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Scandia—A potential biomaterial?

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Biocompatibility is a pre-requisite for all biomaterials used for medical application. During the last two decades significant advances have been made in the development of novel materials and selection and use of these materials has been directly dependent upon their biocompatibility.

Several materials containing calcium or titanium cations demonstrate biocompatibility and are routinely used in various forms within the human body. Due to its position in the periodic table, scandium in the form of its oxide scandia (Sc_2O_3) was studied as the first stage of a wider exploration of the biocompatibility of ceramics. A commercial human osteoblast-like cell line (HOS TE 85) was used to study the biocompatibility of both sintered and abraded scandia surfaces. Scanning electron microscopy was used to examine cell adhesion, the MTT assay was used to measure cell metabolic function and the alamarBlue™ for the assessment of proliferation.

Although the results are only preliminary findings, qualitative observations showed that both sintered and abraded surfaces favoured cell adhesion to the same extent. Quantitatively, a significant increase in cell proliferation was observed on Sc_2O_3 compared to Thermanox™, tissue culture control. Furthermore, Sc_2O_3 has been shown to be non-toxic, able to be maintain cell viability and support cell growth and proliferation.

1. Introduction

Biomaterials in a number of forms and compositions are used in the body, with many new materials under investigation, and many more in various stages of development [1–3]. All materials that are to be used for surgical implants and medical devices must be non-toxic and not cause any deleterious effects, and in addition, should have 'the ability to perform with an appropriate host response in a specific application' [4, 5]

The majority of implants today are made from titanium-based alloy, or alloys made from a mix of cobalt and chromium. Both possess excellent mechanical properties, but are not able to bond with bone. Failure of more traditional materials such as metal and polyethylene for hip prosthetics [6–8] has resulted in ceramics becoming more popular, with the most commonly used ceramic materials being alumina, zirconia and hydroxyapatite [6, 7]. Alumina and zirconia both exhibit high mechanical strength and biocompatibility thus making them suitable for load bearing application [9, 10]. Hydroxyapatite is a synthetic calcium phosphate ceramic that resembles bone mineral. It has excellent biocompatibility properties, but unfortunately due to its poor mechanical properties it has limited use being restricted to non-load bearing applications [9]

Human bone is a composite material made up of collagen and calcium phosphate mineral. Adult bone comprises 10% water, 30–40% collagen and approximately 60–70% mineral deposit. Bone mineral is an apatite calcium phosphate containing carbonate and small amounts of sodium, magnesium, fluoride and other trace elements [11]. The requirement of any bone analogue implant material is to mimic the biological and mechanical properties of natural bone.

This study was prompted by the observation that few candidate materials have been raised to the status of clinically acceptable biomaterials. There may be several reasons for this, for example, currently available materials may be adequate, there may be excessive caution in the use of novel materials, the demands of biological environments may be too great or it may simply be the influence of precedent in research planning.

Since Ca^{2+} and Ti^{4+} are acceptable cations then possibly their neighbour Sc^{3+} deserves examination as scandium has intermediate chemical characteristics (Table I).

The objective of this study was therefore, to evaluate the biological response of scandia using a human osteoblast cell line (HOS TE 85). This osteosarcoma cell line has been shown to have properties similar to

TABLE I Comparison of elements with atomic number 20–22

	Calcium	Scandium	Titanium
Oxidation states	2	3	2,3,4
Relative Atomic mass	40.1	45.0	47.9
Pauling's electronegativity	1.0	1.3	1.5
Ionic Radii/nm	0.099	0.081	0.068 (+4)

primary human osteoblasts; the cells produce alkaline phosphatase and osteocalcin, both of which are markers of osteoblast phenotype. Furthermore, Clover and Gowen [12] have demonstrated that they exhibit similar adhesion properties to human osteoblast-like cells. These cells therefore, provide a suitable model for the study of the interaction of osteoblast-like cells with orthopaedic biomaterials [13–15].

Early cell attachment characteristics were determined using scanning microscopy while cytotoxicity and proliferation were determined using the MTT and alamarBlue™ assays respectively.

No single test method can be used unequivocally to evaluate biocompatibility because the reactions are numerous and highly complex, hence a test schedule has to be determined for individual materials [4, 16]. Furthermore, it is now generally accepted that biocompatibility concerns not only biosafety, but also biofunctionality [4]. The former involves exclusion of any cytotoxic effect and the latter, the ability to perform in a specific biological manner. *In vitro* test regimes are usually carried out in accordance to the International Standardization Organization (ISO) standards, which does not specify a single test, but provides guidelines on controls, extraction conditions, choice of cells and methods for direct and indirect tests [16–18]. Many classic biological assays exist for measuring cell proliferation and include radioisotopes such as ^{3}H-thymidine incorporation [19] and non-radioactive assays based on the reduction of a tetrazolium compounds such as MTT [20], XTT and MTS [21] into their corresponding formazans by mitochondrial enzymes.

In this study we chose to use the MTT assay for the quantitative measurement of a specific cell metabolic function. This test is dependent on the intact activity of a mitochondrial enzyme, succinate dehydrogenase [20]. The function of this enzyme is impaired following exposure of cells to toxic agents. In addition, this assay has shown increased sensitivity compared to other similar test methods [22]. Proliferation was measured using the alamarBlue™assay. This assay incorporates an oxidation-reduction (REDOX) indicator that both fluoresces and changes colour in response to chemical reduction of growth medium resulting from cell proliferation [23].

2. Materials and methods

2.1. Material preparation

Scandia (99.9% purity) was obtained from Pi-Kem Ltd., Shropshire, UK. Forty scandia discs with diameters of ~8 mm and thickness ~4 mm were each made by pressing 1 g of powder at a pressure of 500 MPa held for 60 s and slowly released. These were sintered at 1575 °C and soaked at this temperature for 4 h. Sintered samples were also abraded on a 100 μm SiC grit pad. Abraded surfaces were kept in an ultrasonic bath containing 'Analar' ethanol for 10 ks before reheating to 650 °C for 1.8 ks to remove organic contamination. All scandia samples were γ-irradiated using standard procedures for medical devices and a dose of 0.5 Mrad (Swann Morton Services Ltd., Sheffield, UK) prior to cell culture.

2.2. Cell culture

A human osteosarcoma cell-line (HOS TE85, ECACC No. 87070202) was used. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Paisley UK), supplemented with 10% foetal calf serum (FCS), 1% non-essential amino acids, *L*-ascorbic acid, 0.02 m L-glutamine, 0.01 M HEPES, 100 units $m1^{-1}$ penicillin and 100 μm $m1^{-1}$ streptomycin. Thermanox™ (TMX, Life Technologies, Paisely, UK) was used as the negative (non-toxic) control and commercially available toxic control polyvinylchloride containing an organometallic additive (PVC, 3S Healthcare, UK) was used as the positive control.

For the assessment of proliferation, HOS cells were seeded at a density of 2×10^6 cells/ml on both test materials and controls (8 replicates for both control and test materials). The cells were cultured in DMEM medium (containing 10% FCS) at 37 °C in a humidified atmosphere with 5% CO_2. The cultures were incubated for 1, 5, 7, 14, 21 and 28 days and the culture medium was replaced at intervals chosen to minimise the disturbance of the culture conditions.

Proliferation was determined using the alamarBlue™ assay (Life Technologies), which is a redox indicator that can be used to quantitatively measure proliferation of cells [23]. As the cells grow in culture, their metabolic activity maintains a reducing environment in the surrounding culture medium, whilst growth inhibition produces an oxidised environment. Reduction causes colour change of the alamarBlue™ indicator from non-fluorescent (blue) to fluorescent (red). At the selected time points, medium from all the wells under test was removed and substituted with 1 ml of 10% alamarBlue™, diluted in phenol-red free medium and incubated at 37 °C in a humidified air at 5% CO_2 for a further 4 h. The fluorescence was detected with a Fluoroskan fluorimeter (Ascent, Life Science International) using absorption at 560 nm and emission at 590.

The MTT assay was used as an indirect method to monitor cell metabolic activity following exposure of the cells to any residual agent that has leached out of the test material [20]. The test extracts were prepared by placing the test materials in DMEM and placing on a rotating mixer for the selected time points. Standard DMEM culture medium was replaced with the eluted extracts (100 μl/ml) and plates were incubated for 24 h. 10 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich,

Poole, UK) was added to each well. The plates were incubated for a further 4 h and then the medium was removed by inversion. 100 μl of dimethyl sulphoxide (DMSO, Sigma-Aldrich, Poole, UK) was added to each well, mixed for 20 min until complete dissolution of crystals occurred. Absorbance was measured on a Dynatech MR700 micro plate reader (Japan) (test wavelength: 570 nm; reference wavelength: 630 nm).

2.3. Scanning electron microscopy

For morphological studies, materials were seeded directly at a density of 2×10^5 cells/ml and incu-

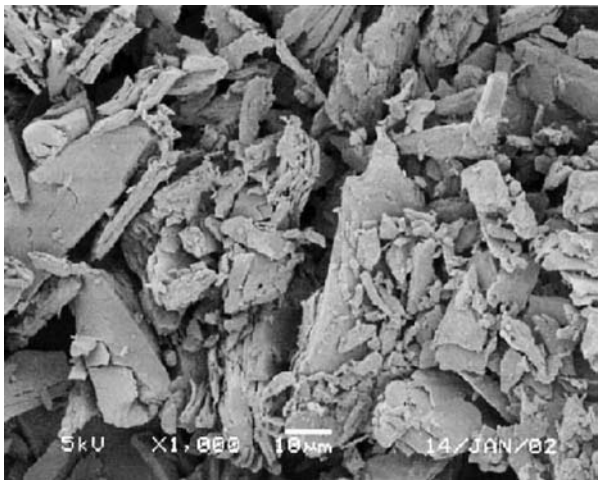

Figure 1 Scandia powder showing a mixed morphology. Some of the granules had a flat "plate-like" appearance.

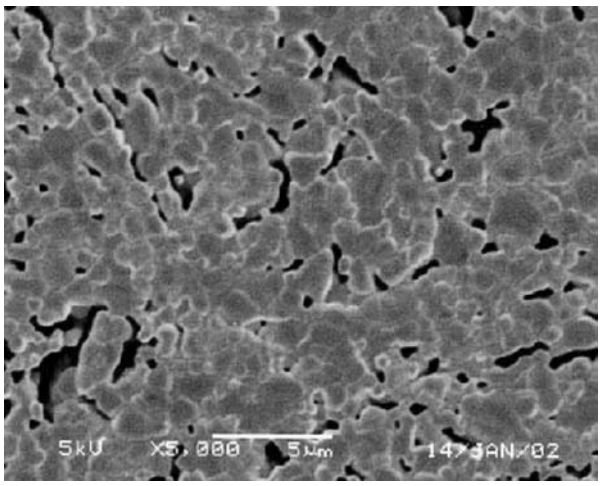

Figure 2 As sintered surface of compacted scandia discs showing a uniform surface morphology with porosity resulting from particle agglomerates.

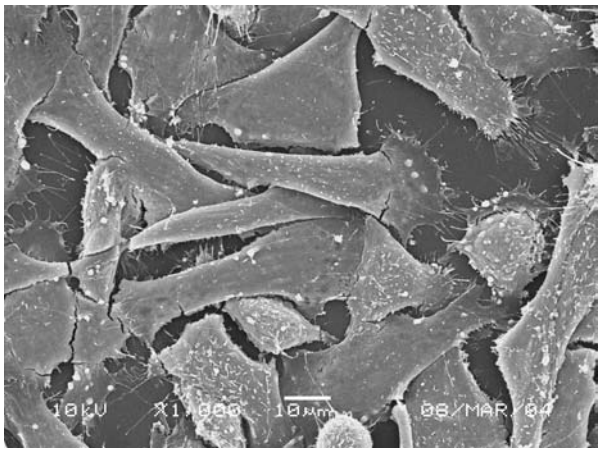

Figure 3 HOS cells attached to the surface of the negative control, TMX. A dense layer of cells can be seen covering the surface with some rounded cells also visible.

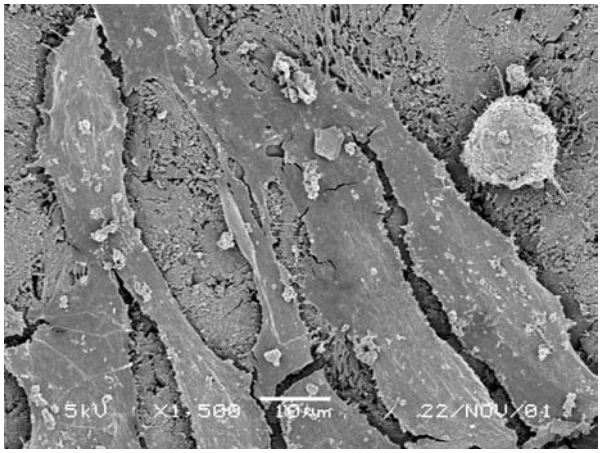

Figure 4 HOS cells attached to the surface of as-sintered scandia. Cells had an elongated appearance with short filopodia, not all the surface was covered with cells.

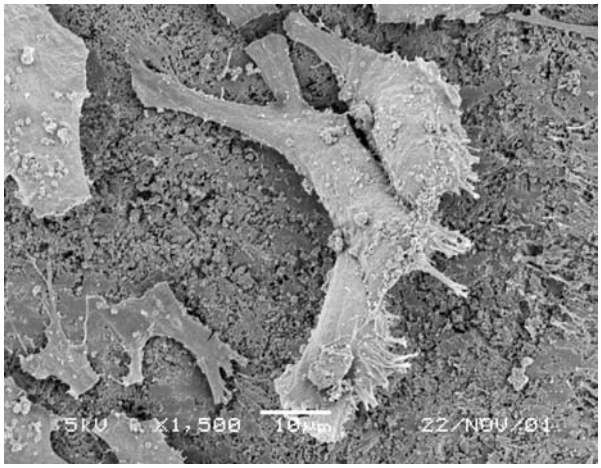

Figure 5 HOS cells attached to the abraded surface of scandia. The cells appeared less elongated and longer filopodia were present.

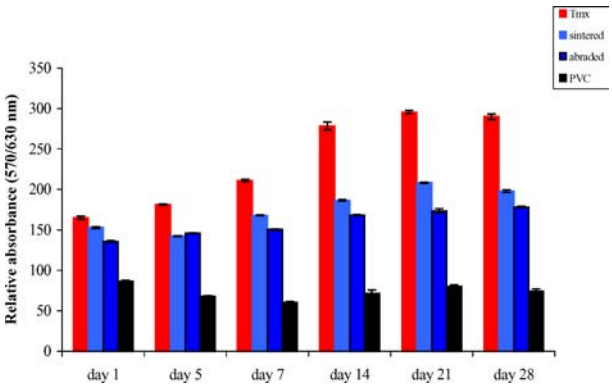

Figure 6 The alamarBlue^{TM} assay proliferation results for the control TMX (negative control) and PVC (positive control) and test materials as-sintered and abraded scandia up to 28 days incubation Replicates $n = 8$, mean data plotted (error bars represent standard deviation).

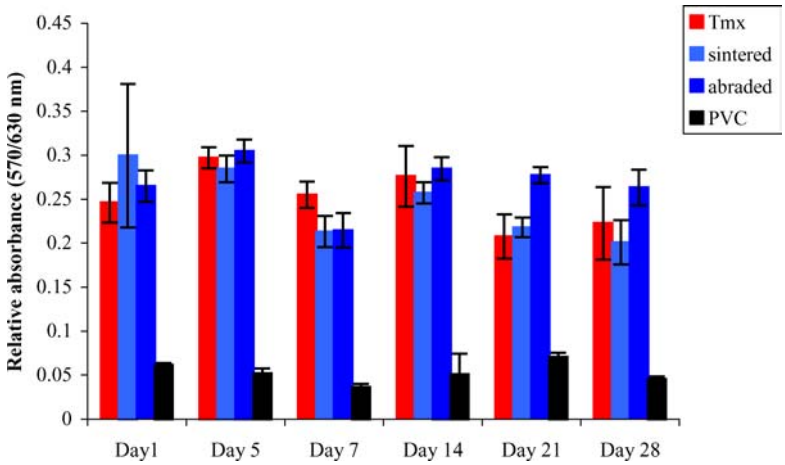

Figure 7 MTT assay results for the negative and positive controls, as-sintered and abraded scandia up to 28 days incubation Replicates $n = 8$, mean data plotted (error bars represent standard deviation).

bated at 37 °C in a humidified air at 5% CO_2. Following a 48 h incubation period, the cultures were fixed with 1.5% glutaraldehyde buffered in 0.1 M sodium cacodylate, stained in 1% osmium tetroxide and 1% tannic acid buffer. The samples were dehydrated using a series of aqueous alcohol solutions starting from 20 to 70% in 10% increments, stained in 0.5% uranyl acetate and further dehydrated in 90, 96 and then in 100% ethyl alcohol (containing Na_2CO_3) and with hexamethyl-di-salazane and finally the samples were air dried. The cultures were coated with a thin layer of palladium before examination of morphology and attachment in the scanning electron microscope (JSM 5500LV).

All the *in-vitro* measurements were carried out on 8 replicates. Statistical analysis was performed using the standard software package of Microsoft Excel. All results were expressed as the means (95% confidence limit). Students *t*-test (significance level $p = 0.05$) was used to determine significant differences between the test materials and the control.

3. Results and discussion

The scandia powder that formed the starting material had a mixed morphology. The particles had a polygranular shape with a mixed flattened morphology which prevented them from pressing to a high density (Fig. 1). The as-sintered samples had a relative density of 94% (theoretical density of scandia = 3860 kgm^{-3}) (Fig. 2). Their surfaces were neither modified nor contaminated after removal from the sintering furnace.

HOS cells attached and completely covered the surface of TMX the negative control after 48 h incubation, cell division was visible (Fig. 3). The cells displayed a typical polygonal morphology with numerous filopodia extensions [2]. In contrast, fewer cells were observed on the as-sintered scandia surface (Fig. 4). In addition, the cell morphology was more elongated with fewer and shorter cell filopodia visible. Cellular attachment on the abraded scandia surface was similar to the as sintered, however, cell filopodia appeared longer and were more numerous (Fig. 5). In general, the HOS cells were able to attach to both the test material surfaces and maintained their normal osteoblast morphology.

In the alamarBlue^{TM} assay, the native, oxidised form of the reagent is readily taken up by the cells and reduced intracellularly by oxidoreductatases and mitochondrial electron transport chain, resulting in a corresponding shift in absorbance in response to the chemical reduction of growth medium resulting from cell growth. Cells on the TMX control showed an increase in proliferation with time (Fig. 6). A significant increase in cell proliferation ($p < 0.05$) was observed at all time points, except day 5, on the sintered surface compared to the abraded.

The MTT assay is dependent on the intact activity of the mitochondrial, enzyme, succinate dehydrogenase, which may be impaired following exposure of cells to a toxic environment. The results of this study showed that within the range of experimental error a similar level of oxidative metabolic activity was observed at days 1, 5, 7, 14 and 28. No significant difference in response was observed between the as-sintered and the abraded Scandia surface for these time points. Thus indicating that no toxic leachable significantly influenced the metabolic activity of the cells. Interestingly, a significant difference in metabolic activity was observed on the abraded surface on day 21 ($p = 0.002$), (Fig. 7). A significant reduction ($p < 0.05$) in metabolic activity was observed for all time points on the positive control surface (PVC); this has been reported elsewhere [23].

4. Conclusions

The results from this preliminary investigation show that scandium oxide is not cytotoxic and does not have a deleterious effect on cell metabolic activity. Attachment of anchorage-dependent cells to a substrate surface is the initial process that occurs in cell-surface interactions and scandium oxide, irrespective of the surface topography promoted HOS cell attachment at a level comparable to the TMX control. It can be argued that more parameters should be studied, especially those concerning biofunctionality. We conclude that scandia shows potential as a novel biomaterial and warrants further investigation.

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