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Fluorapatite-mullite glass sputter coated Ti6Al4V for biomedical applications

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A number of bioactive ceramics have been researched since the development of Bioglass in the 1970's. Fluorapatite mullite has been developed from the dental glass-ceramics used for more general hard tissue replacement. Being brittle in nature, glass-ceramics are currently used mainly as coatings. This paper shows that fluorapatite glass LG112 can be used as a sputtered glass coating on roughened surfaces of Ti6Al4V for possible future use for medical implants. An AFM was used to measure the roughness of the surface before and after coating to determine the change in the topography due to the coating process as this greatly affects cell attachment. The sputter coating partially filled in the artificially roughened surface, changing the prepared topography. Osteoblasts have been successfully grown on the surface of these coatings, showing biocompatibility with bone tissue and therefore potential use in hard tissue repair.

1. Introduction

There is an increasing need for medical implants, due to an increasing aged population. Bone defects resulting from trauma, disease or developmental anomalies can be greatly improved by reconstructive surgery. Small improvements in the design and constituent materials of such implants can greatly reduce the healing time and reduce the need for expensive, traumatic revision surgery. Commonly used bioactive coatings, such as calcium phosphate and hydroxyapatite, reduce healing time but are eventually absorbed by the body, leaving the substrate bare [1]. Hench [2] developed bioactive glass-ceramics in the 1970s that were longer lasting and bonded to bone. Fluorapatite-mullite glasses and glass-ceramics have been developed from dental ionomer glass to try to combat premature absorption. The fluorapatite gives the material good initial bioactivity, whilst the inert mullite extends the lifetime of the material.

Due to the low fracture toughness of these glass-ceramics, these materials are used for non-load bearing components, or more commonly, as coatings. Many different methods of coating [3, 4] with bioactive ceramics have been suggested, with varying degrees of success, including: plasma spray [5], dip coating [6] enamelling [7], sol gel, sputter coating [8, 9] and electrophoretic deposition [10].

In sputter coating, a substrate is coated by ions or neutrals extracted from a target by a gas discharge plasma generated by ion source and accelerated using an electric field. Samples are placed in a high vacuum, usually about 2.8–3.7 $\times$ 10^{-4} Pa. Ions from the ion source hit the target material with such momentum that it causes ions or neutrals from the surface of the target to have sufficient momentum to leave the surface of the target. The type and size of the ions depends on the energy of the ion beam. Calcium phosphate and hydroxyapatite are currently being used as a target materials.

The advantages of this method are that it produces a stable, dense, adherent and homogeneous thin film on the metal substrate. Uniform layers can be formed even with surfaces of a complex geometry [10]. It can be possible to make coatings with variable compositions using this method. The principle disadvantage of this method is the slow deposition rate resulting in a coating thickness of approximately 10 μm in reasonable deposition times, which may not be sufficiently thick for many applications [9]. Another disadvantage is that the surfaces formed are very flat, as the coating in fills small irregularities [9]. This results in a flat surface with few topographic features to encourage cell attachment [9] although the final surface roughness is strongly related to the initial surface roughness prior to coating [9, 11]. The composition of the final coating may differ from that of the initial target, due to loss of volatile phosphorous compounds and energetic interactions with the ion beam [12]. Other factors affecting the stoichmetry of the coating include the elemental composition ratio of the sputter target, the flow rate of the neutral oxygen atoms and the substrate temperature [13]. Similar coating methods have shown a reduction

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in non-bridging oxygen ions, thought to be necessary for bioactivity [14].

Bioactive coatings made by this method have been found to be amorphous, similar to plasma sprayed coatings [8, 15], although post coating annealing can improve the crystallinity [16].

With bioactive materials it is important to measure the cell response to the surface, to determine whether a material is suitable for use in the body. The list of different cell types that can be grown in culture is now quite extensive and includes connective tissue elements such as fibroblasts and skeletal tissue (bone and cartilage) [17]. The type of cell chosen depends on the final use of the coating surface and the type of test required. Bioactivity tests on bioactive glass-ceramics have included the use of fibroblasts, human osteoblasts [18, 19, 20], and protein absorption [21] and *in vivo* tests.

It has been seen that cells can discriminate not only between surfaces of different roughness but also between surfaces of comparable roughness but different topographies [20–23]. Therefore the morphology as well composition of the coating is very important for cell growth. Recent research [24] has shown that cells appreciated smooth surfaces on the cell size scale, but rough isotropic surfaces on macro scale. The ideal surface for adhesion of cells is likely to be a rough surface with numerous bowl-like nests [24].

This paper studies the effect of magnetron sputtering apatite-mullite glass-ceramic LG112 on Ti6Al4V on the topography and cell growth. A polished sample was initially coated to determine the success of the coating prior to study of the effect of coating on the topography. As the topography [20, 25, 26] of a biological surface is important to cell attachment, an atomic force microscope (AFM) was used to analyse the surface, before and after coating. The effect of the surface roughness on the biological properties was analysed by examining osteoblast growth on the surface.

2. Experimental

2.1. Material fabrication

The glass used is Limerick glass, formulation 112 (LG112) which has a composition (in moles) of $4.5SiO_2$ $3.0Al_2O_3$, $3.2P_2O_5$, $3.0CaO$, $1.5CaF_2$, giving a Ca:P of 1:1.40, similar to that of hydroxyapatite.

Feedstock, as shown in Table I, was weighed out using a roughing balance into a 1.5 litre screw lid plastic container. In order to break up any agglomerated powder, one stainless steel rod was placed into each of the containers. The lids were then fastened and the contains mixed on a rotary 'ball' mill and mixed for one hour. The powder was subsequently placed in a 250 cm^3 mullite, Dyson crucible. Lids were placed on these crucibles and heated in a furnace at 1550 °C for two hours. The molten glass was then quenched in room temperature water. The quenched glass was then placed in a grinding dish (ring and puck) set at low amplitude and ground in batches of 300 g for 12 min. After grinding it was sieved down to 35 μm using an Octagon Sieve Shaker. This was then used to make into a target for sputter coating.

TABLE I Raw material feedstock, grades and suppliers

Ion	Source	Grade	Supplier
Si^{4+}	SiO_2	High	Tilcon
Al^{3+}	Al_2O_3	Laboratory	Merck
Ca^{2+}	$CaCO_3$, CaF_2	Analytical	Merck
P^{5+}	P_2O_5	Analytical	Merck
F^-	CaF_2	Laboratory	Fisher
O^-			

For sputter coating, a 10 cm square target was required. Glass powder was compressed in a custom-made stainless steel window frame mould approximately 2.5 mm thick. The compressed powder was sintered, in the window frame mould, at 1000 °C for one hour and furnace cooled. The frame was removed and the resulting target had sufficient strength to be used in the sputter coater.

Ti6Al4V 1 cm discs were used as a substrate. Sputter coated samples were compared to uncoated titanium as a standard. These were roughened using different grades of silicon carbide papers, 120, 240, 400 and 800 grit and some plain unroughened samples. The samples were roughened in both a single direction and in random directions to give different topographies. The samples were ultrasonically cleaned and rinsed in ethanol, and finally dried. The roughness of these samples was measured before and after coating using an atomic force microscope (AFM).

The samples were magnetron sputter coated with the glass-ceramic at 1.5×10^{-4} Torr in an argon atmosphere for 2 h. Larger, polished, Ti6Al4V samples were initially coated, to test the technique using the glass—ceramic. As this was successful, the 1 cm discs were coated using the same parameters. The smaller samples were stuck down with silver paint to keep them in place during coating.

2.2. Cell culture

The cells used were MG63 human osteoblast cells. The cells were washed twice in 5 ml (PBS Dulbeccos). 1 ml of trypsin was added and mixed carefully, incubated at 37 °C for ten minutes followed by cell detachment by tapping the culture flask. Following this 10 ml of cell culture media (Dulbecco's MOD eagle media) was added to resuspend the cells. 0.5 ml of this cell suspension was added to 10 ml culture media in cell culture flask. More media was added to give a cell count of 5000 cells/ml, with approximately 100000 cells per culture well. The cells were cultured for five days. Two wells in each plate were left empty to check for cell confluency and contamination.

After five days, the samples were rinsed with saline, and dried out with differing concentrations of ethanol, up to 100%. The cells were finally dried using hexamethyldisilazane (HMDS) and gold coated before examining in the SEM. Two coated samples of each surface roughness were cultured.

2.3. Substrate characterisation

A number of techniques (optical microscopy, X-ray diffraction, scanning electron microscopy, and

atomic force microscopy) were used to characterise the substrate. Optical microscopy was performed using an Olympus light microscope. For X-ray diffraction (XRD), the substrate was analysed using a Phillips X'pert thin film XRD (Almelo, The Netherlands) set at 0.010° 2θ per second up to 90°. In the scanning electron microscopy (SEM) studies, all samples were coated with gold before placing in a JOEL 6300 SEM. All samples were examined at 20 kV and a working distance of 37 mm. The atomic force microscopy (AFM) was performed on a Thermomicroscopes (Sunnyvale, USA) AFM in contact mode for all experiments. The roughness was measured over a small area, 10 × 10 μm, the approximate area for cell interaction. The roughness was also measured on a larger scale 80 × 80 μm to give a roughness measurement more representative of the whole surface.

3. Results

3.1. Coating characterisation

The initial polished sample was successfully coated. The coating could be seen by eye as a change of colour across the surface of the sample. This indicates that the coating is the approximate thickness of the wavelength of light and that the coating thickness varies slightly across the sample. Light microscopy shows clearly the change in colour across the sample, as well as the residual scratches from polishing, seen through the coating, as shown in Fig. 1. However, the SEM showed a flat, featureless surface, indicating that the residual scratches had been filled in as shown in Fig. 2. AFM of the large sample shows that residual scratches can still be faintly seen, despite the SEM indicating a flat, featureless surface. These faint lines can be seen in Fig. 3. The AFM images of the coated, roughened samples, before and

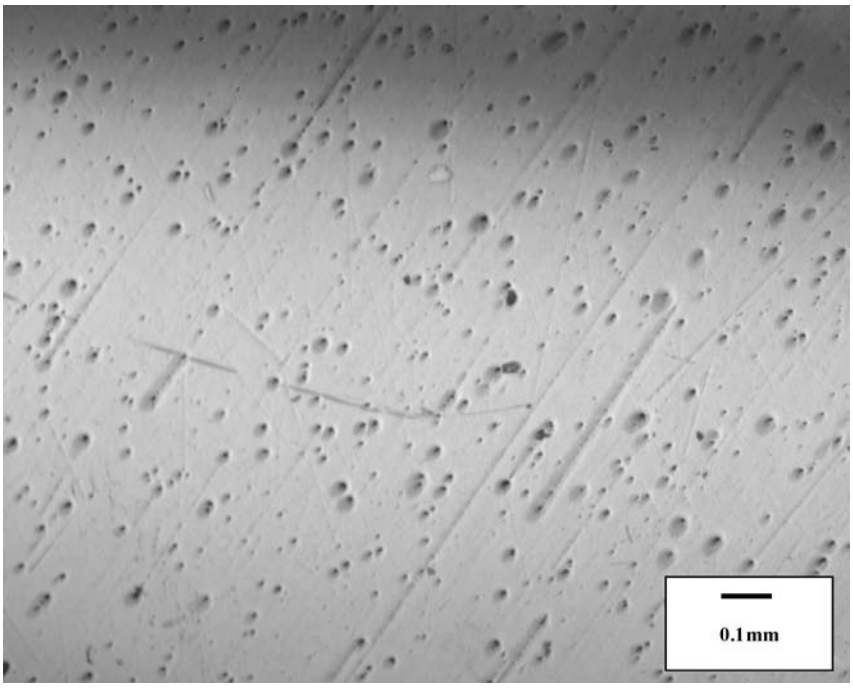

Figure 1 A light microscope image of the polished sputter coated sample, showing a change in colour due to a change in thickness of the coating.

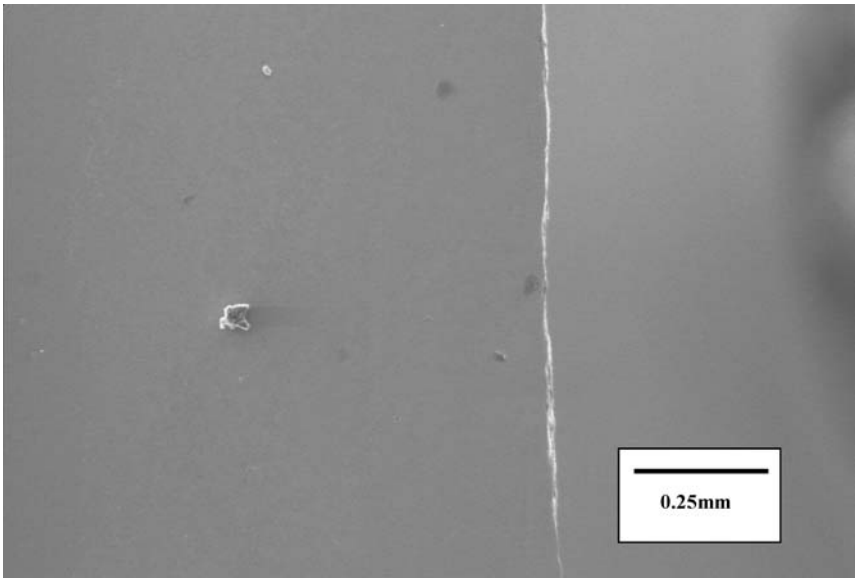

Figure 2 An SEM image of the polished sputter-coated sample showing very little surface topography.

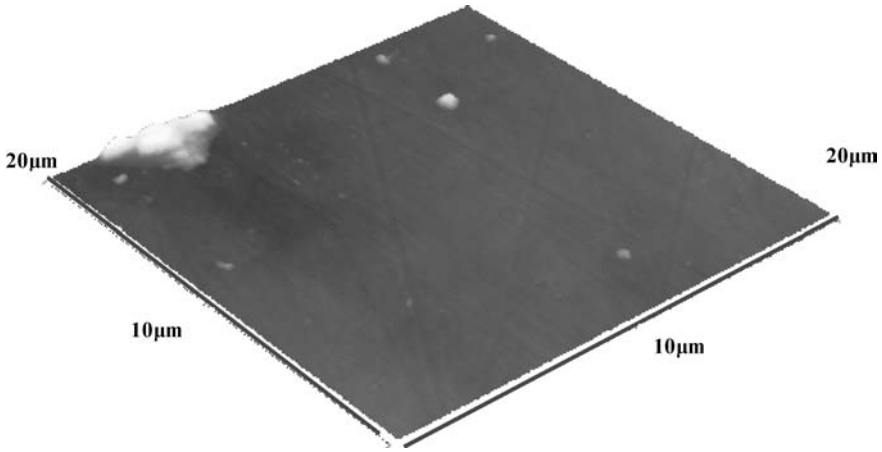

Figure 3 An AFM image of the polished sputter-coated sample showing very faint scratches.

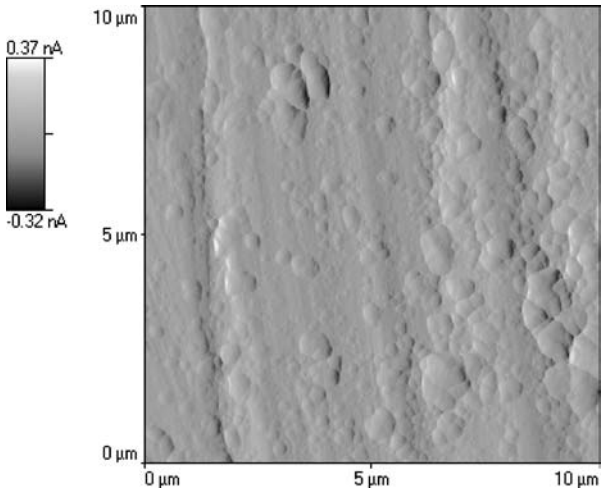

Figure 4 An AFM image of a roughened sputter coated sample with splat-like features.

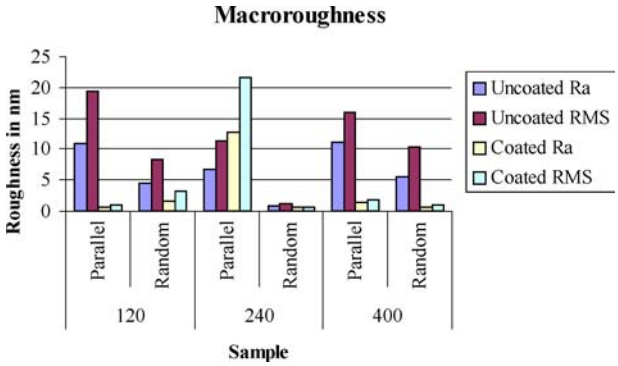

Figure 5 The change in macroroughness due to sputter coating.

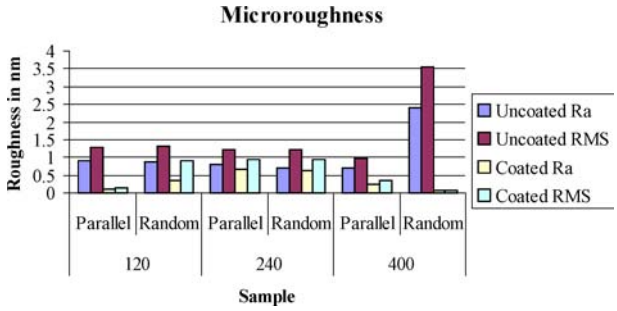

Figure 6 The change in microroughness due to sputter coating.

after coating show very little change. This is shown in Figs. 3 and 4. Although it appears in Fig. 4 that the coating has been deposited in the form of splats, this is also seen in other images of uncoated samples and is due to the grain structure of the underlying titanium alloy substrate. Even though thin film XRD was used, the XRD trace of this sample only gave a trace of the substrate, as the coating was too thin. It can be assumed, by the nature of the coating method, that the coating is amorphous. It is unlikely that an ion beam is sufficiently energetic to remove crystalline material from the substrate.

3.2. Substrate characterisation

Figs. 5 and 6 show the macro and microroughness, both the average (Ra) and the root mean square (RMS) for both the uncoated and coated samples. For ease of clarity, the samples up to 400 grit have been shown. These graphs show that at almost all roughnesses, sputter coating, does make substrate surfaces less rough by infilling defects.

4. Cell culture

4.1. Coated

After five days, the cells on the sputter-coated samples appeared to be confluent as shown in Figs. 7 and 8. The image in Fig. 9 was taken near the edge of the sample where the cells were less dense to show that the cells were spread out and healthy. Cells across the centre of the sample were confluent. Some beginnings of mitosis can be seen on some samples. On inspection, cell growth direction clearly appears to be influenced by the texture of the surface, but the cell quantity does not. Fig. 7 clearly shows cell growth in a parallel direction horizontally across the image. Cell growth show in Fig. 8, a sample roughened in a random direction, shows no directional growth. Both Figs. 7 and 8 were roughened using the same grade of silicon carbide paper and both pictures are at the same scale.

4.2. Titanium standard

The uncoated titanium alloy samples also showed good cell growth, comparable to the coated samples,

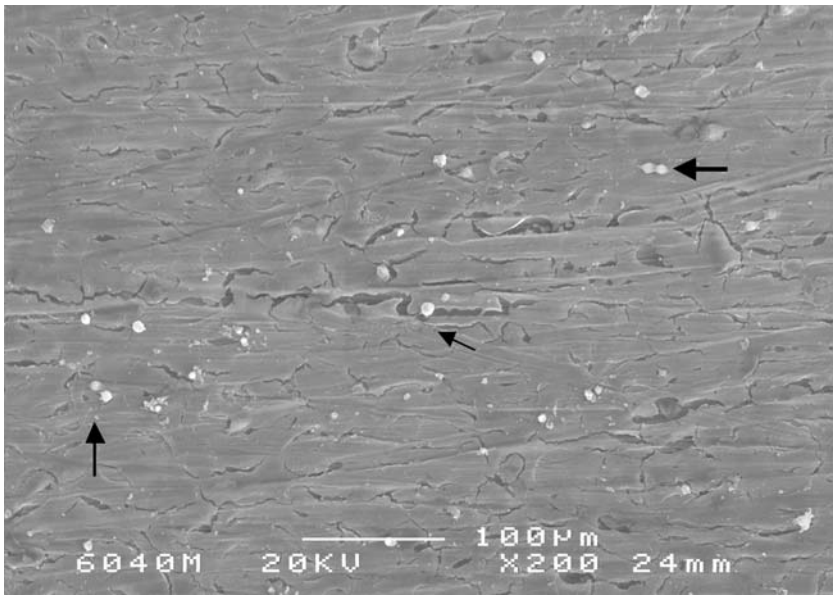

Figure 7 A SEM image of confluent osteoblasts on a coated substrate roughened on 800 grit paper in a single direction showing cell orientation after 5 days. Arrows show cells undergoing mitosis.

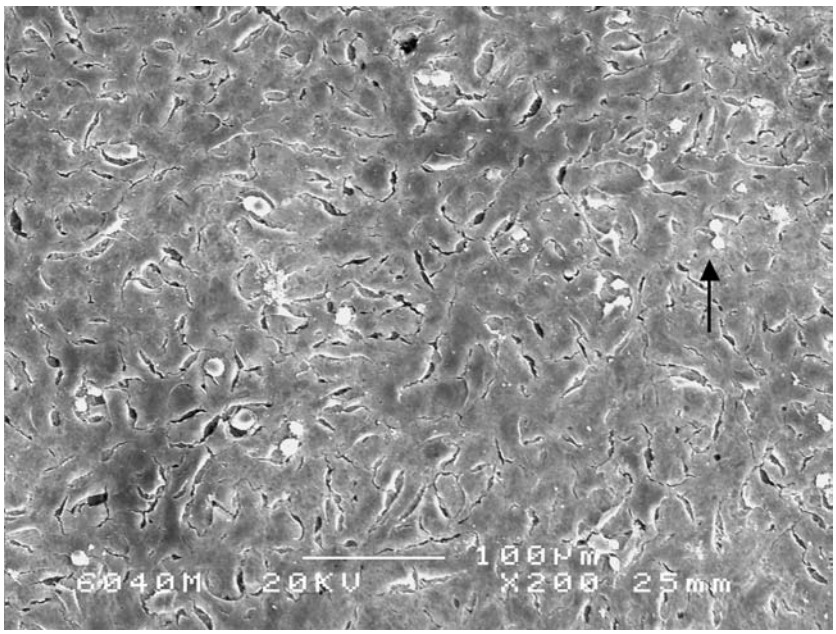

Figure 8 A SEM image of confluent osteoblasts on a coated substrate roughened on 800 grit paper in a random manner showing cell orientation after 5 days. Arrows show cells undergoing mitosis.

including similar orientation effects. Cracks in the titanium oxide layer, present on all titanium alloys, have prevented cell growth. This can be clearly seen in Fig. 10.

5. Discussion

Fluorapatite mullite glass ceramic LG112 can be successfully deposited by sputter coating on Ti6Al4V giving a thin, glassy film after 2 h of coating. This is shown in Figs. 1–4. On a polished surface, the coating does not seem to add any features, shown in Fig. 2, although when compared to Fig. 1 it can be seen the features under the coating have been infilled.

Figs. 5 and 6 shows that using different grades of silicon carbide paper to give reliable varying degrees of roughness were not successful as there is no significant difference in roughness. Figs. 5 and 6 show that sputter coating infills scratches, producing smoother surfaces. This is also confirmed by the SEM and light microscopy images Figs. 1 and 2. The smoother surfaces, the 800 grit sample and the blank (not shown), as received samples, appear to have been roughened by the coating process. Therefore some of the initial topography was only retained in the rougher samples. Although this may be due to a greater variation in surface roughness across the sample, increasing the error in the rougher samples. This change in topography in important when designing medical implants as any artificial surface roughness will be changed by sputter coating.

The AFM images show that this coating method does not add any additional features to the surface.

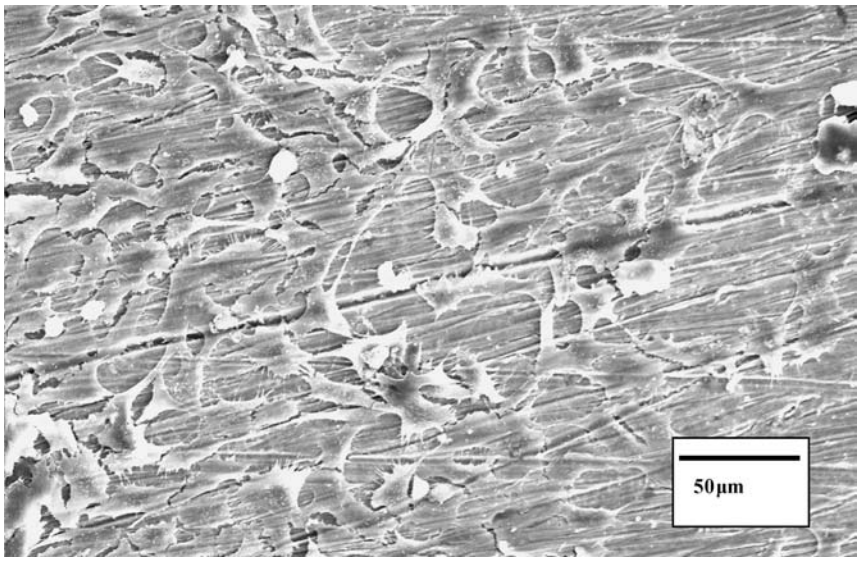

Figure 9 An SEM image of osteoblast cells growing on the sputter-coated surface at the edge of a sample after 5 days.

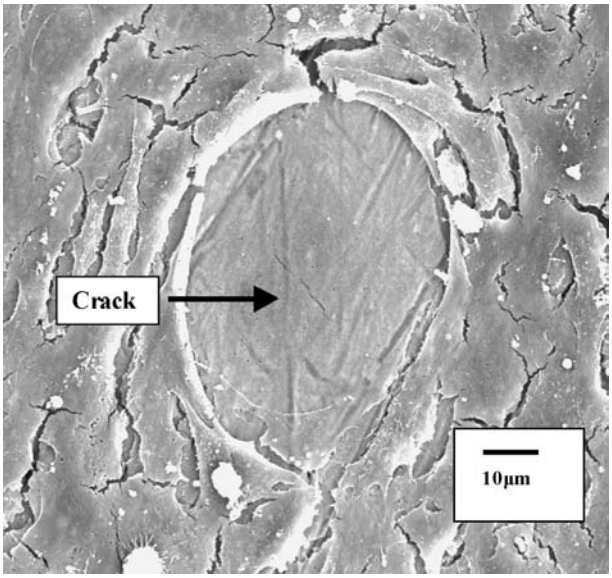

Figure 10 An SEM image of osteoblast growth on Ti6Al4V showing a clear area due to damage to the substrate.

After five days, not only were the cells confluent on the sputter coated samples, but there was some evidence of mitosis of these bone cells. There seemed to be some edge effect on the circumference of the sample, on the growth of the cells caused by the coating. This may be due to the coating either not adhering right to the very edges, or damage to the coating during handling. There did not seen to be any effect on changing the surface roughness, or the topography of the surface on the cell growth.

This work shows that the sputter coating fills in small defects in a titanium surface. Cell culture shows that even small cracks in the surface titanium oxide layer can prevent cell growth, even if the rest of the cell growth in confluent. Sputter coating infills these to give a complete bioactive surface.

6. Conclusions

This paper shows that titanium alloy Ti6Al4V can be successfully sputter coated with fluorapatite-mullite glass-ceramic LG112. This coating can be seen to change the original substrate topography by smoothing fabricated scratches in the substrate. Cells were confluent on this coating after five days. Plain titanium can defects on the surface that prevent cell growth, which can be infilled using have sputter coated LG112.

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