

An in vitro study of electrically active hydroxyapatite-barium titanate ceramics using Saos-2 cells

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Received: 11 February 2009 / Accepted: 16 March 2009 / Published online: 24 March 2009
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Abstract Electrically active ceramics are of interest as bone graft substitute materials. This study investigated the ferroelectric properties of hydroxyapatite-barium titanate (HABT) composites and the behaviour of osteoblast-like cells seeded on their surfaces. A piezoelectric coefficient (d_{33}) of 57.8 pC N^{-1} was observed in HABT discs prepared for cell culture. The attachment, proliferation, viability, morphology and metabolic activity of cells cultured on unpoled HABT were comparable to those observed on commercially available hydroxyapatite at all time points. No indication of the cytotoxicity of HABT was detected. At one day after seeding, cell attachment was modified on both the positive and negative surfaces of poled HABT. After longer incubations, all parameters observed were comparable on poled and unpoled ceramics. The results indicate that HABT ceramics are biocompatible in the short term in vitro and that further investigation of cell responses to these materials under mechanical load and at longer incubation times is warranted.

1 Introduction

There is a growing need for graft materials suitable for the replacement of bone lost both through trauma or disease and as a result of complications arising from joint

replacement surgery. Inherent problems associated with the use of the surgical “gold standards”, autograft and allograft, have resulted in much research into the development and improvement of synthetic bone grafts [1–3]. Growth factors such as transforming growth factor- β , fibroblast growth factor and bone morphogenetic proteins can be synthesised in vitro and included in graft materials [4]. Alternatively, the addition of elements which are commonly found in bone, such as silicon, has been shown to enhance the biological response to bone grafts in vitro [5]. The majority of materials which are commercially available for use as bone graft substitutes are based on ceramics. Of these, hydroxyapatite (HA) based bioceramics are among the most commonly used [6].

Recently, there has been a growing interest in electrically charged and piezoelectric materials which may be relevant in the field of bone grafting [7] on the basis that bone itself is electrically active. Stress generated potentials are induced in loaded bone as a result of streaming potentials (produced when a charged fluid flows over a charged surface) and piezoelectricity (whereby a voltage is produced when a material is compressed). The piezoelectric properties of bone result from the movement of collagen fibres under a mechanical load [8]. As the electrical signals generated in loaded bone have been linked in the literature to mechanotransduction and subsequently to bone regeneration [9], it is possible that the addition of a charged or piezoelectric component to an artificial bone graft may improve the biological response to that graft.

Enhanced responses to electrically charged ceramics have been demonstrated in vivo by Itoh et al., who found that both negative and positive electrical polarisation of HA enhanced osteoblast and decreased osteoclast activity, respectively [10]. These observations were based on studies carried out 3–6 weeks after implantation of charged

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implants in rat calvarial bones. Improved biological responses have also been reported for various materials with piezoelectric charge coefficients between 6 and 17 pC/N⁻¹ [7, 11]. These materials were tested under mechanical loading *in vivo* and compared to electrically inert materials. Tissue growth around the grafts was observed to be significantly different in both studies at one week post implantation. Early results obtained by Park et al., who studied barium titanate (BT), a lead-free ferroelectric material that exhibits good piezoelectric properties, showed no improvement of bone ingrowth or bone-implant interface strength after implantation in canine subjects when compared to unpoled samples. The authors attribute this to a lack of mechanical loading of the samples as a consequence of errors in surgical procedure [12].

BT has also been examined *in vivo* by Gimenes et al., who implanted PVDF-TrFE/BT composites in rabbit tibiae [13]. Although good bone formation was observed around the implants, no unpoled control samples were tested. In addition, Feng et al. carried out an *in vivo* study implanting composites of hydroxyapatite (HA) and barium titanate (BT) into canine jawbones [7]. The implants were loaded and differences were found in bone ingrowth and directionality of tissue growth when compared to electrically inactive HA samples. The results of this study were generally promising, but the scope was limited. Though the authors claim that the results demonstrate a link between osteogenesis and stress generated potentials in the piezoelectric materials, the possible effects of residual surface charge are not considered in their analysis and details of the exact composition of the HABT composite were not given. As with the Gimenes et al. study [13], the biological response to unpoled samples of the material was not reported. On this basis no comparative analysis can be made between the poled and unpoled materials and further work is necessary in order to separate the influence of the chemistry of these materials from that of their electrical state.

There is much published work on the biocompatibility of poled and unpoled HA. In comparison, there is little or no literature encompassing the *in vitro* response to HABT composites. The importance of such *in vitro* studies lies in the possibility of adopting a consistent analytical approach in well controlled conditions. This allows a comparative baseline to be established, against which the effect of individual influences on biological responses may be assessed.

An earlier study by the authors characterised the piezoelectric properties of HABT composite discs containing 0–100% BT by volume [14]. The ceramics produced in this study containing 70% BT or less were found to have no measurable piezoelectric properties while the piezoelectric charge coefficient (d_{33}) of discs containing 80–100% BT

varied between 18 and 108 pC/N⁻¹, dependent on the quantity of HA included. It is therefore reasonable, based on the published literature, to expect that the HABT ceramics containing 80% BT or more may induce an improved biological response under dynamic loading conditions. Samples with 90% BT also exhibit high piezoelectric g_{33} coefficients, a measure of electric field per unit stress [14]. On the basis of the piezoelectric properties of the composites, the ceramic containing 90% BT was selected for *in vitro* testing of biological responses.

It is important to note that it is standard practice to measure the piezoelectric properties, such as d_{33} , of materials with metallic electrodes applied to their major surfaces [15]. In biological applications silver electrodes will not be applied to the ceramics and earlier studies often do not state the conditions in which the piezoelectric properties of the materials were tested [7]. In this work, it is considered desirable to investigate piezoelectric properties of HABT ceramics in the same condition in which they are used for cell culture.

In addition to electrical properties and surface chemistry, substrate topology and roughness are important considerations with respect to the biological response to an implant material [16]. It is therefore necessary to characterise the surface topology and roughness in order to properly determine the impact of changes in chemistry (i.e. the addition of BT) and electrical state of the composite on cell behaviour.

The aim of this study was to compare the attachment, proliferation and morphology of human osteoblast-like cells cultured on commercially available HA and on HABT composite discs containing 90% BT *in vitro* in static, unloaded conditions. The influence of the poling of the 90% BT ceramics on cell behaviour was investigated by testing both unpoled (electrically inert and non-piezoelectric) and poled (piezoelectric) materials. The physical characteristics of the ceramic surfaces, the composition of the sintered HABT discs and their piezoelectric properties were also measured.

2 Materials and methods

2.1 Manufacture and poling of HABT ceramics

Hydroxyapatite (Thermphos BV, UK) and barium titanate (Ferro, UK) powders were combined to produce a mixture containing 90% BT by volume. The mixed powder was measured using a volume gauge (0.353 cm³ per tablet) and pressed in a 15 mm die at 100 MPa for 5 s. Discs were also produced from 100% hydroxyapatite powder. All of the pressed samples were sintered at 1300°C for 2 h. Post-sintering, the discs measured approximately 13 mm in

diameter and 2–3 mm in thickness. The ceramics were ground flat using P600 silicon carbide paper, finished using P1200 silicon carbide paper and were cleaned by sonication in ethanol. Two sets of samples were produced, one of which was poled by corona poling at 130°C with an electric potential of 25 kV and a point source height of 55 mm.

2.2 Determination of piezoelectric properties

In order to replicate the conditions under which biological testing was carried out, discs of 90% BT were stored for 24 h in 70% ethanol at 4°C, rinsed in sterile phosphate buffered saline (PBS; Sigma Aldrich, UK) and soaked in complete culture medium overnight. The piezoelectric coefficients of the discs in the poled direction of the sample (d_{33}) were measured using a Take Control Piezometer System PM25 which applies a small alternating force (0.1 N) and measures the piezoelectric charge generated.

For the measurement of the polarisation-field (P-E) and strain-field (S-E) hysteresis loops of the ceramics, silver electrodes were applied to the surface and samples were poled by corona poling. Measurements were undertaken at 10 Hz with the electric field magnitude gradually increasing in 0.25 kV mm⁻¹ increments up to a maximum of 2 kV mm⁻¹ to observe ferroelectric domain switching. The measurements were made using a system designed and constructed by the National Physics Laboratory, Teddington, UK. A 100% BT sample was also tested for comparison with the 90% BT composite.

2.3 Determination of HABT ceramic composition

The composition of the 90% BT ceramics was analysed using X-Ray Diffraction (XRD). Discs were crushed to powder and studied in a Philips X-Ray Diffractometer using Cu α radiation with a wavelength of 1.54° and a graphite monochromator. X ray intensity was measured for angles in the range 20° < 2 θ < 60° with a step size of 0.01°. The diffraction patterns produced were then compared with existing data using PDFC 2.00 database software. To identify the phases present, the location of peaks in the XRD profiles were compared to reference spectra [17].

2.4 Characterisation of the surfaces used for cell culture

In order to quantify the influence of surface topography and roughness on the behaviour of the cells, three dimensional micro-topography plots of the tablet surfaces were produced using Proscan 2000 non-contact profilometer with a step size of 1 μ m in the lateral x and y axes and resolution of 0.1 μ m height (z axis). The area of the tablet surface to be analysed was selected at random and measured

150 \times 150 μ m. Roughness parameters of average roughness (R_a , the average deviation of the profile from the mean line), maximum roughness (R_{max} , the maximum peak to valley height) and ten-point height (R_z , the average of the five highest peaks and the five lowest valleys) were then calculated. The average roughness, while giving a good general description of height variations, is not sensitive to small changes in profile. It was therefore desirable to include other measures of roughness which were more sensitive to occasional features (R_z and R_{max}) [18].

2.5 Cell culture and seeding

To investigate the influence of the 90% BT material on the cell behaviour, cells were cultured on unpoled (non-piezoelectric) discs of the ceramic as well as on 100% HA discs for comparison. The effect of poling the discs was analysed by seeding cells onto unpoled discs and onto the positive and negative surfaces of poled (piezoelectric) samples.

A human osteosarcoma cell line (Saos-2, ECACC, UK) was used to assess the cellular response to the materials. The Saos-2 cells which display a mature osteoblastic phenotype [19] were cultured in McCoy's 5A medium (Sigma Aldrich, UK) supplemented with 2 mM L-Glutamine (Sigma Aldrich, UK) and 10% fetal calf serum (FCS; Sigma Aldrich, UK). They were seeded at 20,000 cells/cm² in T75 flasks (Triple Red, UK) and grown to confluence at 37°C with 5% CO₂ before being detached from the tissue culture plastic using 0.1% trypsin (Sigma Aldrich, UK) and sub-cultured. The culture medium was changed every 2–3 days.

In preparation for cell culture, the ceramic discs were kept for 24 h in 70% ethanol at 4°C, rinsed in sterile phosphate buffered saline (PBS; Sigma Aldrich, UK) and soaked in complete culture medium overnight. Discs were then allowed to dry in air for 1 h in a 24-well plate (Triple Red, UK) in sterile conditions before cells were seeded at 20,000 cells/cm² in 25 μ l of complete culture medium. Cells were seeded onto tissue culture plastic as a control at 20,000 cells/cm² in 38 μ l of complete culture medium, accounting for the difference in surface area between the control and the sample. The culture plates were incubated for 2 h at 37°C with 5% CO₂ in order to allow cells to adhere to the ceramic surface before 1 ml of complete culture medium was added to each well. Cells were then incubated for 1, 3 or 7 days; culture medium was changed every 2–3 days.

2.6 Cell adhesion and proliferation

After incubation, the culture medium was removed and the wells were rinsed twice using sterile PBS before 500 μ l of trypsin was added to each well, covering the surface of the disc. Samples were incubated for 10 min at 37°C with 5%

CO₂ before 500 µl of complete culture medium was added. The cell suspension in the wells was mixed and a 20 µl sample was taken to be combined in a ratio of 1:1 with trypan blue (Invitrogen, UK). The live and dead cells were then counted using a haemocytometer and total cell numbers and viabilities for the individual samples were calculated. Average cell population doubling times were also calculated.

2.7 Cellular metabolic activity

The metabolic activity of the cells was measured using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay kit (CGD-1, Sigma Aldrich UK). The culture medium was removed from the wells and replaced with 500 µl of serum-free medium. After adding 50 µl of MTT solution to each well, the culture plates were incubated at 37°C with 5% CO₂ for 4 h. The medium was then removed and 200 µl of MTT solvent was added to each well, ensuring all visible crystals formed were dissolved. The solvent was then transferred to a 96-well plate (Fisher Scientific, UK) and a colorimetric measurement was carried out using a Dynex MRX spectrophotometer (Dynex Technologies Ltd., Worthing, UK) at 570 nm with a 690 nm reference wavelength.

2.8 Scanning electron microscopy

The morphology of the culture cells was examined using scanning electron microscopy (SEM). Three samples of each ceramic (HA, unpoled 90% BT and the positive and negative surfaces of poled 90% BT) were studied. Cells were fixed on the surface of the tablets using glutaraldehyde (2.5% by volume) and potassium ferrocyanide (1% by weight) in serum-free McCoy's 5A medium. They were then dehydrated in serial dilutions of acetone and hexamethyldisilazane (Sigma Aldrich, UK). The discs and attached cells were then sputter-coated with gold and images were obtained using a JEOL 6480LN electron microscope with an accelerating voltage of 10–15 kV.

The ×500 magnification micrographs obtained from the SEM were analysed using Image-Pro MC software to measure the longest dimension and the area of the cells growing on the various ceramic surfaces. Two micrographs of each material type were examined, with four cells measured from each one giving a total of eight measurements per composition ($n = 8$).

2.9 Statistical methods

All results are given as mean ± standard deviation and $n = 6$ unless otherwise stated. Statistical analysis was performed on all results using one-way ANOVA and

Kruskal–Wallis tests as appropriate. Statistical significance was determined as $P < 0.05$.

3 Results

3.1 Piezoelectric properties

The piezoelectric d_{33} coefficient of the 90% BT composite prepared for cell culture was 57.8 pC N⁻¹ with a standard deviation of 10.8 pC N⁻¹. The data from the polarisation-field (P-E) and strain-field (S-E) measurements are shown in Fig. 1 where data ranged from low electric fields (small loops) to high electric fields of up to 2 kV mm⁻¹ (large loops). The polarisation-field loop, Fig. 1a, showed that at a maximum applied field of 2 kV mm⁻¹, the mean remnant polarisation (from $P_r(1)$ and $P_r(2)$ in Fig. 1a) was 0.02 C m⁻² and mean coercive field [from $E_c(1)$ and $E_c(2)$] was 0.80 kV mm⁻¹. For comparison, a pure BT sample exhibited a mean P_r of 0.06 C m⁻² and E_c of 0.45 kV mm⁻¹, which compares well with reported values for BT [20]. The P-E loop of the 90 BT composite is asymmetrical since the material was initially poled, leading to a 'biased' loop [20]. The peaks visible on the current-field loops, Fig. 1b, are also shown as they are indicative of domain switching and clearly demonstrate that the HABT composite chosen for this study is ferroelectric in nature. The strain-field loop for a relatively low electric field (1 kV mm⁻¹) is shown in Fig. 1c and the strain-field relationship is relatively linear since there is no domain switching. The (d_{33}) of the 90 BT prepared was calculated as 94.7 pV m⁻¹ (or C N⁻¹) from the gradient of the strain-field loop in Fig. 1c. This is higher than the 57.8 pC N⁻¹ measured by the Piezometer, possibly due to the presence of a silver electrode for the materials tested. At higher electric fields (2 kV mm⁻¹) domain switching occurs and the strain-field response shows a characteristic 'butterfly loop' as shown in Fig. 1d.

3.2 Composition of sintered HABT ceramics

The composition of sintered discs was examined by XRD to assess any chemical reactions taking place between the HA and BT in the 90% BT discs. The majority of the peaks in the spectrum (Fig. 2) corresponded to the main reported peaks for HA and BT [17], as labelled in the figure. However, three peaks were evident in the sintered 90% BT which did not correspond to either of the materials in the original powders (marked with an asterisk in Fig. 2). Though it is not possible to confirm the identity of the compounds producing these peaks, materials containing the same elements as HA and BT and producing peaks at the relevant 2θ angles include β -tricalcium phosphate, barium phosphate, calcium barium hydrogen phosphate and barium titanium phosphate [17].

Fig. 1 Hysteresis loops for poled 90% BT. **a** Polarisation-field loop, **b** Current-field loops for increasing applied electric field, **c** Strain-field loop for low applied field (1 kV mm^{-1}), **d** Strain-field loop for high applied field (2 kV mm^{-1})

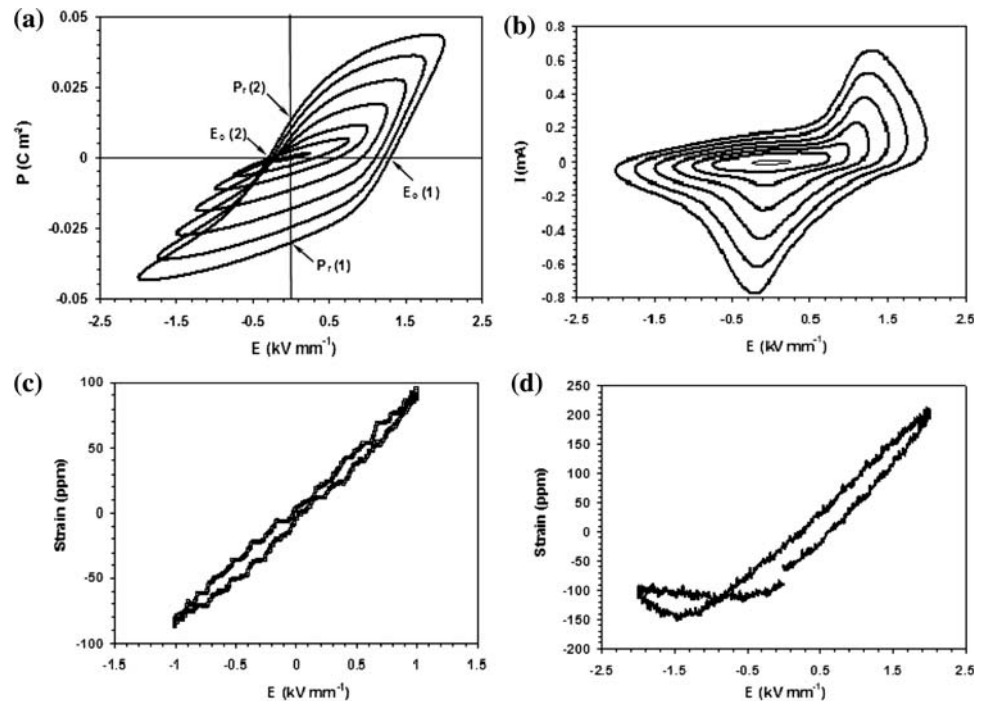
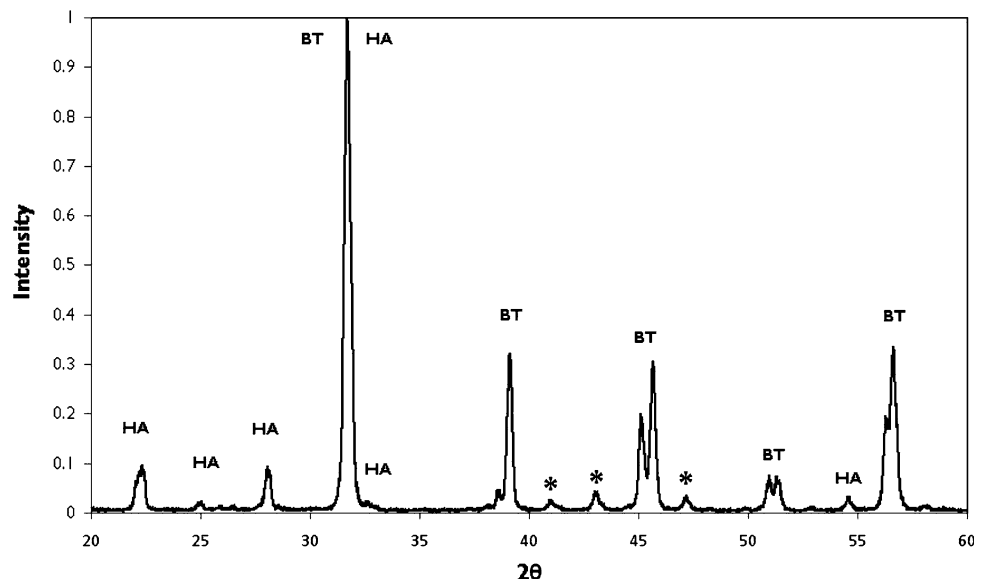


Fig. 2 X-Ray diffraction spectrum of HABT composite containing 90% BT



3.3 Cell culture surfaces

The surface roughness values measured for HA and 90 BT discs are shown in Fig. 3 illustrating the average values for R_a , R_{max} and R_z for the two ceramics examined. No significant statistical difference was found between any of the roughness parameters for the HA and 90% BT discs, which had an average roughness (R_a) around $0.5 \mu\text{m}$, maximum roughness (R_{max}) in the range of $3\text{--}4 \mu\text{m}$ and ten-point roughness (R_z) between 2 and $2.5 \mu\text{m}$. The three dimensional micro-topography plots of the disc surfaces, shown in Fig. 4, demonstrate the presence of similar features for the HA and HABT ceramics.

3.4 Cell adhesion and proliferation

Cell numbers found on the 100% HA and unpoled 90% BT discs, along with tissue culture plastic controls, are given in Fig. 5. No significant difference was found between the numbers of cells on the two different compositions of ceramic at any of the time points tested. The cell population on the ceramics was found to have increased from $15,500\text{--}16,500 \text{ cells/cm}^2$ at 1 day post seeding to $33\text{--}34,000 \text{ cells/cm}^2$ at 3 days and $200,000 \text{ cells/cm}^2$ at 7 days. The proportion of viable cells measured varied between 87 and 97%, with no significant differences found in the viability of cells cultured on the two ceramics.

Fig. 3 Average roughness (Ra), maximum roughness (Rmax) and ten-point height (Rz) of HA and 90% BT

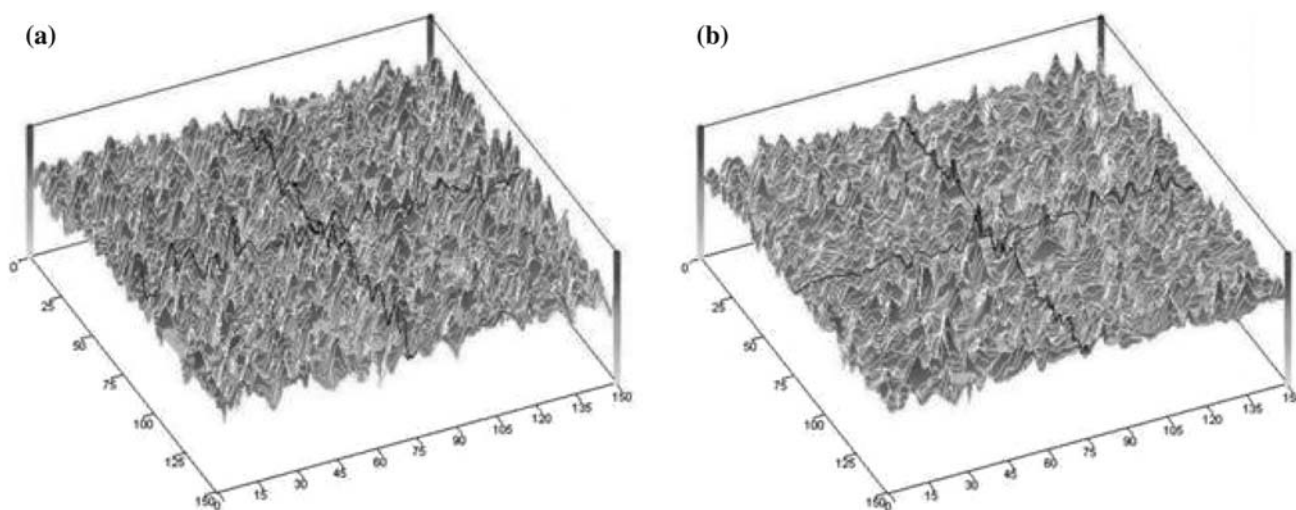
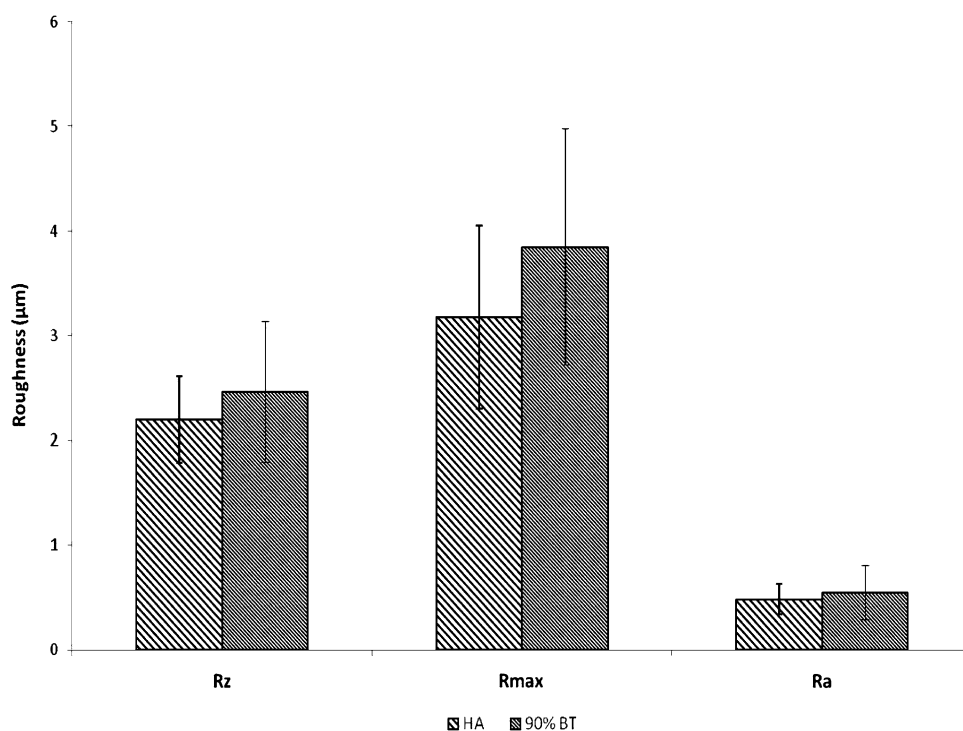


Fig. 4 Surfaces of (i) HA and (ii) 90% BT. **a** Microtopography plots, **b** SEM micrographs (original magnification $\times 500$)

A further investigation was then carried out on the 90% BT ceramics in order to determine the effect of poling condition on the attachment and proliferation of Saos-2 cells on the material. Cells were cultured on unpoled (electrically inert), surfaces and on the positive and negative surfaces of poled discs. It was found that cell viability was not dependent on the electrical state of the culture surface, with mean values varying between 94 and 97%. The numbers of cells found are shown in Fig. 6. The

presence of surface charge, though not the nature of that charge (positive or negative), appeared to increase the numbers of cells found to be attached to the material at one day after seeding, but there was no evidence that this trend was sustained at the later time points. At three days after seeding no significant differences were found between the cell populations on the ceramics. After seven days of incubation the cell populations on all ceramics were comparable to those found on the control.

Fig. 5 Cell proliferation on the control surface (tissue culture plastic), hydroxyapatite (HA) and HABT composite containing 90% BT

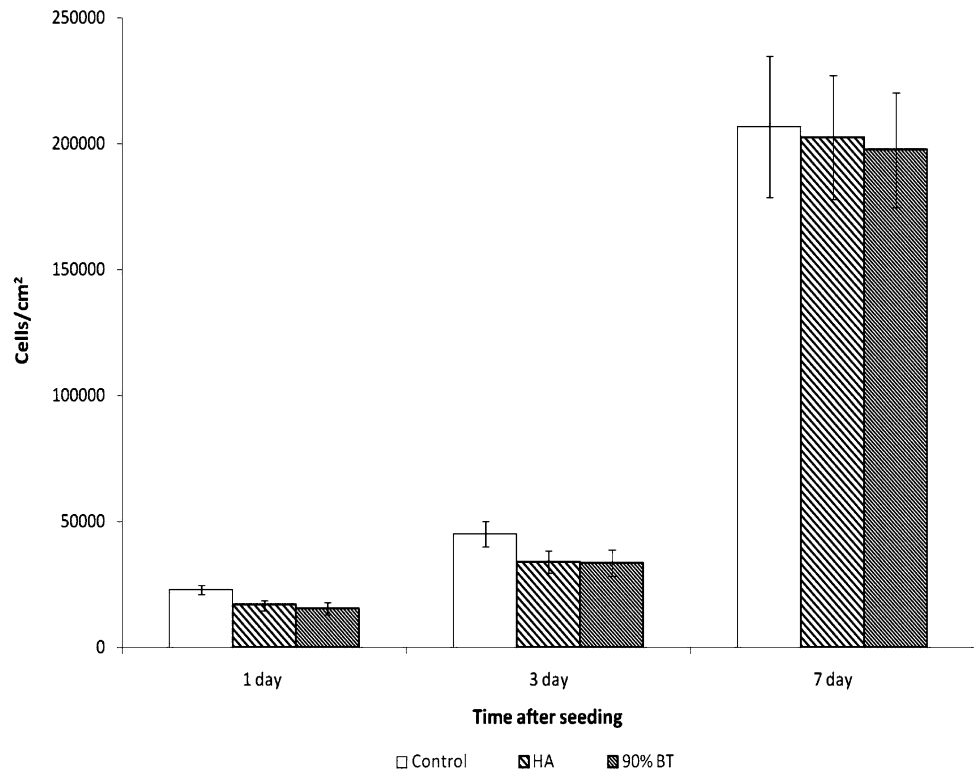
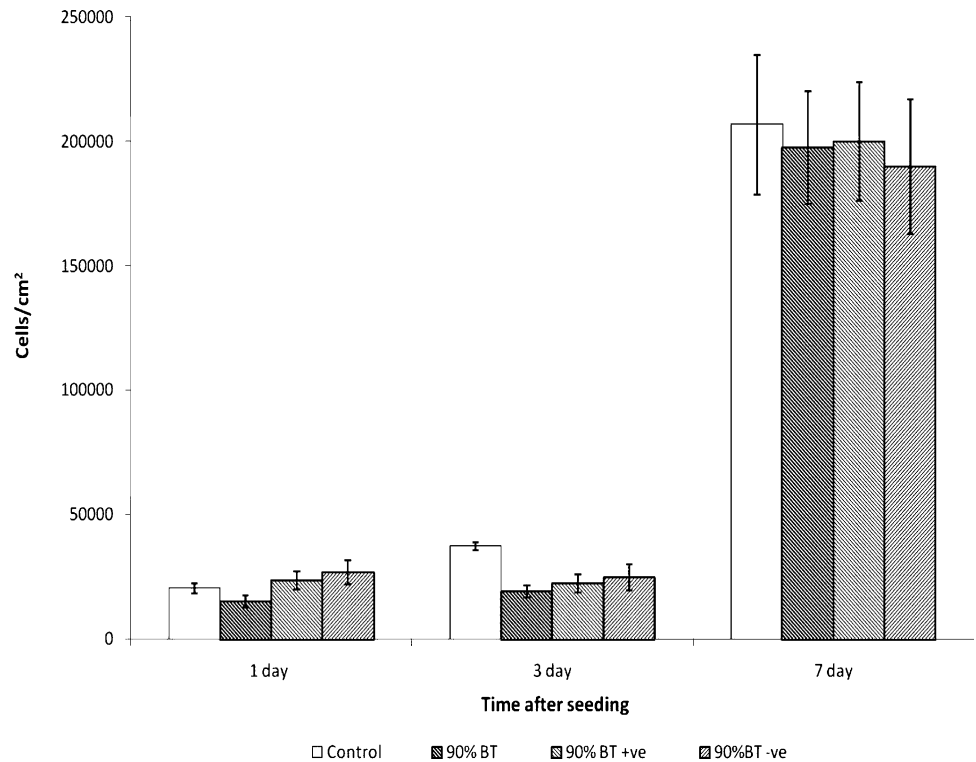


Fig. 6 Cell proliferation on unpoled HABT composite, the positive surface of the poled HABT (90% BT +ve) and the negative surface of the poled HABT (90% BT -ve)



3.5 Cell metabolic activity

The influence of 90% BT discs on the metabolic activity of Saos-2 cells cultured on their surfaces was measured using

an MTT assay. Cells cultured on 100% HA and on tissue culture plastic were used for comparative purposes. The results of this assay are shown in Fig. 7. The metabolic activity of the cells was found to be unrelated to the

Fig. 7 Cell activity on the control surface (tissue culture plastic), hydroxyapatite (HA) and HABT composite containing 90% BT

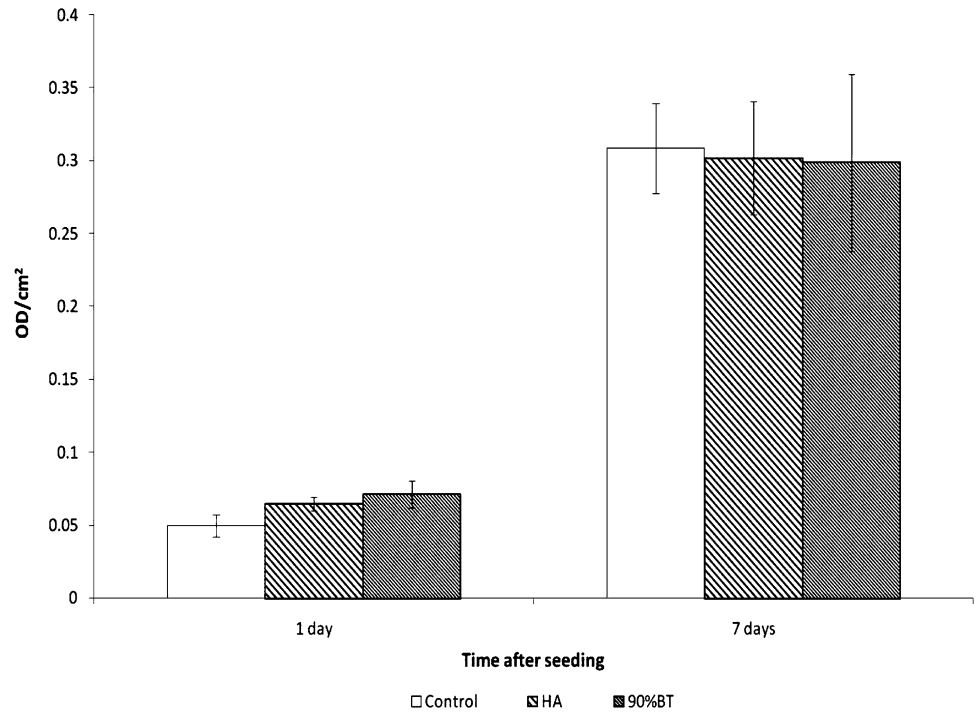
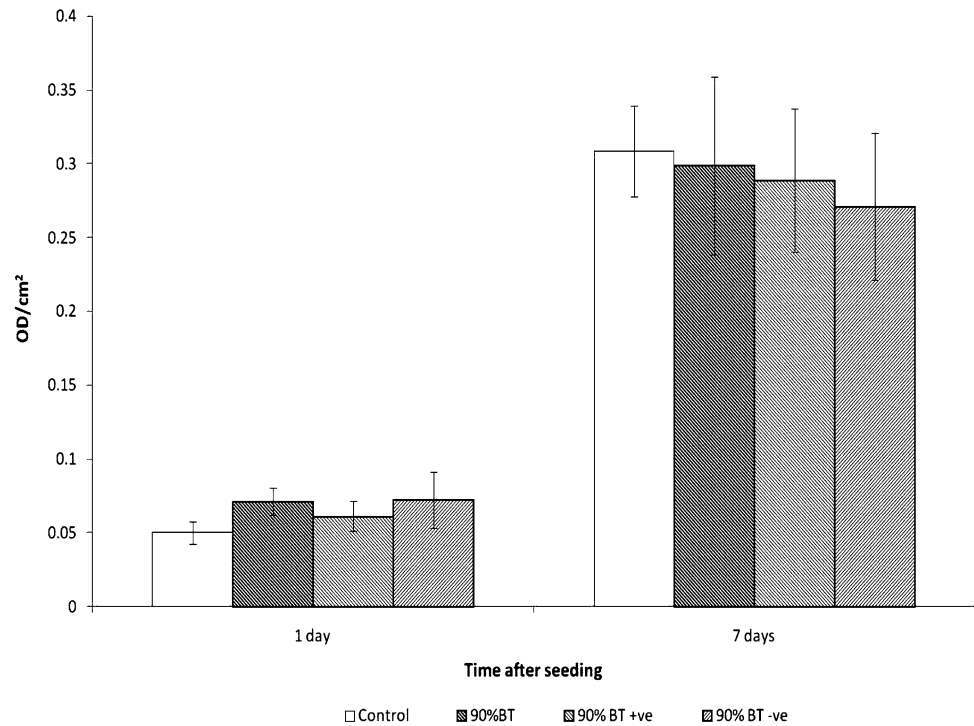


Fig. 8 Cell activity on unpoled HABT composite, the positive surface of the poled HABT (90% BT +ve) and the negative surface of the poled HABT (90% BT -ve)



composition of the ceramic on which they were cultured at both one and seven days after seeding. Cells appeared to be more metabolically active on the ceramics than on the tissue culture plastic controls.

Similarly, the MTT assay was used to examine the effect of the poling of 90% BT on cell metabolic activity. The results, shown in Fig. 8, indicate that there were no significant differences in cell activity on the unpoled, positive

Fig. 9 SEM micrographs showing Saos-2 cells cultured on **a** HA and **b** unpoled 90% BT, 24 h after seeding. The *white arrow* indicates space between cells and the *black arrow* indicate the presence of lamellipodia and filopodia

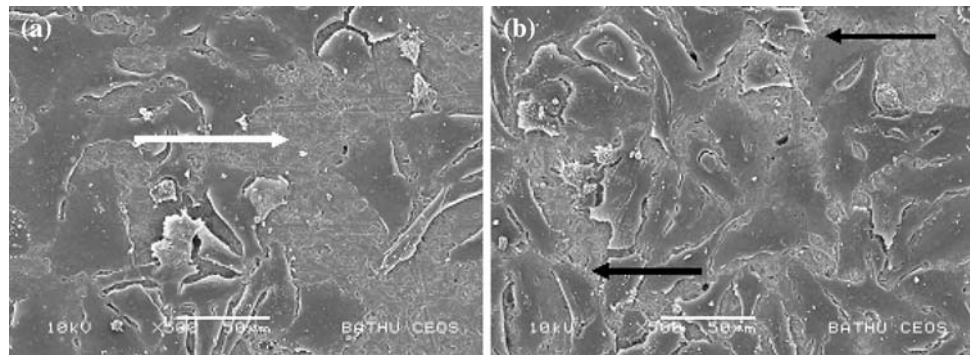
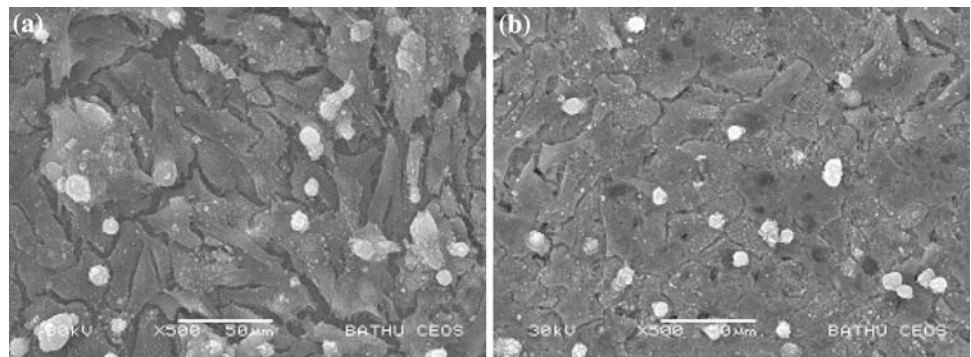


Fig. 10 SEM micrographs of Saos-2 cells cultured on **a** HA and **b** unpoled 90% BT, 7 days after seeding



and negative disc surfaces at either incubation time. This is in contrast with the data found for the cell number at the earlier time point, suggesting that although more cells were present on poled samples, they were less metabolically active than those growing on the unpoled material.

3.6 Cell morphology

The morphology of Saos-2 cells cultured on HA, unpoled 90% BT and the positive and negative surfaces of poled 90% BT was examined using SEM. Examples of images obtained of cells cultured on the HA and unpoled 90% BT discs are shown in Fig. 9. Cells were found to be in intimate contact with the culture surfaces, irrespective of surface chemistry or charge. After one day in culture, cells grown on all materials displayed a flattened morphology. The cell populations were not evenly distributed; some areas of the surface were densely populated, leaving other areas vacant, as indicated by the white arrow in Fig. 9a. The migration of cells on the material surface was demonstrated by the presence of lamellipodia on all the materials studied, as indicated by black arrows in Fig. 9b. By seven days after seeding surfaces were substantially covered (as shown in Fig. 10) and cells displayed attachments both to the ceramic surface and to surrounding cells. The morphology and spreading of cells on unpoled 90% BT were comparable to those observed on both the positive and negative faces of poled 90% BT at both time points. Overall cell lengths were

found to be in the range of 40–60 μm at one day after seeding and approximately 30 μm at seven days after seeding. These sizes were of the same order of magnitude as sizes previously reported for Saos-2 cells [21].

4 Discussion

This study investigated the properties of HABT ceramics for potential use as electrically active bone graft materials. The attachment and development of human osteoblast-like cells cultured on the materials was investigated and the influence of the electrical state of the materials on these cells was assessed.

4.1 Electrical properties

The piezoelectric coefficient (d_{33}) of 57.8 pCN^{-1} for 90% BT ceramics prepared for cell culture was lower than that previously found for samples produced for analysis of piezoelectric properties (i.e. with electrodes on their surfaces). Samples of the same composition with electrodes exhibited a d_{33} of 72.8 CN^{-1} [22]. This phenomenon results from the increased collection of charge on the surface of the material with electrodes. The coefficient of the samples without electrodes remains within the range found by previous studies to influence the biological response to implant materials [7, 11].

The polarisation-field and strain-field loops for the 90% BT composite were typical of a ferroelectric material [20]. This was confirmed by the current-field loop, in which the peaks indicated that switching of ferroelectric domains was taking place. The piezoelectric coefficient obtained from the low-field strain loop concurs with previous measurements of the piezoelectric properties of these materials [14, 22]. The remnant polarization of the surface after poling does not depend on mechanical loading and may affect the behaviour of cells growing on the ceramics in the absence of any stress-generated potential. The remnant polarisation of both the HABT composites (0.021 Cm^{-2}) and the pure BT tested for comparison (0.061 Cm^{-2}) was lower than that reported by Gimenes et al. in their PVDF-TrFE/BT composites (0.33 Cm^{-2}).

The asymmetrical nature of the high-field S-E loop may result from a bias introduced by the initial poling process [20], but may also be linked to the pinning of the ferroelectric domains by inclusions within the ceramic which limit the deformation of the domain walls [23]. A matrix clamping effect, whereby the presence of the non-ferroelectric component may also limit the deformation, and therefore the voltage induced, in the composite for a given applied force [24].

The XRD analysis showed the main phases present in the composite to be HA and BT. Though definitive identification of the other phases present is not possible, the presence of compounds for which the biocompatibility has not previously been determined is likely.

4.2 Surface properties

The topology and roughness of cell culture surfaces have been shown previously to have had an influence on cell attachment, proliferation and maturation [16, 25, 26]. In the current study, the surfaces of the HA and 90% BT ceramics tested were shown to be of comparable topology and average, maximum and ten-point roughness. Any variations in cell behaviour between the ceramics tested may therefore be considered to be the result of the variations in surface chemistry or electrical state.

4.3 Biological responses

The viability of the cells seeded on unpoled 90% BT ceramics was found to be comparable to that of cells cultured on HA, a material widely considered to be biocompatible and suitable for use as a bone graft substitute [1]. This indicated that the 90% BT composite was not toxic to the Saos-2 cell line.

The study of cell numbers on the surface of the HA and unpoled 90% BT ceramics shows that the proliferation of

cells on the composite matches that on the HA during the first seven days after seeding. This result is supported by the analysis of the cell metabolic activity which clearly shows cells to be functioning in a similar manner on the two ceramics. The difference in composition, and therefore surface chemistry, between the HA and HABT ceramics did not alter the attachment or function of human osteoblast-like cells seeded on their surfaces. As the current study is the first *in vitro* assessment of HABT ceramics, it is not possible to compare the findings directly with the published literature. However, they were in broad agreement with the conclusions of Feng et al., who found that HABT ceramics of unspecified composition promoted the growth and repair of bone when implanted in canine subjects *in vivo* [7].

Further evidence of the suitability of 90% BT composites as a substrate for cell culture is provided by the SEM micrographs. Cells were shown to grow in intimate contact with ceramic surfaces in all electrical states examined. The addition of BT to the HA did not adversely affect the initial attachment of Saos-2 cells. The presence of lamellipodia was indicative of cell movement across the surfaces [27]. The similarities observed in uniform cell morphology and spreading between cells cultured on the composite and those cultured on commercially available HA at seven days after seeding indicated that the cells were accepting of the new material.

4.4 Effect of poling

Some influence of poling of 90% BT was found at one day after seeding, when cell attachment and metabolic activity appeared to be modified. In the longer term the influence of poling of 90% BT on cell adhesion, proliferation and viability was shown to be negligible. Previous studies [10] have found altered cell behaviour on charged surfaces in longer term experiments. It may be the case that the surface charges on those materials were higher than those induced on the 90% BT by the poling process, exacerbating the influence of the electrical state on the cells. It is possible that an improvement in biological response will be observed in the poled HABT ceramics when they are examined in loaded conditions resulting in stress generated potentials, as indicated in previous studies on piezoelectric materials with similar piezoelectric coefficients [7]. The incubation times employed in this study (1–7 days) were short in comparison with those used for the *in vivo* studies reported in the literature.

Longer term studies are justified in order to determine if either the surface chemistry or electrical state of 90% BT samples have an influence on the differentiation and function of Saos-2 cells attached to their surfaces.

5 Conclusions

It has been shown that piezoelectric ceramics of specific composition, with known electrical properties can be manufactured and the cell response to these materials measured.

The piezoelectric coefficient of HABT composite ceramics containing 90% BT was found to be within a range which, according to published literature, may be expected to induce an improved biological response in comparison with standard HA graft materials. The ferroelectric nature of the composite was confirmed. The piezoelectric properties of the composite may be limited by domain pinning or matrix clamping resulting from the presence of the HA in the ceramic.

This study is the first to use a human cell line to investigate the biocompatibility of a well characterised HABT composite. The behaviour of Saos-2 cells cultured for seven days on discs of HABT ceramics (90% BT) and 100% HA with similar values of surface roughness was found to be comparable. HABT ceramics were shown not to be cytotoxic to Saos-2 cells. The cellular response to the HABT ceramic was very promising in comparison with that seen on tissue culture plastic controls and on HA, a commercially available bone graft substitute material. No noteworthy variations in cell morphology or spread in relation to different material surfaces were observed. Although poling modified cell attachment and activity immediately post seeding, after three days the biological response was found to be consistent across all compositions and electrical states tested.

The results of this study indicate that the HABT composites are biocompatible with human cells in the short term in vitro. They provide a benchmark with which biological responses to HABT ceramics under mechanical loading may be compared. Overall, the indications are that longer term testing and investigation of the influence of mechanically loading these materials is warranted to establish their potential for clinical applications.

Acknowledgements The authors acknowledge the Engineering and Physical Research Council (EPSRC, UK) for their funding of this project (EP/DO13798/1) and the Centre for Electron Optical Studies at the University of Bath for the use of their facilities.

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