

1993

Bone Growth on Sol-Gel Calcium Phosphate Thin Films In Vitro

Q. Qiu

Queen's University, Kingston

P. Vincent

Queen's University, Kingston

B. Lowenberg

University of Toronto

M. Sayer

Queen's University, Kingston

J. E. Davies

University of Toronto

Follow this and additional works at: <https://digitalcommons.usu.edu/cellsandmaterials>



Part of the [Biomedical Engineering and Bioengineering Commons](#)

Recommended Citation

Qiu, Q.; Vincent, P.; Lowenberg, B.; Sayer, M.; and Davies, J. E. (1993) "Bone Growth on Sol-Gel Calcium Phosphate Thin Films In Vitro," *Cells and Materials*: Vol. 3 : No. 4 , Article 2.

Available at: <https://digitalcommons.usu.edu/cellsandmaterials/vol3/iss4/2>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Cells and Materials by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



BONE GROWTH ON SOL-GEL CALCIUM PHOSPHATE THIN FILMS *IN VITRO*

Q. Qiu, P. Vincent, B. Lowenberg¹, M. Sayer and J.E. Davies¹

Applied Solid State Group, Department of Physics, Queen's University,
Stirling Hall, Kingston, Ontario, Canada, K7L 3N6

¹Centre for Biomaterials, University of Toronto, 170 College Street, Toronto, Ontario, Canada, M5S 1A1

(Received for publication May 12, 1993, and in revised form October 26, 1993)

Abstract

Thin, sub-micron, films of calcium phosphate were fabricated on either glass or quartz supports by a colloidal suspension sol-gel method. These films, which varied in both surface chemistry and topography were then employed as culture substrata for osteogenic rat bone marrow cells. During an 18 day culture period, the cells elaborated a morphologically distinguishable bone matrix on all substrata which was similar to that reported earlier on tissue culture polystyrene. Selected samples of the culture substrata were fractured, critical point dried, and observed by scanning electron microscopy. Particular attention was paid to the morphologies of the interface between the sol-gel layer and the underlying support, and that between the sol-gel layer and the elaborated bone tissue. The mechanical disruption of both tissue and thin films resulting from critical point drying affected the morphology of both interfaces dependent upon the film processing conditions. The interfacial bone matrix, which was a cement-line like matrix, interdigitated with the surface of the films. This mechanical interdigitation created a bond which remained intact during tissue processing. With films processed at 1000°C on quartz supports, but not with those processed at lower temperatures on glass, fracture of the interface revealed pitting in the quartz surface which was associated with areas of adherence of the overlying calcium phosphate film. These preliminary studies demonstrate the intimate relationship which can be established between such thin calcium phosphate thin films and bone matrix.

Key Words: Bone, thin film ceramics, sol-gel, interface, grain size, *in vitro*.

*Address for correspondence:

J.E. Davies,
Centre for Biomaterials,
University of Toronto,
170 College Street,
Toronto, Ontario, Canada M5S 1A1

Telephone number: (416) 978-1471

FAX number: (416) 978-1462

Introduction

It is still unclear whether the improved bone healing response obtained with calcium phosphate coated bone implants is the result of the chemical or surface topographical characteristics of the coating. The majority of coatings employed by the implant industry are created by plasma-spraying techniques which, commonly, result in the deposition of multiphasic calcium phosphates with a varying, and generally uncontrollable, surface chemistry and roughness. Although there is considerable reported evidence that calcium phosphate coatings increase the implant/bone bond strength (Geesink *et al.*, 1987; Oonishi *et al.*, 1989; Rivero *et al.*, 1988) and accelerate early bone healing in the implantation bed (Ducheyne *et al.*, 1980, 1990; Rivero *et al.*, 1988; Luckey *et al.*, 1992), deconvolution of the effects of surface chemistry and surface roughness of the coating on this phenomenon remain unexplored. Furthermore, some plasma-sprayed coatings have demonstrated, either or both, delamination from the underlying metal, or particulate degeneration of the coating, during an extended implantation period (Oonishi *et al.*, 1989; Rivero *et al.*, 1988; Luckey *et al.*, 1992). Many initial reports of such mechanical problems were associated with relatively thick, approximately 200 μm , plasma-sprayed coatings; but even with currently fabricated coatings of 50-100 μm thickness, concern is still expressed with regard to potential delamination or fracture occurrence within the bulk of the coating layer.

The advantages of calcium phosphate coatings may be related to the ability of healing bone tissue to bond to their surfaces (Ducheyne *et al.*, 1992) and thus their designation as "bone-bonding" or "bioactive" materials. However, despite early hypotheses related to surface chemical interactions (Hench *et al.*, 1971) and morphological evidence indicating micro-mechanical interlocking (Ricci *et al.*, 1991), a mechanistic explanation of the bone bonding phenomenon has yet to emerge due to the difficulty in designing experiments to clearly differentiate between surface chemical and topographical factors (Orr *et al.*, 1993).

Clearly, the possibility of harnessing the accelerated bony healing, and bone bonding, attributed to

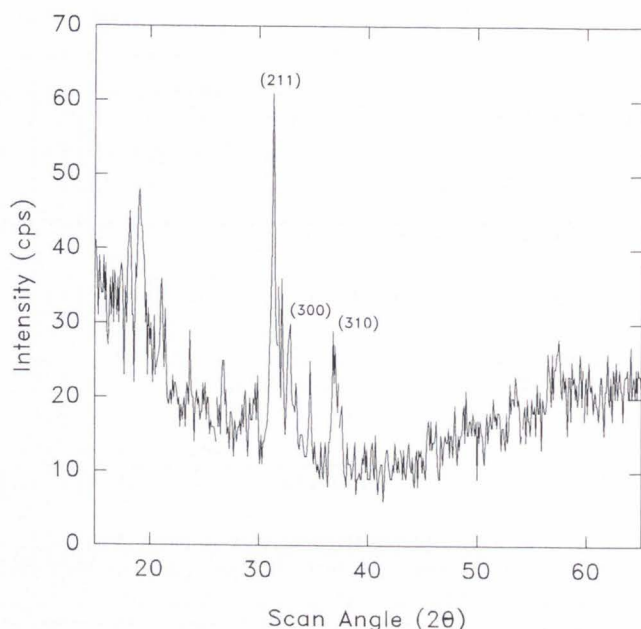


Figure 1. X-ray spectrum of an 1 μm thick HA film fired at 1000°C for 2 hours. This spectrum was derived by glancing angle geometry.

calcium phosphate coatings while diminishing the risks of mechanical failure of the coatings, at either the bone/coating or coating/metal interfaces, would improve the engineering design of bone implants. Hypothetically, the major problems could be overcome by a reduction in coating thickness and developing coating methods which provide the possibility of chemical bonding between the coating and the underlying metal as well as permitting the formation of a biological bond between bone and the coating surface.

To address these possibilities, we describe herein the fabrication of thin films of calcium phosphates by a sol-gel route, and report preliminary data on both the fabrication and characterization of such films and bone matrix elaboration on their surfaces. To assess the suitability of these films to support bone growth, and generate a "bonding" interface, we have employed an osteogenic rat femoral bone marrow cell culture system.

Materials and Methods

Preparation and characterization of thin films

Either glass [1 inch (~ 2.5 cm) square pieces cut from 2 x 2 inch (~ 5 x 5 cm) 0.48 inch (~ 1.2 cm) thick plates supplied by Corning Glass Works, Corning, New York] or fused quartz plaques [1 inch (~ 2.5 cm) diameter, 1/8 inch (~ 0.3 cm) thick, G1 commercial grade, from Esco Products, Oak Ridge, NJ] were used as supports for the creation of sol-gel thin film layers. They were cleaned by boiling, for one hour, in double distilled and deionized water (DDH₂O) containing 2% (volume/volume, v/v) detergent, following which they were sonicated for one hour and then rinsed in DDH₂O,

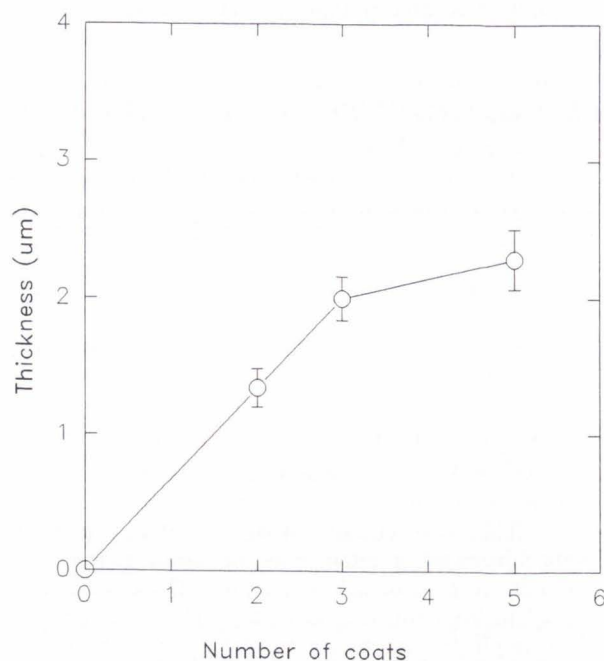
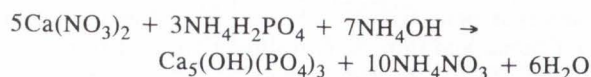


Figure 2. Sol-gel thin film thickness as a function of the number of coatings.

sonicated for two further periods of one hour and then blow-dried in nitrogen. The supports were stored in 35 mm petri dishes until ready for coating. Calcium phosphate films were created by the reaction:



A solution of 2.361 grams of calcium nitrate [(Ca(NO₃)₂·4H₂O, 99.94%, Fisher Scientific Company, Unionville, ON, Canada) in 20 ml DDH₂O was brought to pH ~ 12 with about 1 ml of concentrated ammonium hydroxide (NH₃ $\sim 29.5\%$) and diluted to 40 ml in a 250 ml round bottom flask which sat on a magnetic stirrer. Into this solution was added, drop by drop slowly and with vigorous stirring, a solution of 0.691 g ammonium dihydrogen phosphate (NH₄H₂PO₄, 99.9%, Fisher Scientific Company) in 30 ml DDH₂O which had been brought to pH ~ 12 with 20 ml of concentrated ammonium hydroxide (NH₃ $\sim 29.5\%$) and thereafter diluted to 80 ml in a 150 ml funnel. The mixture was stirred at room temperature for 24 hours and, after two days, used to fabricate films by a dip-coating method. After drying in air at room temperature, the resultant films were fired at 400°C, 600°C, 800°C, 900°C, 1000°C and 1100°C respectively for 2 hours, those at 800°C and over on the fused quartz supports and the remainder on glass supports. Films of over 0.5 μm thickness were obtained by applying multiple coatings. A total of over 100 films were made in this study.

X-ray diffraction analysis of the films was performed using a Rigaku "Miniflex" X-ray diffractometer (Model CN2005) furnished with a Cu anode, or a 12 kV

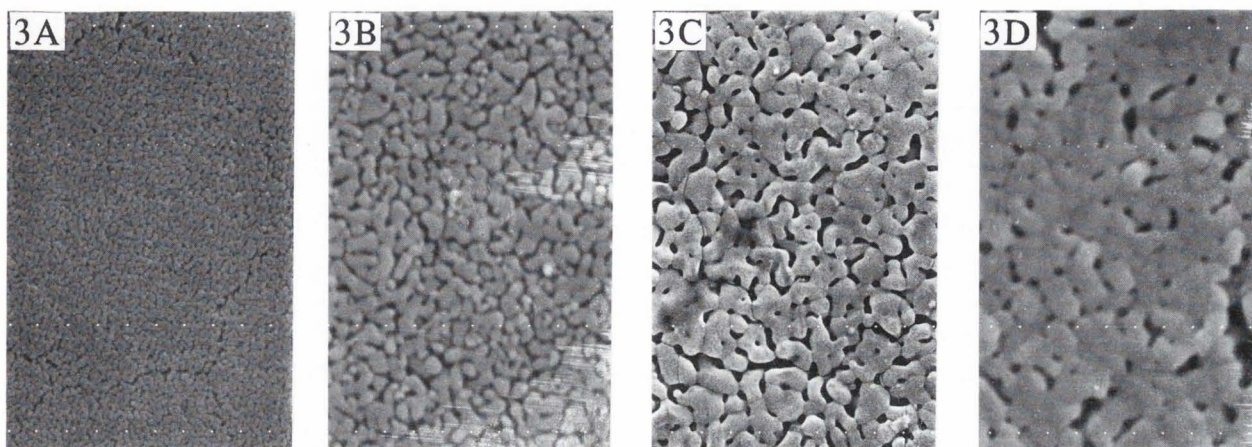


Figure 3. Scanning electron micrographs of the surfaces of the thin films as a function of annealing temperatures (A: 800°C; B: 900°C; C: 1000°C; and D: 1100°C), the annealing time was 2 hours in all four cases. Note: the increase in grain size with temperature. Field Widths [FW] = 5 μm (Figs. 3A-3D are at same magnification).

Rigaku rotating anode X-ray generator (Model RU-200 BH) fitted with a glancing angle attachment. Diffraction peaks were compared with JCPDS (Joint Committee on Powder Diffraction Standards) file data, and were consistent for 10 samples selected from the total number.

The surface morphologies of the hydroxyapatite (HA) films as a function of the time and temperature of annealing were studied by preparing films of 1 μm thickness and annealing them at temperatures ranging from 800-1100°C for periods of 2 minutes to 4 hours. These films were then examined using a JEOL 840 scanning electron microscope (SEM) and the grain size measured using a 3-circle linear intercept technique (ASTM, 1978). The total circumference of the three circles was 500 μm. The average number of grains per unit distance 'n' was calculated by:

$$n = (N/L) \cdot \text{Mag} \quad (1)$$

where: N = number of grain intersections counted; Mag = the magnification employed; and L = the length of the intercept lines used.

The grain size was thus: $G = (1-P)/n$, where P = porosity. The porosity was calculated by measuring the size of each pore along two 79.5 μm lines and dividing the total pore length by the total line length.

Finally, a simple assessment of adhesion of the films to the underlying supports was carried out by immersing the films perpendicularly in DDH₂O in a beaker placed in an ultrasonic cleaner for a period of 1 hour.

Rat marrow cell culture

Bone marrow cells were obtained from young adult male Wistar rats (approximately 120 gm) and cultured as described previously (Davies *et al.*, 1991a, b). Briefly, femora were removed and washed with α-Minimal Essential Medium (α-MEM) containing 1.0 mg/ml penicillin G, 0.5 mg/ml gentamicin and 3.0 μg/ml fungizone. The epiphyses were removed and the marrow

washed out using α-MEM supplemented with 15% foetal bovine serum, 50 μg/ml of freshly-prepared ascorbic acid (added as 1% of a 5 mg/ml stock solution in phosphate buffered saline), 10 mM Na β-glycerophosphate (added as 1% of a 1 M stock solution in DDH₂O), 10⁻⁸ M dexamethasone and antibiotics at 1/10th of the concentration described above. The calcium phosphate thin films on glass or quartz supports, described above, were placed individually in 35 mm diameter dishes and sterilized by irradiation (2.5 MRads from ⁶⁰Co). Aliquots (0.9 ml) of the rat bone marrow cell suspension were inoculated onto the test substrata. Non-adherent cells were aspirated the following day, and each dish was inoculated with 2 ml of fully supplemented medium. Subsequently, the medium was renewed three times a week and the cultures maintained for 18 days in a humidified atmosphere of 95% air with 5% CO₂. Cultures were prepared for either light microscopy (LM) or SEM at this stage.

Scanning electron microscopy

Cultures were fixed in 2.0% paraformaldehyde in 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer (pH 7.2-7.4), and postfixed in a 2% solution of osmium tetroxide in the same buffer. Following this, they were dehydrated in graded alcohols, critical-point dried from CO₂ (Ladd Research Industries Inc., Burlington, VT), sputter-coated with gold (approximately 10 nm) (Polaron Instrument Inc., Doylestown, PA) and examined in an Hitachi 2500 SEM. Some samples were freeze-fractured in liquid nitrogen in order to examine vertical cross-sections of the interfaces between the film and underlying support or elaborated bone matrix.

Results

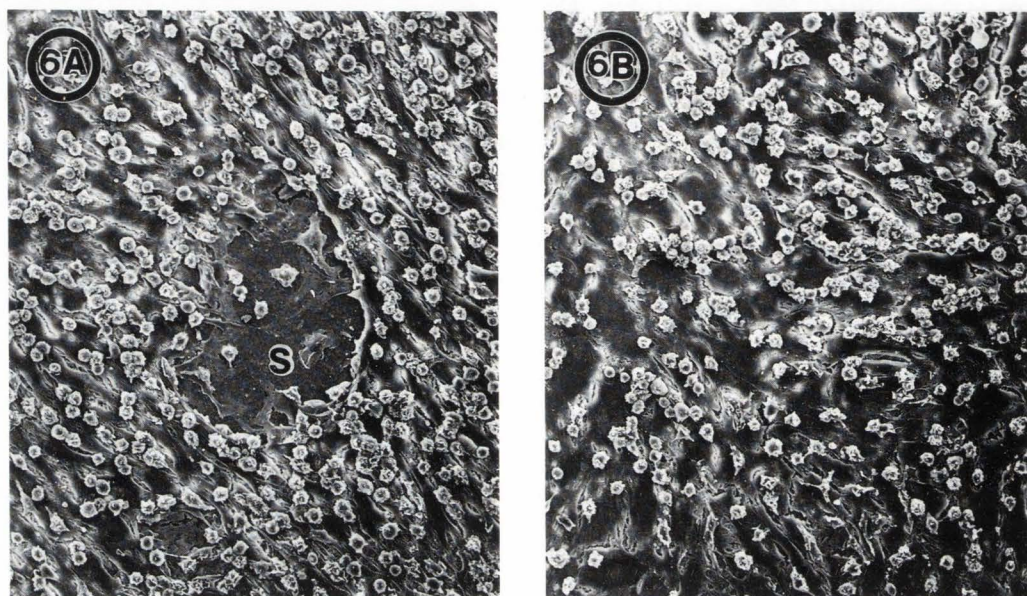
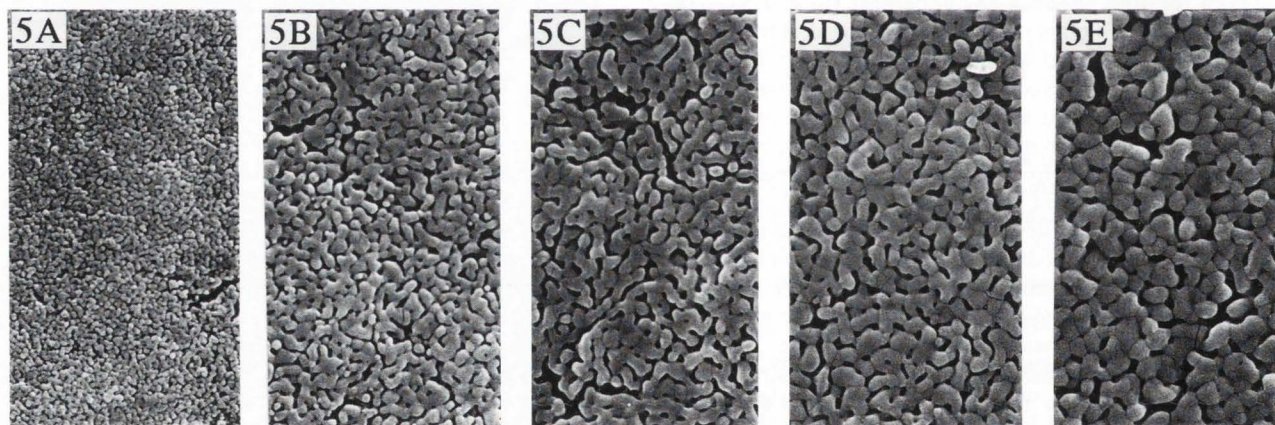
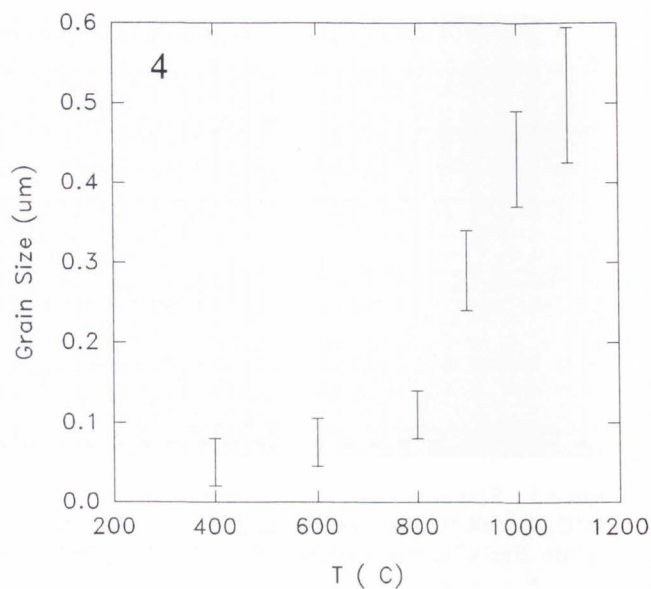
Crystalline structure of the thin films

X-ray diffraction analysis of the 400°C, 600°C, 800°C, and 1000°C annealed films not only showed

Figure 4. Grain size, as calculated by the 3-circle linear intercept technique, as a function of annealing temperature.

Figure 5. Scanning electron micrographs of the surfaces of the thin films as a function of annealing time (A: 2 minutes; B: 5 minutes; C: 10 minutes; D: 1 hour; and E: 4 hours). Note: the increase in grain size with time at 1000°C. FW = 5 μm (all five figures are at identical magnifications).

Figure 6. Dorsal views of cells cultured on A) 400°C thin film on glass; and B) 1000°C thin film on quartz. Note: Although, in (A), the underlying substratum (S) can be seen in the centre of the field of view, such exposed areas were found on all substrata. No evident differences were observed in the morphologies of cells grown on films of different annealing temperatures. FW = 440 μm (in A and B).



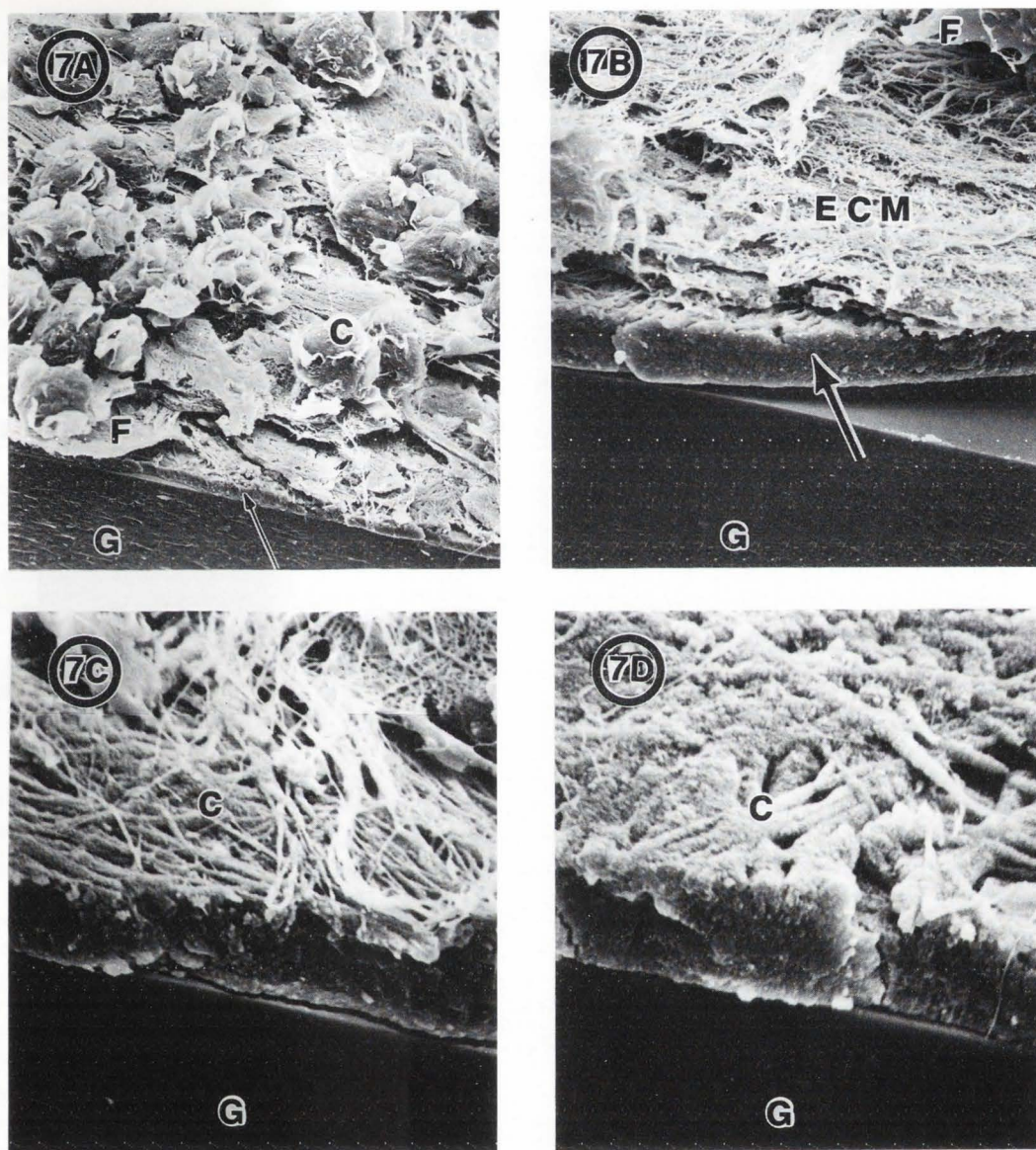
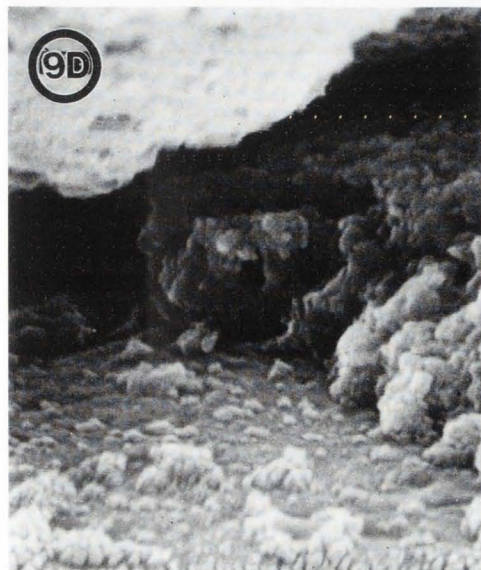
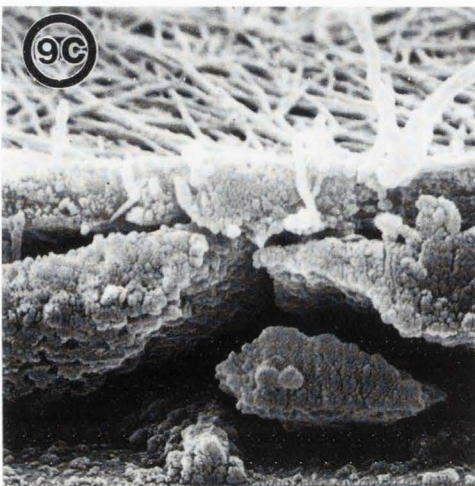
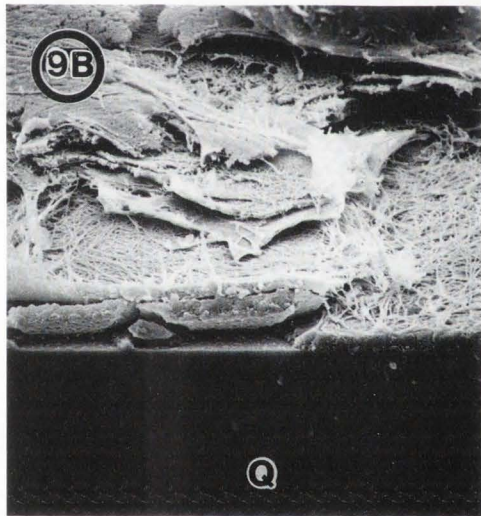
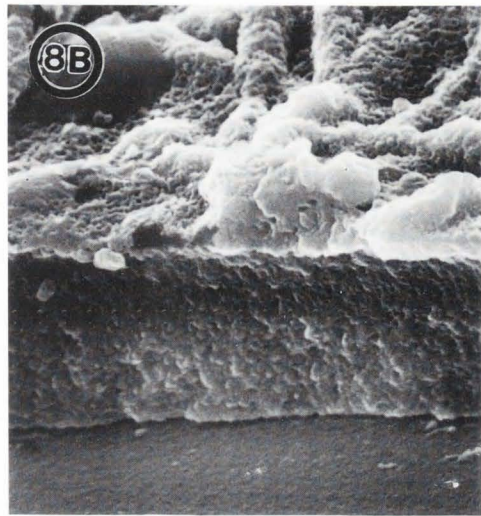
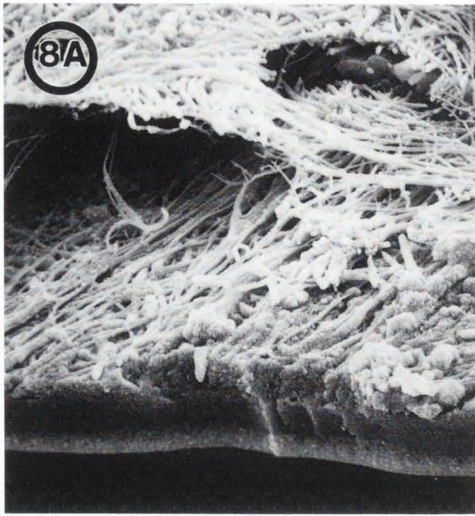


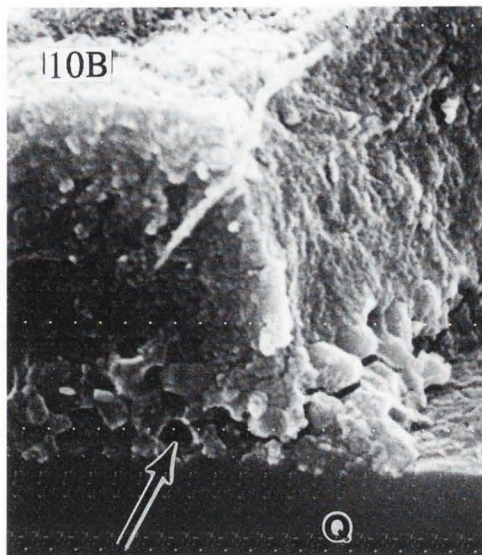
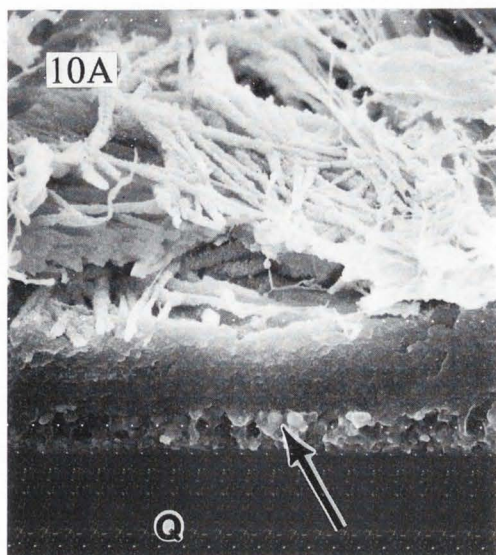
Figure 7. Scanning electron micrographs of bone culture on films fired at 400°C. While at low magnification both the glass (G)/film (arrow) interface and the film/cell (F and C) would seem intact (A), at higher magnifications (B) it is clear that the film was detached from the glass support. Cells nearest the film are flat (F) while the surface cells are more rounded. In (C) and (D), the collagen fibres (C) of the extracellular matrix can be seen to be embedded in the surface layers of mineralization. FW = 62 μm (in A); 19 μm (in B); 9 μm (in C); and 8 μm (in D).

close agreement between the experimental data for these films and the JCPDS file data for hydroxyapatite (Fig. 1) but also that higher temperature samples had higher crystallinity than those fired at lower temperatures. This is consistent with results obtained on ceramic coatings such as zirconia (Pascual *et al.*, 1991). The peak heights occurring in the X-ray spectra of the films were lower than those observed in the spectra of bulk ceramic. This was due to the limited thickness of the coating and possibly to less well developed crystallinity in the films.

Film thickness and surface morphology

The thickness of the films varied as a linear function of the number of coatings for the first three layers, as shown in Figure 2. The surface morphologies of the HA films as a function of annealing temperatures are shown in Figure 3. The four samples are all crack-free, the surface of the 1000°C sample seems most uniform, and the series shows that the grain size of the film surface increased with annealing temperature, which is confirmed in Figure 4. Grain size also increased with





Figures 8 and 9 on facing page.

Figure 8. Scanning electron micrographs of bone culture on films fired at 600°C. Two distinct layers can be seen in this sol-gel film which is covered by an extracellular matrix comprising multiple layers of mineralizing collagen fibres (A). (B) The collagen fibres closest to the film are completely buried in biologically produced mineral which seems to merge with the surface of the sol gel thin film. FW = 19 μm (in A); and 3.5 μm (in B).

Figure 9. Scanning electron micrographs of bone culture on films fired at 800°C. Clearly, at all magnifications, the film has separated from the underlying quartz (Q) support, while the biological matrix has remained in continuity with the surface of the film. However, fracture of the film during processing (critical point drying and specimen fracture) has occurred within the film and some grains have been left tightly adherent to the underlying support. FW = 94 μm (in A); 30 μm (in B); 6 μm (in C); and = 1.9 μm (in D).

annealing time, as shown in Figure 5, with a resultant change in surface morphology.

Cell culture

Initial monitoring, during culture, of the viability of cells on the sol-gel substrata described was not possible, when viewed by inverted phase microscopy, due to optical interference created by the thin film. Nevertheless, with increase in culture time the multilayering of cells became evident and randomly distributed, diffuse, patches of extracellular matrix formation, typical of this culture system (Maniopoulos *et al.*, 1988; Davies *et al.*, 1991a,b) appeared distinct from the thin film both in texture and plane of focus. After the 18 day culture period, the culture units were prepared for examination by SEM, as described above, but were also fractured prior to gold coating and re-examined by LM to visualize the random distribution of darker areas (due to

Figure 10 (above). Scanning electron micrographs of bone culture on films fired at 1000°C. A clear distinction is seen between the upper and lower layers of this film. The lower layer, in contact with the quartz support (Q), shows a clear grain structure (arrow in A) with intergranular voids (arrow in B). Some of the smaller grains seemed to have remained adherent to the underlying quartz while evidence of fracture within the granular layer is seen in (B). FW = 9 μm (in A); and 3 μm (in B).

the osmication procedure) which represented developing bone nodules (not shown). In these areas, both the cell layers and the quartz support as well as the increase in thickness of the interfacial region concomitant with the presence of elaborated bone tissue, could be clearly seen. While such differences in the cell and matrix layers were evident at LM levels, and also in the SEM views of the fractured surfaces (see below), no discernable differences were evident between the morphology of either the cell layers or individual cells grown on films fired at different temperatures, as illustrated in Figures 6A and 6B.

Interfacial extracellular matrix elaboration

Figures 7 to 10 provide a series of scanning electron micrographs of the freeze-fractured appearances of extracellular matrix elaborated by the cultured osteogenic cells on films of different annealing temperatures. In all cases, a dense collagenous matrix was evident on the apparent surface of the thin films. Figures 7A-7D show a series of magnifications of a film fired at 400°C on glass. At low magnification, both the dorsal cells of the culture and the underlying glass support were seen (Fig. 7A). The cells nearest the thin film were evidently more flattened than the dorsal cells which exhibited a rounded morphology. From Figure 7B, it is evident that the preparative procedures used to examine this sample in the SEM have led to the separation of the thin film

from the underlying support. This appearance was consistently different from that associated with films fired at higher temperatures on quartz (see below). If the first layer of collagen was assumed to represent the film/matrix interface, then several layers of matrix were visible at this low magnification. Figure 7C illustrates the dense appearance of the collagenous extracellular matrix on the underlying thin film. At this magnification, the collagen fibres nearest the thin film appeared thicker than those laid down above the immediate interfacial zone. In Figure 7D it is evident that these interfacial collagen fibres were mineralized, as judged by their typical encrusted appearance. Several layers of overlapping fibres were evident, which seemed to be buried in the surface of the thin film. The latter comprised a fine granular structure which, in cross-section, was indistinguishable from the granular appearance of the collagen fibres. No morphological features distinguished these two layers from either each other, or mineral which may have been elaborated by cellular mechanisms.

Similar appearances were noted with the thin films fired at 600°C, as shown in Figures 8A and 8B. However, in this sample, an apparent distinction could be made between the bi-layered thin film and an overlying granular layer which was more intimately associated with the interfacial collagen fibres. Damage to the biological extracellular matrix, as a result of sample preparation, was evident as shown in Figure 8A. However, it was equally clear that multiple layers of mineralized collagen fibres existed on these samples. The layer most closely adapted to the underlying thin film was almost completely buried in a mineralized matrix which, at higher magnification (Fig. 8B) was indistinguishable from the thin film itself. The structure of the film was similar to that seen in samples fired at 400°C (see above) with no clear distinction of either individual grains within the sol-gel layer, nor evidence of adhesion of the thin film to the underlying glass support.

The freeze-fractured films fired at higher temperatures on quartz showed consistently different appearances at the interface with the underlying support than those fired on glass. First: in the 800°C and 1000°C films, within the range of magnifications used for these SEM observations, individual grains were seen in the thin film layer near to the underlying support. These grains were of variable dimensions and more clearly seen with higher firing temperature. Figures 9A-9D show a series of micrographs at increasing magnifications of the interface between the cell elaborated extracellular matrix and the underlying thin film which was fired at 800°C. At low magnification, Figure 9A, there was little discernable difference in appearance in comparison to the lower temperature films (see Figure 7A). Film/support separation was again evident, as well as the differences in the appearance of the cells at the interface compared to those exposed to the culture medium, and multiple layers of extracellular matrix (Fig. 9B). However, in some areas, the film did not separate from the underlying support which resulted in fracture of the film

itself (Figs. 9B and 9C) and the adhesion of the thin film to the underlying support (Fig. 9C). Furthermore, the mineralized matrix containing collagen fibres also fractured from the underlying thin film indicating a segregation between these two areas which was not evident on morphological appearance alone. At higher magnification, the quartz support surface was roughened by the attachment of individual grains of the sol-gel thin film.

This difference in appearance of the thin film layer in contact with the quartz compared to that in contact with the biological matrix was most clearly seen on the samples fired at 1000°C (Figs. 10A and 10B). Here, the film/support interface was occupied by a layer of distinct grains separated by intergranular voids. The grains were of variable size, the smallest of which were apparently in intimate contact with, and adhered to, the underlying support. Sample preparation has caused small fracture planes to appear at this level in the thin film while adhesion to the support remained intact.

Adhesion of the thin films to the underlying support

The results of the ultrasonic test were, at first, surprising. Those films fired at 400 and 600°C for 2 hours showed no apparent change after 1 hour of sonication. However, parts of the films fired at 800 and 1000°C for 2 hours peeled off their quartz supports after the same treatment. However, on examination by both LM and SEM, it was found that an adherent layer of material was still evident on the quartz supports which correlated with the appearances described above following cell culture.

Discussion

We have made no attempt in the work reported herein to quantify the cell attachment to, or proliferation and migration on, the sol-gel substrata described. It was, however, our intention to provide some initial characterization of these calcium phosphate thin films; to examine the relationship between the thin film and underlying support; and compare bone extracellular matrix elaboration on these substrata. Subjective assessment of the rate of development of bone nodules on these surfaces indicated a similar behaviour to that which we have already described on both polymer and metallic surfaces (Davies *et al.*, 1991a,b; Lowenberg *et al.*, 1991).

The films demonstrated chemical and physical characteristics which were expected from the processing route employed. We have observed an increase in grain size with temperature and time in similar work on zirconia ceramic films (Pascual *et al.*, 1991). In thin films, the bulk three dimensional theory which should describe this process applies only to the initial stages of sintering where the particle size is still small compared to the thickness of the film (Thompson, 1990). Once the grain size is comparable to the thickness, growth occurs only in two dimensions and this alters the growth kinetics significantly. An additional factor is the constraining forces which stem from the interaction with the support

(Garino and Bowen, 1990). It is logical that during sintering, when the film material is slowly coalescing and moving across the support, there will be a strong force resisting this. These constraining forces will have the effect of reducing the driving forces, enhancing stagnation of the growth, and in general, reducing the long term densification rates over time and temperature. The kinetics then take on characteristics intermediate between free three-dimensional sintering and linear one-dimensional sintering where only shrinkage in film thickness is unconstrained. The sintering kinetics of calcium hydroxyapatite displayed some correlation with such theoretical models of two dimensional sintering (Coble, 1961; Scherer and Garino, 1985). The form of the sintering behaviour of the films with variations in temperature, time and thickness was consistent and predictable. The particle size appears to be more sensitive to variations in temperature than to sintering time. The variation of porosity with temperature exhibits the general form of the normal densification behaviour particularly if consideration is given to two-dimensional effects.

The structure of many of the high temperature films consisted of a high density, small grain size, highly adherent layer at the interface between the support and the film, and a larger grain size, more porous structure at the surface of the film. The adherence of these films to the quartz is assumed to be chemical in nature since the quartz discs showed no surface features before processing which would result in a mechanical bond. A possibility that cannot be discounted at this time is that of diffusion of silica from the quartz at high temperature to form a glassy adhesion layer at the quartz surface. The transparent glass and quartz supports were employed in the present work to facilitate our monitoring of the cell cultures. The indication of chemical bonding to the underlying support, in the case of high sintering temperatures, agrees with our observations of epitaxial growth of sol-gel films from underlying supports in other systems (Pascual *et al.*, 1991). It also indicates the possibility of using similar processing routes to achieve chemically bonded films on metallic bone implants, which is not routinely possible with plasma sprayed calcium phosphate coatings.

The biological results suggest that cells interact with the surface layer and the elaborated extracellular matrix bonds with the film surface. If the nature of this interaction is influenced by the grain size and purity of the apatite film, it would seem reasonable that initiation of the cell response would be optimum for calcium hydroxyapatites having a grain size which is close to that produced by natural bone growth processes. The biological interfacial matrix was that which we have previously described as a cement line-like material (Davies *et al.*, 1991a, b) which comprises a calcified organic extracellular matrix which is devoid of assembled collagen fibres. We have recently demonstrated that the organic component of this matrix contains bone cell derived proteins (Shen *et al.*, 1993) and we would assume that these proteins would be adsorbed to the surface microporosity

of the thin films used in the work reported here. This organic matrix is calcified by the seeding of biological derived calcium phosphate nanocrystals (Davies *et al.*, 1993) which increase in size as the cement line matrix matures. It would be expected that, if this biological matrix was forming on a stable microporous surface containing intergranular voids of the same order of magnitude as biological calcium phosphate crystals, then it would be difficult to distinguish between the biological and non-biological matrices within the interfacial zone. This would, indeed, seem to be the case in the films employed in the present work. This interdigitation of the biological matrix with the film surface created, in all cases, an interface which withstood the mechanical disruption caused by differential shrinkage associated with the critical point drying procedures employed to examine these specimens in the SEM. The collagen, laid down by the bone cells, was integrated with the surface of the cement line matrix and, as this was indistinguishable from the underlying sol-gel films, no evidence was seen for interdigitation of the collagen fibres with the latter.

Conclusions

These results indicate that it is possible to control the characteristics of sol-gel thin films of calcium hydroxyapatite by varying the dipping and sintering parameters. Higher annealing temperatures induce higher crystallinity and a larger grain size; longer annealing times also produce larger particle sizes. The sol-gel process, with its associated control of particle size, as-deposited particle density, and capability for the production of multilayer coatings, therefore provides a process route in which the film grain size, porosity and morphology may be optimally engineered not only for interfacial bonding with bone to implants but also to design experiments to unravel the mechanisms of bone bonding. This is a capability which is unlikely to be possible with conventional plasma sprayed coatings.

Acknowledgements

Financial support of this work from the Ontario Centre for Materials Research (OCMR) is gratefully acknowledged.

References

- ASTM (American Society for Testing and Materials) (1978) Annual Book of ASTM Standards, Part 11, ASTM Philadelphia. E 112-77, p. 205.
- Coble RL (1961) Sintering of crystalline solids 1. Intermediate and final stage diffusion models. *J. Applied Phys.* **32**, 787-792.
- Davies JE, Chernecky R, Lowenberg B, Shiga A (1991a) Deposition and resorption of calcified matrix *in vitro* by rat bone marrow cells. *Cells and Materials* **1**, 3-15.

Davies JE, Ottensmeyer P, Shen X, Hashimoto M, Peel SAF (1991b) Early extracellular matrix synthesis. In: *The Bone-Biomaterial Interface*. Davies, JE (ed.). University of Toronto Press, Toronto, pp. 214-228.

Davies JE, Perovic DD, Shen X (1993) Characterization of early bone mineral crystallites found at the substratum interface *in vitro*. In: Proc. 25th Annual Meeting of the Society for Biomaterials (Minneapolis, MN). p. 247 (abstract).

Ducheyne P, Cuckler J, Radin S, Nazar E (1990) Plasma sprayed calcium phosphate ceramic linings on porous metal coatings for bone ingrowth. In: *CRC Handbook of Bioactive Ceramics Vol. 2*. Yamamuro T, Hench LL, Wilson J (eds.). CRC Press, Boca Raton, FL. pp. 123-131.

Ducheyne P, Hench LL, Kagan A, Martens M, Bursens A, Mulier JC (1980) Effect of hydroxyapatite impregnation on skeletal bonding or porous coated implants. *J. Biomed. Mat. Res.* **14**, 225-237.

Ducheyne P, Bianco PD, Cheolsang K (1992) Bone tissue growth enhancement by calcium phosphate coatings on porous titanium alloy: the effects of shielding metal dissolution product. *Biomaterials* **25**, 617-624.

Garino T, Bowen HK (1990) Kinetics of Constrained film sintering. *J. Amer. Cer. Soc.* **73**, 251-257.

Geesink R, de Groot K, Klein C (1987) Chemical implant fixation using hydroxyl-apatite coatings. *Clin. Orthop. Rel. Res.* **225**, 147-170.

Hench LL, Splinter RJ, Allen WC (1971) Bonding mechanisms at the interface of ceramic prosthetic materials. *J. Biomed. Mat. Res. Symp.* **2**, 117-141.

Luckey HA, Lamprecht EG, Walt MJ (1992) Bone apposition to plasma-sprayed cobalt-chromium alloy. *J. Biomed. Mat. Res.* **26**, 557-575.

Lowenberg B, Chernecky R, Shiga A, Davies JE (1991) Mineralized matrix production by osteoblasts on solid titanium *in vitro*. *Cells and Materials* **1**, 177-187.

Maniopoulos C, Sodek J, Melcher AH (1988) Bone formation *in vitro* by stromal cells obtained from bone marrow of young adult rats. *Cell Tiss. Res.* **254**, 317-330.

Oonishi H, Yamamoto M, Ishimaru H, Tsuji E, Kushitani S (1989) Comparison of bone ingrowth into porous Ti6Al4V beads coated and uncoated with hydroxyapatite. In: *Bioceramics*. Oonishi H, Aoki H, Sawai K (eds.). Ishiyaku EuroAmerica Inc. Tokyo. pp. 400-405.

Orr RD, de Bruijn JD, Davies JE (1993) Scanning electron microscopy of the bone interface with titanium, titanium alloy and hydroxyapatite. *Cells and Materials* **2**, 241-251.

Pascual P, Sayer M, Yi G, Baker C (1991) Phase transformation kinetics in thin film zirconia. *J. Can. Ceram. Soc.* **60**, 43-46.

Ricci JL, Spivak JM, Blumenthal NC, Alexander H (1991) Modulation of bone ingrowth by surface chemistry and roughness. In: *The Bone-Biomaterial Interface*. Davies JE (ed.). University of Toronto Press, Toronto. pp. 334-349.

Rivero DP, Fox J, Skipor AK, Urban RM, Galante JO (1988) Calcium phosphate-coated porous titanium implants for enhanced skeletal fixation. *J. Biomed. Mat. Res.* **22**, 191-201.

Scherer GW, Garino T (1985) Viscous sintering on a rigid substrate. *J. Am. Ceram. Soc.* **68**, 216-220.

Shen X, Roberts E, Peel SAF, Davies JE (1993) Organic extracellular matrix components at the bone cell/substratum interface. *Cells and Materials* **3**, 257-272.

Thompson CV (1990) Grain growth in thin films. *Annu. Rev. Mater. Sci.* **20**, 245-268.

Discussion with Reviewers

G. Daculsi: To observe bone nodules is an important result. Why do you not show the nodule? What is your proof of calcified bone nodules?

Authors: In this paper we have focussed on the fabrication of the thin films and the bone interface. The culture technique which we employ to obtain high yields of calcified bone nodules has been described in detail previously (Davies *et al.*, 1991a, b) and, for this reason, these morphological findings are not repeated herein.

G. Daculsi: The appearance of fibres cannot be related to a mineralization process. This is speculative. You must perform energy dispersive X-ray spectroscopy and Fourier-transformed infrared spectroscopy.

Authors: We believe the morphologies illustrated are clear evidence that the collagen fibres were mineralized. However, we would agree that this does not shed light on the process of mineralization which would have to be investigated using other techniques. Indeed, the elaboration of the interfacial cement-like matrix, which is collagen fibre free, lends support to the general notion that collagen *per se* is not required for this biological mineralization process to occur but that certain non-collagenous proteins are strongly implicated (see Shen *et al.*, 1993).

P. Ducheyne: There is only one essential change needed in my opinion: the thin films were not deposited by a sol-gel technique.

Authors: Although the most common route for fabrication of sol-gel thin films uses alkoxide preparations, the particulate colloidal suspension route employed herein is indeed considered a sol-gel technique and is described as such in standard texts [Brinker CJ, Scherer GW (1990) *Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing*, Academic Press. pp 235-301].

G. Daculsi: You talk of 400, 600, 800, and 1000°C in text, and 800, 900, 1000, 1100°C on Fig. 3!

Authors: There is no inconsistency here. We fired samples, as stated, at several different temperatures. Some were used for comparison of morphology with annealing times, others were used for comparisons of morphology with annealing temperatures.