J., Saunders, L. S., & Ward, I. M. 2000 Fatigue properties of isotropic and hydrostatically extruded HAPEX™. *J. Mater. Sci. Letters*, **19**, 1787-1788. (doi:10.1023/A:100672180210)

Meijer, A. G. W., Segenhout, H. M., Albers, F. W. J., van de Want, H. J. L. 2002 Histopathology of biocompatible hydroxylapatite-polyethylene composite in ossiculoplasty. *ORL: J Oto-Rhino-Laryngology Rel. Specialities*, **64**, 173-179.

Nazhat, S. N., Joseph, R., Wang, M., Smith, R., Tanner, K. E. & Bonfield, W. 2000 Dynamic mechanical characterisation of hydroxyapatite reinforced polyethylene: Effect of particle size. *J. Mater. Sci. Mater. Med.* **11**, 621-628. (doi:10.1023/A:1008957729512)

Nazhat, S. N., Kellomäki, M., Törmälä, P., Tanner, K. E. & Bonfield, W. 2001 Dynamic mechanical characterization of biodegradable composites of hydroxyapatite and polylactides. *J. Biomed. Mater. Res. (Appl. Biomater)*, **58**, 335-343.

Parkhill C. 1897 A new apparatus for the fixation of bones after resection and in fractures with a tendency to displace. Trans Am surg Ass. 15 pp 251-254 reprinted Clin Orth RR 180 pp 3-6

Quinn, J., Joyner, C., Triffitt, J. T. & Athanasou, N. A. 1992 Polymethylmethacrylate-induced inflammatory macrophages resorb bone. *J. Bone Jt. Surg*. **74-B**, 652–658.

Rea, S. M., Best, S. M. & Bonfield, W. 2004a Bioactivity of ceramic-polymer composites with varied composition and surface topography. *J. Mater. Sci. Mater. Med.* **15**, 997-1005.

Rea, S. M., Brooks, R. A., Best, S. M., Kokubo, T. & Bonfield, W. 2004b Proliferation and differentiation of osteoblast-like cells on apatite-wollastonite/polyethylene composites. *Biomaterials* **25**, 4503-4512.

Rea, S. M., Brooks, R. A., Schneider, A., Best, S. M. & Bonfield, W. 2004c Osteoblast-like cell response to bioactive composites surface-topography and composition effects. *J. Biomed. Mater. Res. B Appl. Biomater* **70B**, 250-261.

Revell, P. A., Tanner, K. E. & Bonfield, W. 1997 Bone growth into implants of hydroxyapatite-coated titanium and hydroxyapatite-polyethylene composite implants, *J. Path*. **181**, A12.

Rich, J., Tuominen, J., Kylmö, J., Seppälä, J., Nazhat, S. N. & Tanner K. E. 2002 Lactic acid based PEU/HA and PEU/BCP composites: dynamic mechanical characterisation of hydrolysis *J. Biomed. Mater. Res*., **63**, 346-353. doi: 10.1002/jbm.10232

Sabokbar, A., Fujikawa, Y., Brett, J., Murray, D. W. & Athanasou, N. A. 1996 Increased osteoclastic differentiation by PMMA particle-associated macrophages – inhibitory effect by interleukin 4 and leukemia inhibitory factor. *Acta Orthop. Scand*. **67**, 593–598.

Sanginario, V., Ginebra, M. P., Tanner, K. E., Planell, J. A. & Ambrosio, L. 2006 Biodegradable and semi-biodegradable

composite hydrogels as bone substitutes: Morphology and mechanical characterization. *J. Mater. Sci. Mater. Med.*, **17**, 447-454. doi:10.1007/s10856-006-8472-y

Sarmiento, A., Zych, G. A., Latta, L.L. & Tarr, R. R. 1979 Clinical experiences with a titanium alloy total hip-prosthesis – posterior approach. *Clin. Orthop. Rel. Res*. **144,** 166-173**.**

Savalani, M. M., Hao, L., & Harris, R. A. 2006 Evaluation of CO_2 and Nd:YAG lasers for the selective laser sintering of HAPEX *Proc. IMechE Part B: J. Eng. Manufacture* **220-B** , 171-182.

Savalani, M. M., Hao, L., Zhang, Y., Tanner, K. E. & Harris, R. A. 2007 Fabrication of porous bioactive structures using the selective laser sintering technique. *Proc. Inst. Mech. Eng.: Part H J. Eng. Med.*, **221-H**, 873-886. (doi:10.1243/09544119JEIM232)

Suwanprateeb, J., Tanner K. E., Turner, S. & Bonfield, W. 1995 Creep in polytheylene and hydroxyapatite reinforced polyethylene. *J. Mater. Sci. Mater. Medicine*, **6**, 804-807. (doi:10.1007/BF00134321)

Suwanprateeb, J., Tanner, K. E., Turner, S. & Bonfield, W. 1997 Influence of Ringer's solution on creep resistance of hydroxyapatite reinforced polyethylene composites. *J. Mater. Sci. Mater. Med.*, **8**, 469-472. (doi:10.1023/A:1018522025474)

Suwanprateeb, J., Tanner, K. E., Turner, S. & Bonfield, W. 1998 Influence of sterilisation by gamma-irradiation and of thermal annealing on creep of hydroxyapatite reinforced polyethylene composites. *J. Biomed. Mater. Res.* **39**, 16-22. (doi:10.1002/(SICI)1097-4636(199801)39:1<16::AID-JBM3>3.0.CO;2-L)

Tanner K. E., Downes R. N., & Bonfield, W. 1994 Clinical application of hydroxyapatite reinforced polyethylene. *Brit. Ceram. Trans.* **93**, 104-107.

Tanner K. E., Doyle, C. & Bonfield, W. 1990 The Strength of the interface developed between biomaterials and bone. In "Clinical Implant Materials", Edited by G. Heimke, U. Soltèsz and A.J.C. Lee, Published by Elsevier, pp. 149-154.

Ton That, P.T., Tanner, K. E. & Bonfield, W. 2000a Fatigue characterisation of a hydroxyapatite reinforced polyethylene composite: Part I: Uniaxial fatigue. *J. Biomed. Mater. Res.*, **51**, 453-460. (doi:10.1002/1097-4636(20000905)51:3<453::AID-JBM20>3.0.CO;2-Q)

Ton That, P.T., Tanner, K. E. & Bonfield, W. 2000b Fatigue characterisation of a hydroxyapatite reinforced polyethylene composite: Part II: Biaxial fatigue. *J. Biomed. Mater. Res.*, **51**, 461-468. (doi:10.1002/1097-4636(20000905)51:3<461::AID-JBM21>3.0.CO;2-M)

Törmälä, P., Kellomäki, M., Bonfield, W. & Tanner, K. E. 1997 Bioactive and biodegradable composites of polymers and ceramics or glasses and method to manufacture such composites. USA Patent Application No 08/921,533.

Wang, M. & Bonfield, W. 2001 Chemically coupled hydroxyapatite-polyethylene composites: structure and properties. *Biomaterials* **22**, 1311-1320.

Wang, M., Deb, S. & Bonfield, W. 2000 Chemically coupled hydroxyapatite-polyethylene composites: processing and characterisation. *Materials Letters* **44**, 119-124.

Wang, M., Hench, L. L. & Bonfield, W. 1998a Bioglass® high density polyethylene composite for soft tissue applications: Preparation and evaluation. *J. Biomed. Mater. Res.* **42**, 577-586.

Wang, M., Joseph, R. & Bonfield, W. 1998b Hydroxyapatite-polyethylene composites for bone substitution: effects of ceramic particle size and morphology. *Biomaterials* **19**, 2357-2366.

Wang, M., Ladizesky, N.H., Tanner, K. E., Ward, I. M. & Bonfield, W. 2000 Hydrostatically extruded HAPEX™. *J. Mater. Sci.*, **35**, 1023-1030. (doi:10.1023/A:1004731315328)

Wang, M., Porter, D. & Bonfield, W. 1994 Processing, characterization and evaluation of hydroxyapatite-reinforced polyethylene composites. *Br. Ceram. Trans.* **93**, 91-95.

Waris, T., Pohjonen, T. & Törmälä, P 1994 Self-reinforced absorbable polylactide (SR-PLLA) plates in craniofacial surgery – A preliminary report on 14 patients. *Euro J Plast Surg*, **17**, 236-238.

Westbroek, P. & Marin, F. 1998 A marriage of bone and nacre. *Nature*, **392**, 861-862.

Zhang, Y. & Tanner, K. E. 2003 Impact behaviour of hydroxyapatite reinforced polyethylene composites. *J. Mater. Sci. Mater. Med.*, **14**, 63-68. (doi:10.1023/A:1021553504549)

Zhang, Y. & Tanner, K. E. 2008 Effect of filler surface morphology on the impact behaviour of hydroxyapatite reinforced high density polyethylene composites. *J.*

Mater. Sci. Mater. Med., **19**, 761-766. (doi:10.1007/s10856-007-3119-1)

Zhang, Y., Hao, L., Savalani, M. M., Harris, R. A., Di Silvio, L. & Tanner, K. E. 2009 *In vitro* biocompatibility of hydroxyapatite reinforced polymeric composites manufactured by selective laser sintering. *J. Biomed. Mater. Res. A*, **91-A**, 1018-1027. (doi: 10.1002/jbm.a.32298)

Zhang, Y., Hao, L., Savalani, M. M., Harris, R.A. & Tanner, K. E. 2008 Characterization and dynamic mechanical analysis of selective laser sintered hydroxyapatite filled polymeric composites. *J. Biomed. Mater. Res. A*, **86-A**, 606-616. (doi:10.1002/jbm.a.31622)

Zhang, Y., Tanner, K. E., Gurav, N. & Di Silvio, L. 2007 *In vitro* osteoblastic response to 30vol% hydroxyapatite polyethylene composite. *J. Biomed. Mater. Res. A*, **81-A**, 409-417. (doi:10.1002/jbm.a.31078)