Short communication

# Biological performance of titania containing phosphate-based glasses for bone tissue engineering applications ☆

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## ARTICLE INFO

## ABSTRACT

The interplay between glass chemistry, structure, degradation kinetics, and biological activity provides flexibility for the development of scaffolds with highly specific cellular response. The aim of this study was therefore to investigate the role of titania inclusion into the phosphate-based glass on its ability to stimulate osteoblast-like human osteosarcoma (HOS) cells to adhere, proliferate and differentiate. In depth morphological and biochemical characterisation was performed on HOS cells cultured on the surface of glass discs. Cell proliferation was also studied in the presence of the glass extract. Cell differentiation, through osteoblast phenotype genes, alkaline phosphatase (ALP) activity and osteocalcin production, was carried out using normal or osteogenic media. Both Thermanox® and titania free glass were used as controls. The data demonstrated that titania inclusion provides desired cytocompatible surface that supported initial cell attachment, sustained viability, and increased cell proliferation similar or significantly higher than Thermanox®. The modified glasses regulated osteoblastic cell differentiation as detected by osteoblast phenotype gene transcription and upregulated ALP and osteocalcin expression. Using osteogenic media had no significant effect on ALP activity and osteocalcin expression. Therefore, titania modified phosphate glasses may have future use as bone tissue engineering scaffolds.

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## 1. Introduction

Bone regeneration is a natural process that occurs during bone remodelling. Where the defects are too large, however, the bone cannot heal itself. Bone tissue engineering has emerged as a promising route to stimulate the regenerative capacity of host bone cells to form new bone [1]. Bone tissue engineering utilises three-dimensional natural or synthetic scaffolds to provide the suitable environment for host cells to grow, and hence, proceed in tissue regeneration. These scaffolds are required to provide structural support for the host bone cells and should favourably affect bone formation by stimulating rapid cell adhesion, proliferation and finally regulate osteoblastic differentiation [2].

The fact that the major components of most phosphate-based glasses (e.g., phosphorus, sodium and calcium oxides) are also found in the inorganic phase of bone is a major contributing factor for the

bioactivity of these glasses. Ions released from degradable glasses can modulate the host bone cells response [3–9], and they could be involved in various cellular processes at different levels [10]. For example, calcium "Ca" supports osteoblast proliferation, differentiation and extracellular matrix mineralisation; it also stimulates the Ca-sensing receptors in bone cells and subsequently increases growth factor expression e.g., IGF-I and IGF-II [11]. Inorganic phosphate "P" stimulates the expression of Gla protein (MGP) which is a key regulator in bone formation [12]. Accordingly, the control of the glass dissolution rate and hence the metallic ion release could be an attractive approach to drive the biological response in the desired route [10].

The interplay between glass chemistry, degradation kinetics, and biological response provides a wealth of information for the development of scaffolds with highly specific cellular response [13,14]. Titanium is a transition metal; it is highly reactive as an element and spontaneously forms a stable 'native' oxide layer (~3–7 nm thick) on its surface [15]. This oxide layer acts as an inorganic substrate that attracts molecules, proteins and then cells to attach and finally form bone [16]. Hence this oxide layer has been credited to the ability of titanium implants to facilitate bone apposition. Thus titanium-containing materials/implants have been successfully used in various craniofacial and orthopaedic applications [17,18]. Also other phosphate glasses of more complex composition have been investigated and found suitable for tissue engineering applications [9,19,20].

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Inclusion of titanium oxide, namely titania, into slowly degradable phosphate-based glasses [50 mol% $P_2O_5$, 40 mol% CaO, $Na_2O$ (10 − x), and $TiO_2$ (x) where x varied from 1 to 5 mol%] has been reported to encourage attachment and viability of human osteosarcoma (HOS) cells. Increasing titania content (from 1 to 5 mol%) had a profound effect on cytoskeleton organisation, spreading and maturation of primary osteoblasts [5,21]. Currently, glass microspheres of approximately 10–200 μm in diameter based on the same system but with higher titania content (3–7 mol%) show considerable potential for use in a whole host of biomedical applications ranging from cancer radiotherapy and thermotherapy, to drug and protein delivery or bone tissue engineering applications [8]. The inclusion of titania into another relatively more degradable glass system [50 mol% $P_2O_5$, 30 mol% CaO, $Na_2O$ (20 − x), and $TiO_2$ (x) where x varied from 1 to 5 mol%] that released higher level of Ti ions (e.g., 5 mol% titania containing glass of this system release at 0.0085 $ppm \cdot h^{-1}$ instead of 0.0051 $ppm \cdot h^{-1}$ for the same titania content in the previous system) also improved MG63 cell attachment, proliferation and osteoblast phenotype gene expression as well as the in vivo bioactivity of these glasses [5]. Inclusion of up to 15 mol% titania, the maximum possible content, has been attempted with this glass system to produce further control on glass structure and properties. The network structure and properties of this system containing up to 15 mol% titania have been elucidated using diverse analytical techniques [3], but its cytocompatibility has not yet been assessed. From a materials science perspective, slight changes in glass composition produce major changes in glass structure, degradation, ion release and consequently biological response [6,22]. The aim of this study was therefore to, for the first time, assess the biocompatibility of phosphate-based glasses doped with the maximum possible titania content [50 mol% $P_2O_5$, 30 mol% CaO, $Na_2O$ (20 − x), and $TiO_2$ (x) where x varied from 5 to 15 mol%]. The influence of several glass characteristics and its degradation products on viability, proliferation and differentiation of osteoblast-like human osteosarcoma (HOS) cells has been investigated.

## 2. Materials and methods

### 2.1. Production of glasses

Using the precursor materials $NaH_2PO_4$, $CaCO_3$, $P_2O_5$, and $TiO_2$ (BDH, Poole, UK, all chemicals were > 98% purity), four glass compositions with different titania ($TiO_2$) contents were produced by melt-quenching as described previously [3,4]. The $P_2O_5$ and CaO contents were fixed at 50 and 30 mol% respectively, while the $Na_2O$ and $TiO_2$ contents varied. $TiO_2$ contents were 5, 10 or 15 mol% and accordingly the $Na_2O$ contents were 15, 10, or 5 mol% respectively. The melting temperature of the as-prepared glasses was 1300–1350 °C. The melted glass was poured into a preheated graphite mould and annealed at 420 °C for 1 h to remove any stresses due to preparation and then slowly cooled to room temperature. These glasses were coded as PCN, PCNT5, PCNT10 and PCNT15 respectively. Discs of 15 mm in diameter and approximately 1 mm in thickness were used. The discs were polished using waterproof silicon carbide papers P# 120 for 30 s at 300 RPM, then P# 500, 1000 and 2400 for 1 min each and finally P# 4000 for 2 min to get a mirror-like surface on a Struers Rotopol-11 (Struers, UK).

### 2.2. Human osteosarcoma cell culture

Human osteosarcoma (HOS) cells were cultured at 37 °C in a humidified atmosphere of 5% $CO_2$ in air, in growth medium [Dulbecco's modified Eagle's Medium (DMEM, Gibco), supplemented with 10% foetal calf serum, and 1% penicillin and streptomycin solution (Gibco)]. The medium was changed at 2 day intervals.

All samples for biocompatibility tests were sterilised by dry heating at 180 °C for 3 h, and pre-incubated in 2 ml of growth medium for 24 h at a 37 °C humidified atmosphere of 5% $CO_2$ in air. Both titania free glass

(PCN) and Thermanox® were used as controls. Cells were plated at a density of $3 \times 10^4$ cells/well in 24-well culture plates that provided a snug fit of the discs, in a 50 μl aliquot of medium (for initial attachment) prior to the addition of 1.5 ml of growth medium.

### 2.3. Cell viability and live dead staining

Determination of cell viability was carried out by incubating the specimens for 1 h in a standard growth medium containing 1 μl/ml calcein AM (acetomethoxy) to stain the live cells, and propidium iodide to stain the dead cells. The assessment of cell viability in three dimensions was performed using confocal laser scanning microscopy (CLSM, Bio-Rad, USA).

### 2.4. Cell proliferation assay

This study was carried out by growing HOS cells either directly on glass discs or in the presence of glass extracts. The extracts were produced by incubating glass discs in growth medium for 24 h. For the extract study, normal growth medium was used as a control. The proliferation assay was conducted up to 21 days for the direct study but only 7 days for the indirect one. At the required time point, the cultured cells were incubated in 10% alamarBlue® (Invitrogen, UK) for 4 h according to the manufacturer's instructions. The absorbance of the samples (n = 3 and duplicate reading for each one) was measured at 530 nm ($A_{530}$) and 590 nm ($A_{590}$) as excitation and emission wavelength using a Fluoroskan Ascent plate reader (Labsystems, Helsinki, Finland). The cell growth was presented as the average intensity of six replicate wells that was base line corrected and compared to the positive control cells.

### 2.5. Cellular differentiation

#### 2.5.1. Gene expression

For this study, only titania containing glasses, coded as PCNT5, PCNT10 and PCNT15, were used and compared with Thermanox®. At 1 and 14 days, the total RNA was isolated from the lysed [with RLT-buffer (Qiagen, Germany) and β mercaptoethanol (Sigma)] and homogenised cells using RNeasy minikit (Qiagen) and eluted with 30 μl RNase-free water. RNA concentration was calculated by using Quanti IT™ RNA assay kit with the Qubit™ flourometer (Molecular Probes™, Invitrogen). Subsequently, the extracted total RNA was reverse-transcribed into cDNA using a high capacity cDNA archive kit (Applied Biosystems, Cheshire, UK) and an Eppendorf thermal cycler (Mastercycler, Eppendorf UK Ltd., Cambridge, UK). Samples were stored at −70 °C for further analysis.

cDNA for each sample was then amplified by real-time PCR-ABI PRISM® 7300 sequence detection system (Applied Biosystems, Cheshire, UK) using human TaqMan® gene expression assay (Applied Biosystems, Cheshire, UK). Conjugated to FAM (6-carboxyfluroescein) reporter dye, TaqMan® probes (Applied Biosystems, Cheshire, UK) were used to target corresponding nucleotide sequences for osteoblast phenotype genes [alkaline phosphatase (ALP), collagen type I alpha subunit I (COLIaI), core binding protein factor alpha 1 (Cbfa1) and osteonectin (Sparc)] in the cDNA single strands. Relative quantification Q-PCR, using the 7300 SDS software, was conducted against the expression of rRNA encoding housekeeping gene, 18s.

#### 2.5.2. Alkaline phosphatase activity

For cell culture used in this study, both aforementioned normal growth medium (non-osteogenic), and osteogenic medium (prepared by adding 50 μg/ml ascorbic acid and 5 mM Na-β glycerophosphate to the normal growth medium) were used.

After 1, 7 and 14 days, the culture supernatants were removed and used for osteocalcin assay; samples were washed with phosphate buffer solution (PBS) (Gibco, UK) and then solubilised with 1% Triton-X by

gentle agitation. 50 μl of each test sample (solubilised cells) and 50 μl of the freshly prepared assay buffer solution (mixture of 0.4 mM diethanolamine-HCl (pH 10.0), distilled water, 2 mM magnesium chloride and 4 mM paranitrophenolphosphate (p-NPP) that was used as a substrate) were added to each well in a 96-well plate. After 30 min of incubation at 37 °C, the enzymatic reaction was terminated by adding 150 μl/well of 1 M NaOH. Enzyme activity was quantified by absorbance measurements at 405 nm using a 96-well microplate reader (Fluoroskan). Optical density was measured in reference to a control sample containing the substrate solution (50 μl p-NPP), Triton-X (50 μl) and 1 M NaOH (150 μl). The ratio of enzyme activity (nmol of p-NPP/ml) to the mass of protein (μg) was determined [21].

### 2.5.3. Osteocalcin assay

The concentration of osteocalcin in the isolated culture supernatants was obtained using Gla-type osteocalcin EIA (TaKaRa Bio) assay with lyophilised Gla-OC (glutamic acid, osteocalcin) standard according to the manufacturer instructions. The absorbance was spectrophotometrically measured and the total osteocalcin content was evaluated using calibration curve. The assay is calibrated using human osteocalcin standards (0–32 ng/ml) [21].

### 2.6. Statistical analysis

Statistical analysis was applied using a one way ANOVA test using SPSS 16.0 (UK Ltd., UK) followed by Dunnett (2-sided) $t$-test that treated Thermanox® and day 1 as a control. The mean difference was considered to be significant at 0.05 and 95% confidence interval.

## 3. Results

### 3.1. Cell viability and live dead staining

Fig. 1 shows confocal laser scanning microscopy (CLSM) images of live/dead stained HOS cells attached to the surface of the tested glass compositions and Thermanox® after 1 and 7 days of culture. After 1 day of culture, there were fewer live cells attached to the surface of PCN glasses than those on the positive control surface. In contrast, a higher number of viable cells attached to the surfaces of PCNT5, PCNT10 and PCNT15, indicated by higher cell density, than those attached to the positive control. After 7 days of culture, the number of live cells remained attached to the surface of PCN glass, and has been reduced, but no dead cells were detected. On all titania modified glasses,
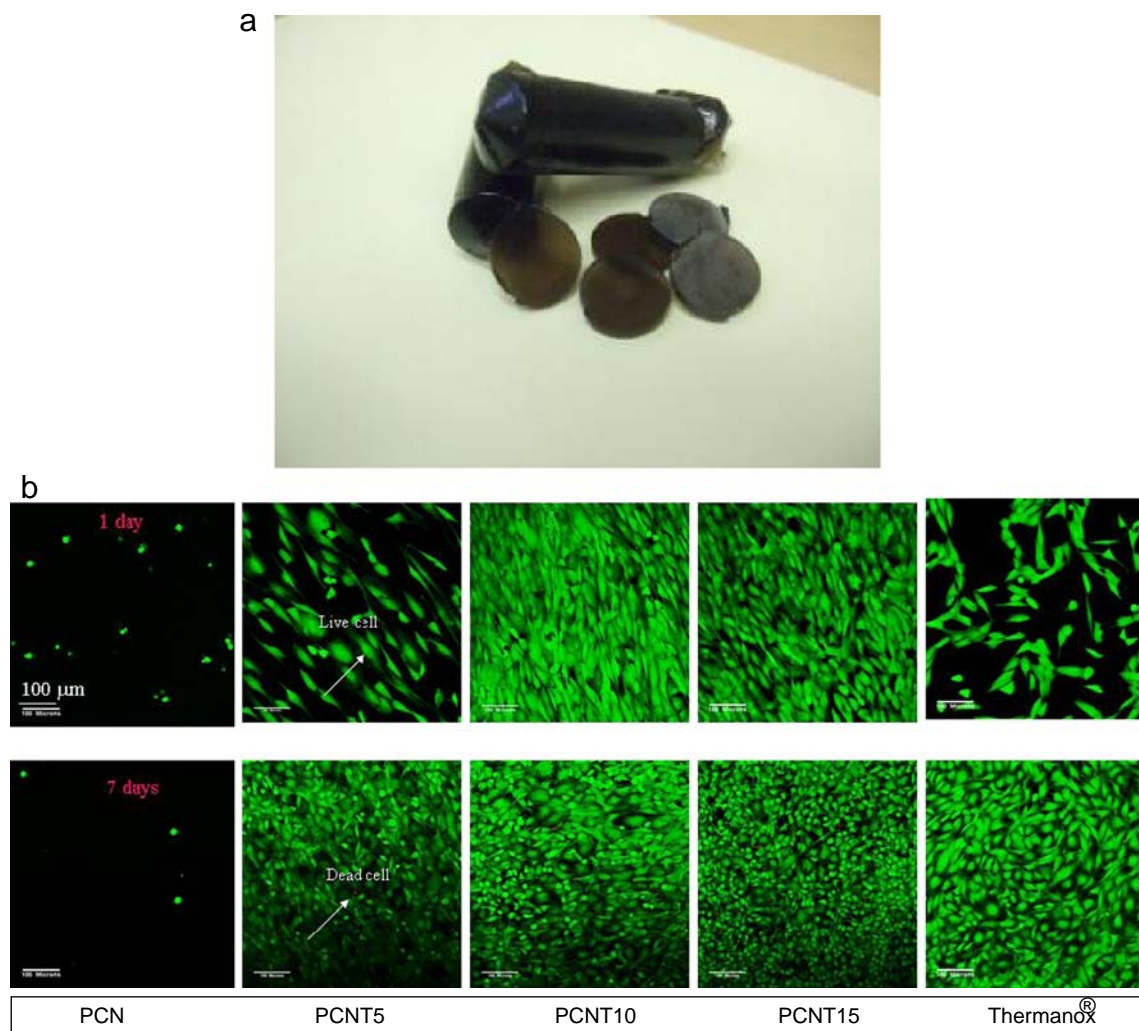


**Fig. 1.** (a) Photograph showing glass discs and rods. (b) CLSM images showing the viability of HOS cells cultured on the surface of different glass compositions compared to Thermanox® positive control. Live cells are stained green, while dead cells are stained red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HOS cells showed a tightly packed flattened morphology forming a three dimensional network covering the whole glass surface in a manner comparable to the positive control sample. Few dead cells were also seen attached to the surface of titania modified glasses.

### 3.2. Cell proliferation assay

Fig. 2 shows the overall metabolic activity of HOS cells grown directly on the pre-treated surface of tested and control samples (Fig. 2a) as well as in the presence of glass extract or the normal tissue culture media as a control (Fig. 2b). Generally PCN showed significantly lower cell growth than Thermanox® at the studied time points ($p < 0.0001$). All titania containing glasses, however, showed no significant differences from Thermanox® at all time points except at day 5 where PCNT5 showed significantly higher cell growth than Thermanox® ($p < 0.0001$). Moreover, the cell number increased with time on titania containing glasses and Thermanox®. At a later time point, 14 and 21 days in particular, there was no significant difference observed among titania containing samples (Fig. 2a). This could be associated with the fact that the cells reached confluence by day 7 of culture as reflected from confocal images. This finding demonstrates high efficacy of the titania glass to support proliferation of the cells.

Regardless of the significant ($p < 0.0001$) lower cell growth at day 1, the extracts of titania containing glasses supported a similar or significantly higher cell number than the control cells at days 5 and 7. The extract of PCN glass, however, resulted in significantly lower cell growth till 7 days of culture (Fig. 2b).

### 3.3. HOS cell differentiation

#### 3.3.1. Gene expression

Fig. 3a shows the relative quantification of ALP gene expression for cells seeded on PCNT5, PCNT10 and PCNT15 and Thermanox® after 1 and 14 days in culture. There was an increase in the transcription level of ALP at day 14 compared to day 1; this increase was only significant ($p < 0.05$) for the positive control and PCNT5. COL1a1, was also up-regulated at day 14; this up-regulation was statistically significant for all tested glasses but insignificant ($p \geq 0.05$) for the positive control (Fig. 3b). Cbfa1 showed an exactly similar trend to ALP — Fig. 3c. Although the transcription of Sparc increased at day 14 on all tested glasses and positive control, but this increase was not statistically significant (Fig. 4d).

Comparing the gene transcription level on titania containing glasses with Thermanox®, there was no statistical significant difference ($p \geq 0.05$) between the tested glasses and positive control except for PCNT5 at day 1 that showed a significantly higher ALP transcription level. A similar finding was also observed for COL 1a1, but PCNT5 showed a significantly higher level than Thermanox® at day 14. For Cbfa1, however, all tested glasses showed a statistically significantly higher level than positive control at day 1, but not at day 14. Sparc showed the opposite trend to Cbfa1 at day 1, but similar trend at day 14.

#### 3.3.2. Alkaline phosphatase activity

Fig. 4a–b show ALP expression after 1, 7 and 14 days of culture in both non-osteogenic and osteogenic media. As seen, there was no significant difference in ALP activity between osteogenic or non-osteogenic at different times of culture. Furthermore, there was a significant increase in ALP activity at days 7 and 14 compared to day 1; the difference however was not significant between ALP expression at days 7 and 14 for tested glasses and control. The ALP expression of HOS cells grown on PCNT5 and PCNT10 was not significantly different from that on Thermanox® at days 7 and 14. At day 1, however, ALP expression on these glasses was significantly lower than that of Thermanox®. ALP expression on PCNT15 was not significantly different from that on Thermanox® at the studied time point.

#### 3.3.3. Osteocalcin expression

Fig. 4c–d show osteocalcin expression after 1, 7 and 14 days of culture in both non-osteogenic and osteogenic media. Generally, osteocalcin expression in the presence of non-osteogenic media was higher (but does not necessarily prove to be significant) or comparable to that in osteogenic media i.e., there was no clear difference in osteocalcin expression in the presence of either osteogenic or non-osteogenic media. Moreover, there was no significant difference in osteocalcin expression on tested glasses and control when both osteogenic and non-osteogenic media were used for culture. Osteocalcin was mostly expressed at day 1 and then its amount slightly decreased with time but this was not significant; this was true for tested glasses and Thermanox®.

## 4. Discussion

An ideal biomaterial for bone tissue engineering should provide the appropriate signals for osteogenesis i.e., cell attachment/proliferation/differentiation, extracellular matrix formation and mineralisation. This study evaluated the biocompatibility of titania containing phosphate-based glasses, developed for potential bone tissue engineering application. For the evaluation of their biocompatibility and efficacy to promote cell proliferation and differentiation, HOS cells were cultured on their surface. Cell viability was investigated by live/dead staining and confocal laser scanning microscopy. For the evaluation of cell proliferation, HOS cells were incubated in vitro either in direct contact with the
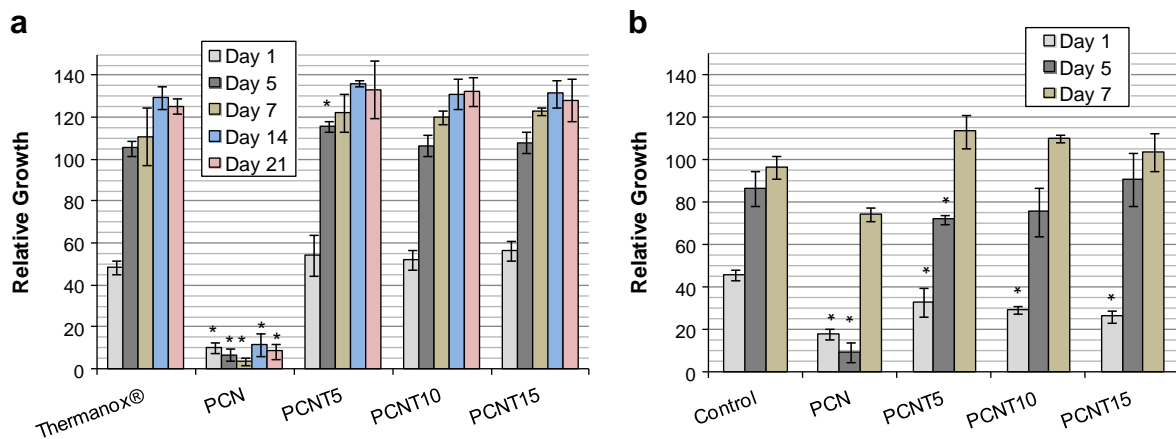


**Fig. 2.** Relative growth of HOS cells (a) seeded on the surface of different glasses or (b) in the presence of glass extracts. The unit represents the mean absorbance of the positive control cells. The ratios of the mean absorbance of test cells to that of positive control cells were presented as the relative growth. All values are presented as the mean ± standard deviation. * showed the significant difference from Thermanox®; the significance level was set at 0.05.
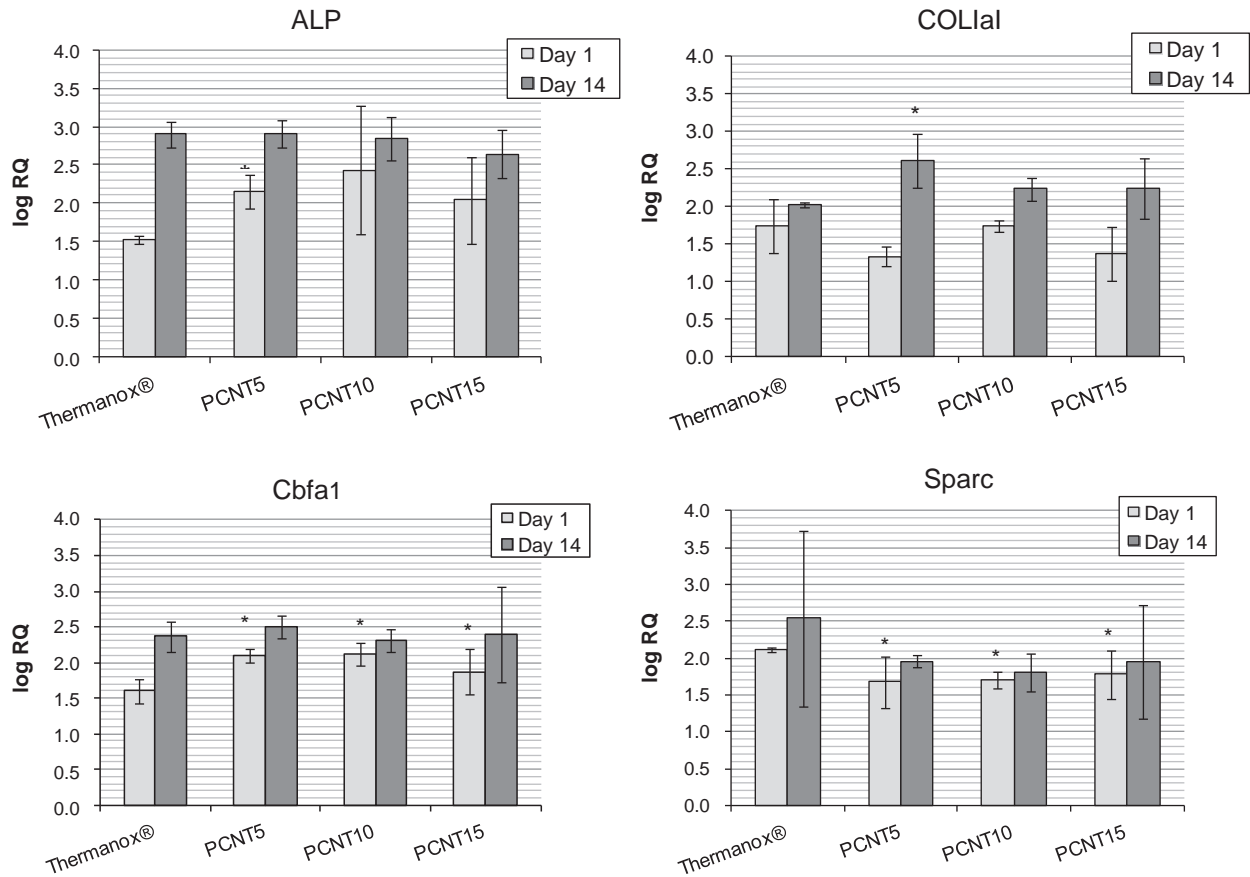
Fig. 3. Expression of osteoblast phenotype genes ALP (a), COL1a1 (b), Cbfa1 (c) and Sparc (d) of HOS cells grown on the surface of titania containing glasses and Thermanox® over a period of 14 days relative to 18s. All values are presented as the mean ± standard deviation. * showed the significant difference from Thermanox®; the significance level was set at 0.05.

glass surface or in the presence of glass extracts (indirect contact). This was considered as a model to simulate the in vivo environment where the material surface and its degradation products, which lead to a change in the concentration of ions in the surrounding environment, have a profound effect on cellular response. The difference between direct and indirect test should be, however, taken into consideration during data analysis and designing of the material for tissue engineering application. In direct proliferation, the cell response could largely be influenced by substrate properties such as surface roughness, stiffness, chemistry, free energy, and charge; all these factors are absent in the case of the extracts. Furthermore, the local conditions such as pH and ionic concentration are different in both cases [23]. The contact angle of these glasses was around 35° as reported previously [3]. The roughness of those samples however was <0.5 nm [3].

A number of studies have shown that the ionic product of degradation has an effect on cell proliferation and gene expression–differentiation. The ionic products may result in a gradient of pH in the culture medium with a significant shift near the surface as a consequence undesired cell response could occur [24]. Pre-immersion treatment of implantable devices including bioactive glasses in culture medium has been shown to improve cell response predominantly by pre-adsorbed proteins and inorganic component from the medium, which form a thin film on the surface of the device [25]. This film is capable of modulating cell behaviours e.g., cell-adhering fibronectin [26,27]. Furthermore, pre-immersion in culture media allows the release of the undesirable ionic products from the material and the adjustment of the surrounding pH [28]. Pre-conditioning of glass samples in the culture medium for 24 h has been therefore attempted before any cell culture study. Yet, the effect of the ionic products released from these glasses during pre-conditioning on cell proliferation has been considered throughout this study.

Previous studies on these glasses have employed osteoblast cell lines, MG63, and the results showed that these compositions were biocompatible with MG63 for the time of the experiment (7 days) [3]. Hereby, HOS cells were employed due to their extensive use as a model for the investigation of osteoblastic response to various biomaterials, and the fact that they express a number of features characteristic to osteoblasts [29–31]. The high viability of HOS cells grown on titania containing glasses indicated the suitability of these surfaces for cells to attach and grow. The PCN surface however could not support the attachment of HOS cells. Limited cell attachment and subsequent proliferation are likely to be caused by the high degradation rate of this glass system, which prevents stable anchoring of the cells on the surface. These findings are in agreement with previous studies [3,32]. Accordingly, PCN glasses were excluded from further experiment.

The improvement of the overall metabolic activity of HOS cells grown on the surface of titania containing glasses could be due to the early enhanced adhesion of HOS cells onto the glass surface. Cell adhesion and spreading are the initial events during cell-biomaterial interactions, which affect the long term function such as cell proliferation, synthesis of protein and calcium mineral deposition [33]. Cell adhesion could be also correlated with the hydrophilic nature of the glass surface [3] as well as its degradation [3,4]. Accordingly, the mobile surface of PCN did not support cell adhesion and growth. This finding was also supported by the absence of live cells attached to PCN glasses at day 7 of culture. The incorporation of titania provides a control on the glass degradation; this was suggested to be due to the formation of hydration resistant Ti–O–P instead of hydration susceptible P–O–P bonds [3,4].

The mobile surface is not the only factor that could affect cell attachment and proliferation but the ions released from the material as well. The extract of PCN glasses did not support the proliferation of HOS cells unlike the rest of the tested glasses and the normal tissue culture
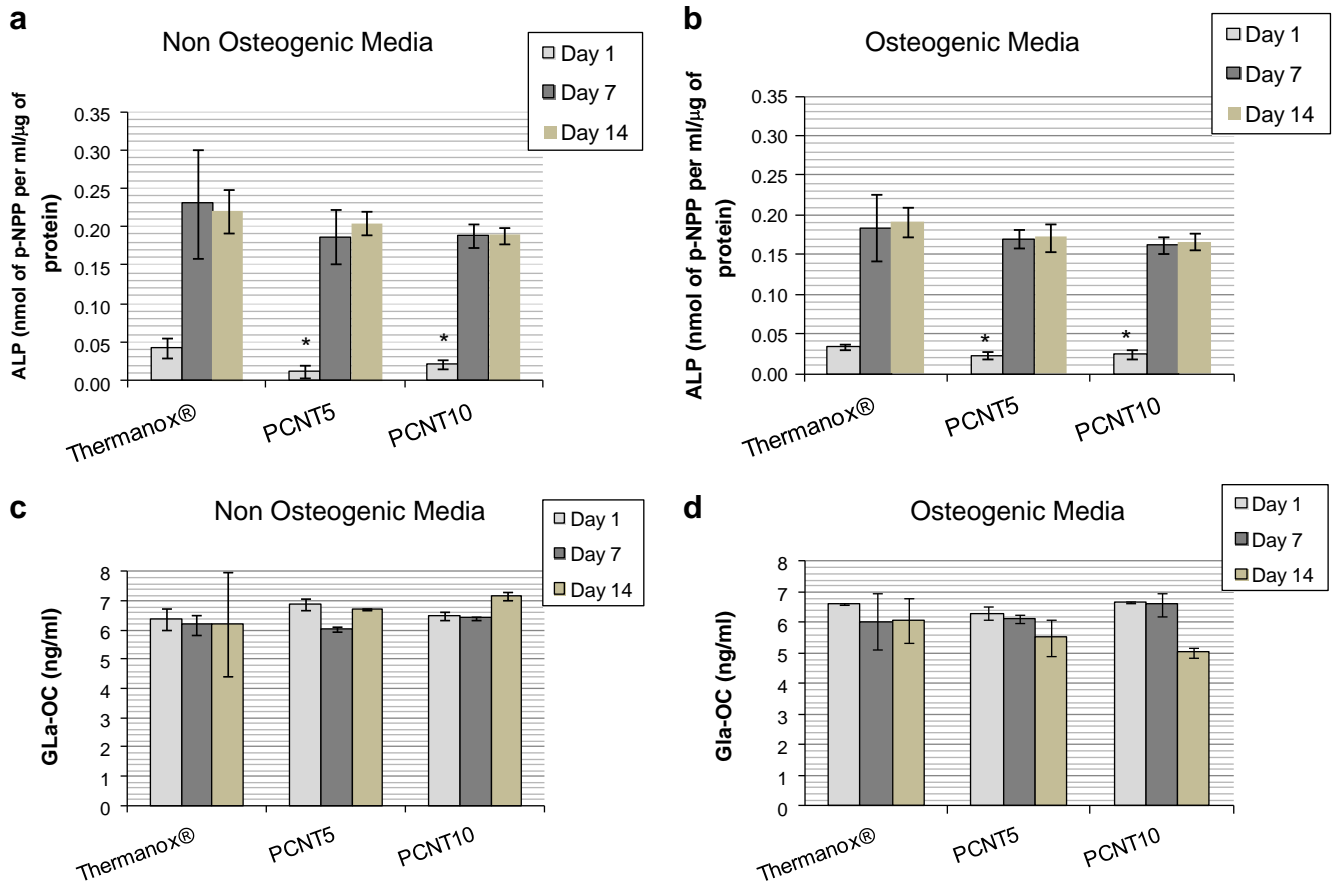
**Fig. 4.** ALP activity for HOS cells grown on the surface of titania containing glasses in normal tissue culture medium (a) and in osteogenic medium (b). GLa-OC expression for cells grown in normal tissue culture medium (c) and in osteogenic medium (d). Cells grown on Thermanox® were used as positive control cells. All values are presented as the mean ± standard deviation. * showed the significant difference from Thermanox®; the significance level was set at 0.05.

growth media. This may suggest that the titanium (Ti) ions released from PCNT5, PCNT10 and PCNT15 glasses were responsible for the enhancement of cell growth in these glass extracts, similar to the normal tissue culture medium. As mentioned above, the control of the glass dissolution rate and hence the metallic ion release can be considered as an attractive approach to drive the biological response in the desired route [10].

The role of Ti in bone formation and mineralisation has been researched in a number of studies [6,34,35]. It has been shown that Ti ions at a specific concentration stimulated the ALP, osteopontin and osteonectin i.e., acts as promoters of osteoblast differentiation. Above this concentration, however, Ti will have a cytotoxic effect on bone cells. It has been reported that 200 μmol/l of Ti was considered cytotoxic to ROS 17/2.8 osteoblast-like cell line [36]. In another study, ≥10 ppm of Ti ions, which is equivalent to ≥200 μmol/l, in the tissue culture medium inhibited proliferation of rat calvarial osteoblasts. While, ≤5 ppm Ti ions, which is equivalent to ≤100 μmol/l, either had no effect or stimulated rat calvarial osteoblast proliferation but did not affect the number of formed nodules. The optimum Ti ion concentration for optimum osteoblast proliferation was 1 ppm, which is equivalent to 20 μmol/l [37]. It has been also shown that Ti has an effect on bone formation indirectly by affecting the behaviour of osteoclasts. For example, Ti ions in a concentration which is 30–40 times higher than that required to affect osteoblasts produced an apoptosis-linked preferential degradation effect on osteoclasts [37]. In a more recent study, 1–9 ppm of Ti ions had no significant effect on osteoblastic MC3T3-E1, osteoclastic RAW264.7 and epithelial cell-like GE-1 viability, but 20 ppm produced a significant reduction in their viability [38]. As reported previously, the Ti release from titania modified glasses used in this study

reached a maximum of 3 ppm after 3 weeks in deionised water [3]. Accordingly, this level of Ti release falls in the stimulatory range as highlighted above; this could explain the significant improvement in cell proliferation observed with titania containing glasses compared to the parent titania free ones.

In addition to the proliferation characteristics, an analysis of the osteogenic potential of HOS cells using gene expression, ALP activity and osteocalcin protein expression helps to determine the extent of their differentiation. Osteocalcin is also known as GLa protein; it is a bone specific protein and therefore has been recognised as a sensitive and specific marker of osteoblast activity [39]. Osteocalcin has high affinity to Ca or hydroxyapatite [40]. The osteocalcin assay was used to determine the osteogenic differentiation of the supernatant culture medium removed from each studied glass and Thermanox®. This assay is a competitive immunoassay that uses osteocalcin coated strips, a mouse anti-osteocalcin antibody, an anti mouse IgG-alkaline phosphatase conjugate and a p-nitrophenyl phosphate (pNPP) substrate to measure "de novo" intact osteocalcin. Titania modified glasses showed comparable ALP activity and osteocalcin expression to the positive control surface. This indicated that titania modified glasses supported differentiation of osteoblasts starting from increased cell density and then upregulated expression of bone markers (osteoblast phenotype genes, ALP and osteocalcin protein). The final stage of differentiation, mineralisation, was beyond the scope of this study. The absence of osteogenic media had no effect on ALP activity and osteocalcin expression; a similar finding was also observed for HOS cells cultured on Ni–Ti alloy surface exposed to different modifications [21]. This could be due to the nature of human osteosarcoma (HOS) cells. Although these cells maintain their phenotype at high subpassages, they do not express all

the characteristic features of osteoblasts e.g., they are not capable of producing mineralised matrix, the final stage of differentiation [41]. However, due to their versatility and extensive use in research studying the osteoblasts' response to various biomaterials [21,42–44] they have been used in this study as highlighted above.

## 5. Conclusion

Modification of the glass network with 5 to 15 mol% titania produced generally cytocompatible substrates that allow the desired cell adhesion, sustained viability and high proliferation, and promote differentiation of human osteosarcoma (HOS) cells. This improved biological response of titania containing glasses could be associated with the release of Ti ions which has been previously shown to affect bone cell function. The Ti release from these glasses falls in the range that has been reported to stimulate osteoblasts or osteoblast-like cell function. This finding suggested that titania modified phosphate-based glasses may have future implications as a scaffold for bone tissue engineering applications.

## Acknowledgement

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