Journal of the European Ceramic Society 18 (1998) 1931–1937

PII: S0955-2219(98)00132-0
0955-2219/98/$—see front matter

Preparation of TCP–TiO_2 Biocomposites and Study of Their Cytocompatibility

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(Received 26 June 1997; accepted 20 May 1998)

Abstract

Ceramic composites made of a core of dense titania and a surface of porous tricalcium phosphate granules were prepared using a three-stage process of slip casting, pre-sintering, and co-sintering. A cytocompatibility study has shown that the composites might offer potential for medical applications. © 1998 Elsevier Science Limited. All rights reserved

1 Introduction

Surgical and odontological implants or other prosthetic devices are made from materials whose first requirement is to be harmless to the human body. However, other requirements concern optimal mechanical, biological, and chemical characteristics for particular applications. In many cases, a point of particular importance is the implant-tissue attachment.[1] Bioinert materials, for example alumina, do not help for attachment but lead to a non-adherent encapsulation of the implant by fibrous tissue. In contrast, bioactive materials can allow bioactive fixation, with direct attachment by chemical bonding with the bone.[1–3] This advantage can be balanced by sensitivity to chemical and biological corrosion.

Titanium is widely used for prosthetic applications due to its excellent biocompatibility and high mechanical characteristics.[2–4] In physiological conditions, there is formation of a thin layer of titania (TiO_2) on the surface of the metal,[5–8] which implies that the surface reactivity behavior of titanium is mainly controlled by the presence of titania. Although titanium and titania are generally considered as bioinert, it was recently shown[9–13] that surface treatments of titanium-based materials can induce bioactivity, gel-derived titania being a hydroxyapatite inducer because of its abundant TiOH groups. Bioactive materials are typified by bioactive glasses[1,14] and calcium phosphates.[15–19] Among the phosphates, tricalcium phosphate $Ca_3(PO_4)_2$ (designated as TCP) is very reactive, which means it can be progressively 'digested' by surrounding tissues.[18,19]

An implanted device does not necessarily need the same properties for its bulk and its surface. This suggests the use of graded parts, for example of titanium coated with calcium phosphate[20] which combines the high mechanical properties of the metal with the bioactivity of the phosphate. Hip prostheses are examples where high mechanical properties are required, but there are other applications (such as the filling of bone cavities in odontology) that are not so mechanically demanding. In this spirit, the present work has investigated the potential of a TCP–TiO_2 ceramic composite. As far as we know, there were no previous studies about this kind of composite.

The aim was processing of a graded composite made of TCP granules co-sintered with the surface of a TiO_2 core. The progressive digestion of the granules by living tissues can promote their ingrowth and, therefore, can allow implant-tissue attachment by 'biological fixation' according to Hench's terminology.[21] The TiO_2 core must be dense to ensure reasonable mechanical properties, whereas the reactivity of the TCP surface must be enhanced by using a finely porous material. Moreover, the tissues ingrowth needs vascularization, which requires the use of TCP granules with large diameter (100–200 μm).[22,23]

Once implanted, biomaterials are in contact with tissues, which leads to a complex interfacial zone.

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In a first approach, the *in vivo* interface can be studied from *in vitro* experiments, using organotypic culture.[24,25] In comparison with a dispersed cell culture, organ cultures are able to maintain the interactions between the different cell types necessary for tissue function integrity. In the present work, the use of explant culture has been set up to assess the cytocompatibility of materials and to study their influence on bone cells (migration, multiplication, adhesion and viability). The study was conducted by using *in vitro* organotypic culture, previously successfully applied to vascular prostheses[26] and dental implants.[27]

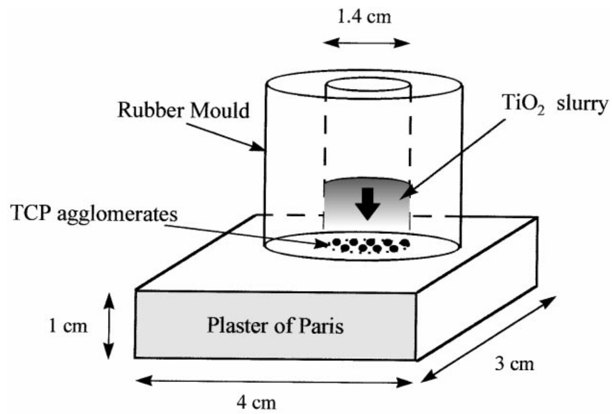


Fig. 1. Schematic illustration of the slip casting unit.

2 Experimental

2.1 Preparation of materials in the TCP–TiO_2 system

TiO_2 green bodies with TCP-granule enriched surfaces were processed using an original slip casting technique. Slip casting leads to homogeneous, high-density cakes that offer nice conditions for sintering. The problem of matching sintering shrinkage of TCP with that of TiO_2 was solved by using a two-stage procedure, first making very porous TCP granules, then pre-sintering them to required density. Pre-sintering also favors the successful percolation of slurry through the TCP-granule layer. The whole procedure was as follows:

(1) The TiO_2 powders were attrition ground (350 rpm for 2 h in ethyl alcohol) using dissociated-zircon balls. Deflocculation was obtained by adding 0·8 wt% of a phosphate-based surfactant. Deionized water was added to the slurry, which was subsequently heated to 80°C to evaporate ethyl alcohol. The aqueous slurry was electrostatically stabilized at pH $\approx$ 10 by addition of NH_4OH.[28] Particle size was controlled using a sedimentation analyzer. The solid-to-liquid ratio of the slurry was $\approx$ 20 vol%.

(2) The slurry was poured into a cylindrical rubber mould posed on a plaster-of-Paris slab (Fig. 1). The cast cake and mould were dried at 60°C for 3 days, then the cake was removed from the mould and sintered at 1200–1400°C for 2 h. Relative densities were measured by the Archimedes technique. SEM observations were conducted on polished and thermally etched (50°C below the sintering temperature) samples.

(3) The TCP powders were gravitationally sedimented in deionized water. The relative density of the dried sediments was $\approx$ 28%; 4 wt% of polyvinyl alcohol was added as binder to reduce the fragility of the sediments and facilitate their manipulation. The sediments were presintered at various temperatures (from 500 to 1100°C for 2 h) to change their porosity, as explained later on, then they were ground with agate mortar and pestle to form coarse granules which were screened to 120–180 μm quasi-spheroidal granules. Dilatometry experiments (heating rate: 5°C min^{-1}) were carried out on TCP sediments (0·3×0·3×0·5 cm specimens) presintered at 1000°C.

(4) The TCP granules were placed in the rubber mould in order to build a layer with apparent surface density of 0·03 g cm^{-2}. Then, the TiO_2 slurry was poured into the mould. After final sintering at 1300°C for 2 h, the composite was cut, polished and etched for SEM microstructural observations.

2.2 Organotypic culture

Tissues were harvested from tibias of 19-day old chick embryos. White cartilaginous ends were removed; only red ends of the tibia, containing cortical bone and a small quantity of trabecular bone, were used for explant preparation. The explant (about 1 mm^2) was then placed on a semi-solid agar nutrient medium and was covered with a 1 cm^2 disc of biomaterial, the trabecular bone side facing the material surface to be tested. The nutrient medium was adapted to bone cell culture.[29] Reference samples were also cultured: a non-toxic reference, labeled as T(–), was inert Thermanox® plastic (Lux Corp.).

(1) Cytocompatibility assessment was carried out as follows. After 14 days incubation at 37°C, which is enough to allow osteoblast cells to invade all the specimen surface, and neutral red staining, the area of the cell layer was measured with a stereomicroscope fitted with camera and digitizing tablet connected to a computer. After trypsin dissociation, the cells were counted with a Multisizer® (Coultronics) and the cellular density was calculated. Cell densities were plotted versus cell migration surfaces, each dot being an average over 36 samples.

(2) To evaluate cell adhesion to the material, the cells developed on the specimen surface were enzymatically detached using trypsin-EDTA to establish the curve of the percentage of released cells

versus time. The area between the curve and x-axis was determined by integration. This area is inversely proportional to the cell-biomaterial adhesion.

(3) The cell viability was estimated by using a colorant (Trypan blue) exclusion test. To determine the percentage of viable cells, the cell suspension was counted using a Mallassez unit.

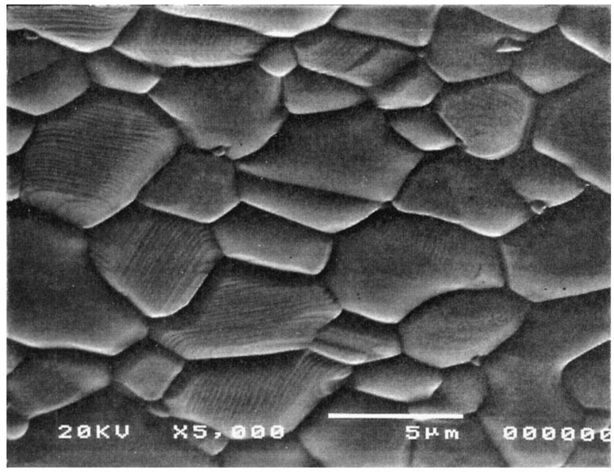
3 Results and Discussion

3.1 Materials

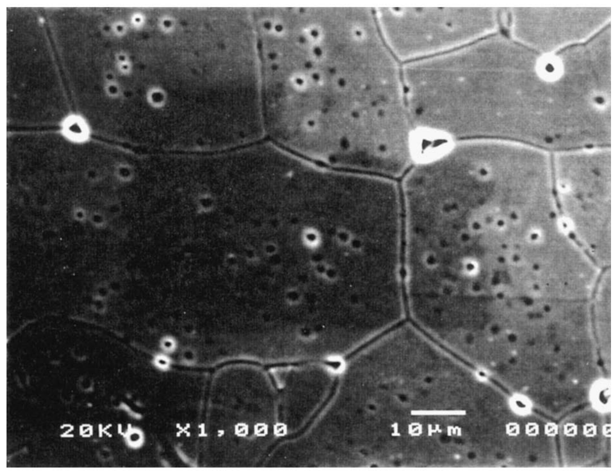
Attrition grinding decreases the mean particle size of the TiO_2 powders from 1·6 to 1·0 μm and also eliminates agglomerates. The beneficial influence of grinding is demonstrated by the fact that the relative density of green cakes is of 60% when using an attrited slurry but of 33% only when using as-received powders. Figure 2 shows the relative density of sintered TiO_2 ceramics versus sintering temperature. A final temperature of 1300–1350°C is required to sinter to nearly-theoretical density (4·23 g cm^{-3}).

Figures 3(a) and (b) show the microstructures of two specimens sintered at 1300 and 1400°C, respectively. The former is fully dense, with a mean grain size of 6·5 μm, whereas the latter has experienced dedensification and exaggerated grain growth. Volatilization phenomena are thought to be responsible for these detrimental effects. This shows that the sintering temperature must be chosen between 1300 and 1350°C, with preference for the lower temperature that yields smaller grains and, therefore, higher mechanical strength.

When the TCP sediments are sintered in the same conditions as the TiO_2 ceramics (1300°C for 2 h), their relative density increases from 28 to 60%. This large change leads to a linear shrinkage of 26%, much greater than that of TiO2 (16%, which corresponds to a density increase from 60 to 100%). As previously said, this suggests that the TCP sediments require a presintering treatment to partially densify them and reduce shrinkage during cosintering with TiO_2. Figure 4 shows the shrinkage experienced by a TCP sediment during the cosintering treatment at 1300°C for 2 h versus presintering temperature (from 900 to 1100°C).



(a)



(b)

Fig. 3. Microstructures of TiO_2 ceramics sintered for 2 h at (a) 1300°C and (b) 1400°C.

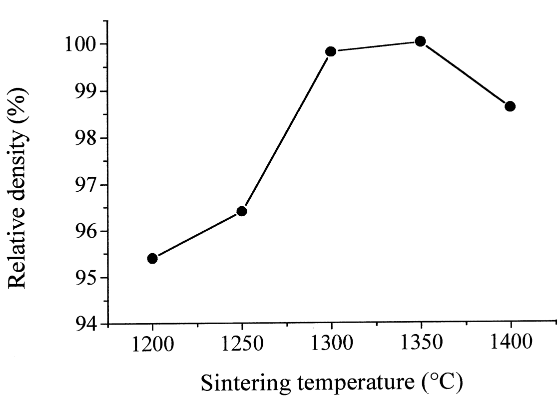


Fig. 2. Densification of TiO_2 ceramics versus sintering temperature.

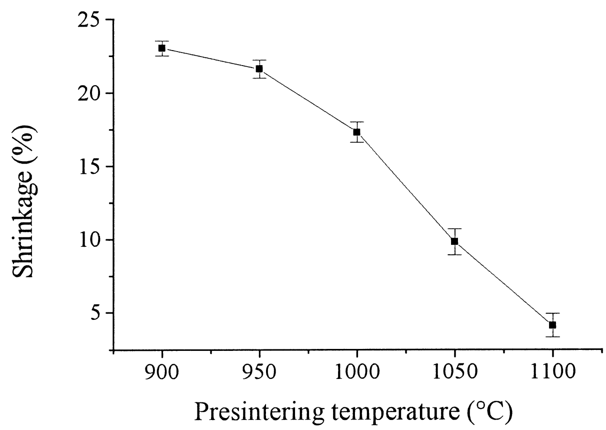


Fig. 4. Shrinkage of TCP sediments sintered for 2 h at 1300°C versus presintering temperature.

Presintering the TCP at 1000°C leads to a cosintering shrinkage of $\approx 16\%$, which matches the shrinkage of TiO_2. Figures 5(a) and (b) compare the dilatometric behavior of 1000°C-presintered TCP with that of TiO_2. The shrinkage rate reaches its maximum at ≈ 1100°C for TCP and at ≈ 1250°C for TiO_2. The shrinkage curves are very similar for both materials, with a final shrinkage mismatch of $\approx 1\%$ only.

Another cause of mismatch is the difference of thermal expansion coefficient (α) between the two materials. By taking handbook data ($\alpha_{20\text{–}1000°C} = 7\text{–}9\times10^{-6}\,K^{-1}$ for TiO_2 and $11\text{–}14\times10^{-6}\,K^{-1}$ for calcium phosphate) we have $\Delta\alpha \approx 5\times10^{-6}\,K^{-1}$ and, therefore, $\Delta l/l_0 \approx 0{\cdot}5\times10^{-2}$ if $\Delta T = 1000$°C (at $T > 1000$°C, diffusion and plasticity are expected to relax elastic mismatch). The total mismatch (cosintering shrinkage plus thermal expansion) is, therefore, less than 2%.

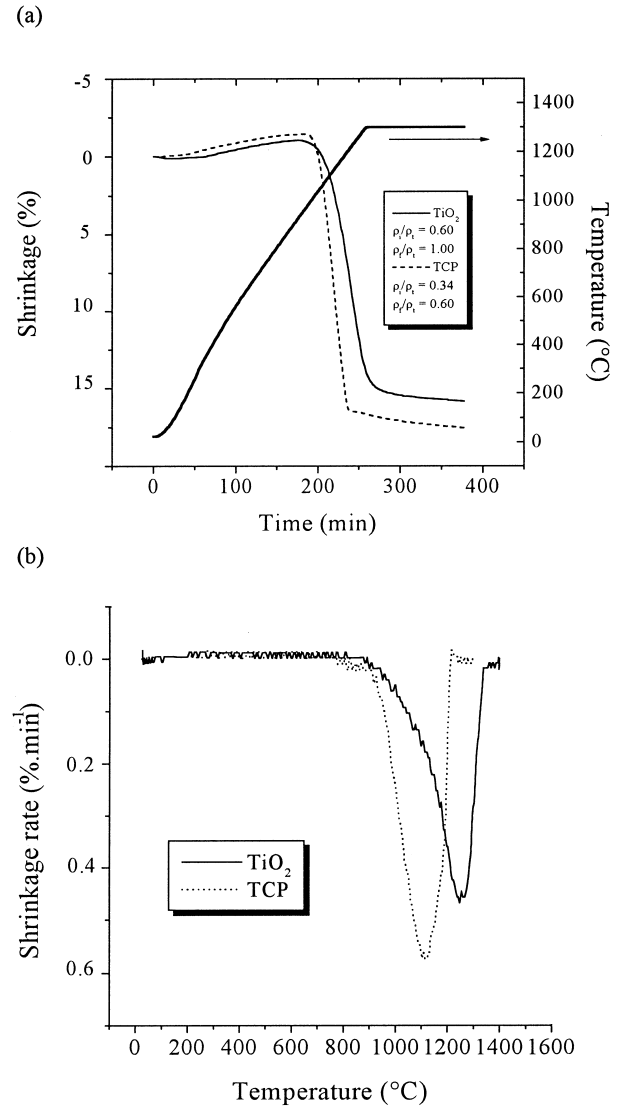


Fig. 5. TiO_2 and 1000°C-presintered TCP materials: (a) shrinkage and temperature versus time (heating rate: 5°C min^{-1} up to 1300°C, then soaking time of 2 h at 1300°C); (b) shrinkage rate versus temperature (heating rate: 5°C min^{-1}).

The transition from β-TCP to α-TCP occurs at 1125°C but the rate of transformation is relatively slow. This means that a mixture of the two phases might expected even after sintering at 1300°C. XRD experiments show that the 1300°C sintered TCP contains about 75% of α phase and 25% of β phase. As seen later on, organotypic culture tests show that α and β exhibit similar behavior with respect to cell culture. This suggests that the α-to-β ratio is a second-order parameter from this point of view.

Figures 6 and 7 illustrate the porous morphology of TCP granules and the microstructure of a composite made with TCP granules presintered at 1000°C, respectively. Figure 7 shows that the spheroidal TCP granules are homogeneously distributed in the superficial zone and that they are well cosintered with the dense titania matrix. In contrast, Fig. 8 illustrates the microstructure of a composite made with granules presintered at 500°C. Now, the granules have packed together to form a continuous coating on the surface of titania

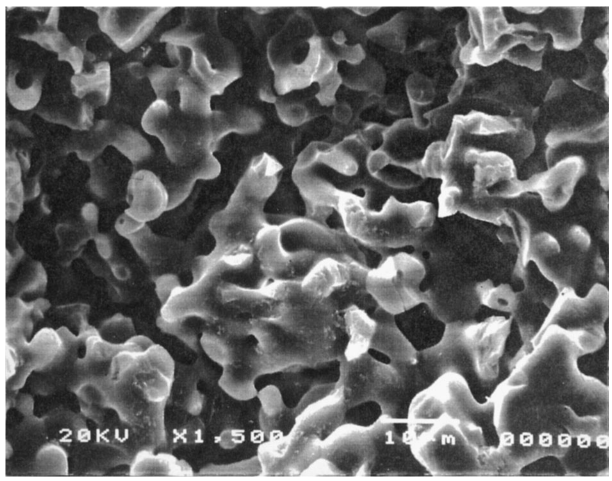


Fig. 6. Porous morphology of a TCP granule presintered at 1000°C.

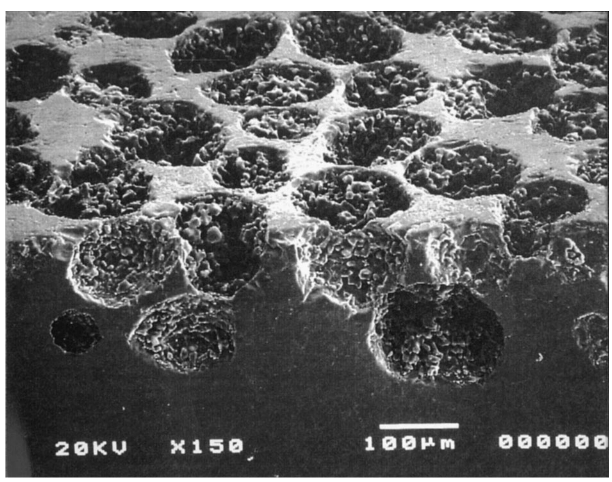


Fig. 7. Microstructure of a composite made with TCP granules presintered at 1000°C.

and the mean thickness of the TCP-containing zone is of $\approx$ 300 μm whereas it is of $\approx$ 450 μm in Fig. 7.

The point seems to be dependent on the facility or difficulty with which the TiO_2-slurry can percolate through the TCP granules during the slip casting stage. Figure 9 suggests a possible mechanism, where the water in the slurry is absorbed by the porous TCP granules, which leads to early formation of a TiO_2 shell that encases the granules, which hinders further slurry percolation. The TCP granules presintered at 500°C [Fig. 9(a)] are more porous than those treated at 1000°C [Fig. 9(b)], which implies that they can absorb more water and are, therefore, encased with a thicker TiO_2 protective shell. The validity of this explanation was verified by using porous, but non-absorbing TCP granules, prepared by presintering at 500°C only but subsequent impregnation with *Vaseline* to make them unwettable. Figure 10 shows that the TCP granule distribution in a composite made with those highly porous but vaselinated granules is similar to that in a composite made with less porous granules (Fig. 7). However, the shrinkage mismatch due to the use of very porous TCP granules has induced decohesion between the granules and the matrix, as visible in the bottom right of Fig. 10 (arrow).

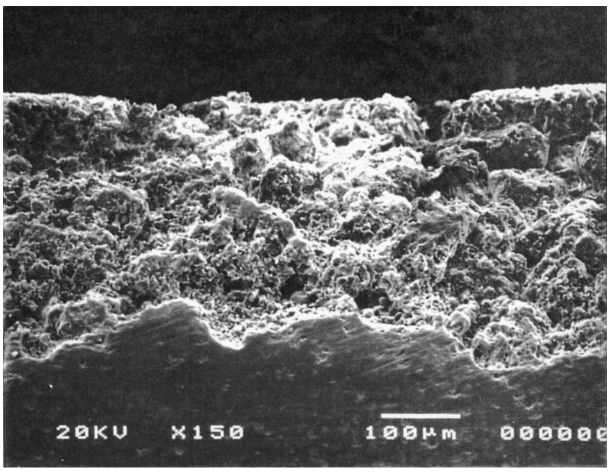


Fig. 8. Microstructure of a composite made with TCP granules presintered at 500°C.

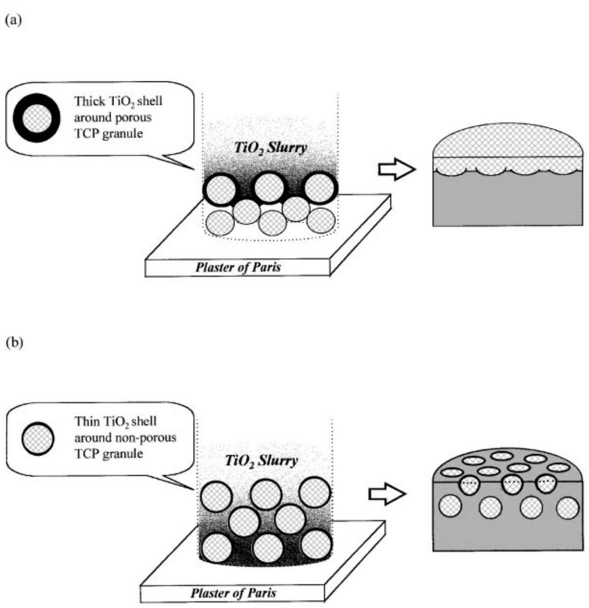


Fig. 9. Possible mechanism for percolation of the TiO_2 slurry between the TCP granules: (a) thick TiO_2 shell cast encasing water-absorbing granules and (b) thin TiO_2 shell cast encasing non-water-absorbing granules.

All these data indicate that the presintering temperature of the TCP granules is a useful parameter for controlling the microstructure of the composite. A high temperature of 1000°C leads to well-dispersed granules of TCP, tightly-cosintered with the TiO_2 matrix, whereas a low temperature of 500°C leads to a TCP coating deposited on the surface of the TCP-free TiO_2. One can assume that such a coating does not exhibit strong adhesion to the core, which indicates that a high presintering temperature should be preferred.

3.2 Organotypic culture

The tests were carried out using seven different materials, namely: (i) negative reference T(−), (ii) porous (β-TCP, (iii) dense β-TCP, (iv) dense α-TCP, (v) porous TiO_2, (vi) dense TiO_2, and (vii) 38 vol% TCP–TiO_2 composite. Table 1 brings information about the materials other than the reference. The use of two forms of TCP (the stable β form and the metastable α form) was initiated in a previous study[30] and is justified by the existence of a phase transformation at high temperatures.

Figure 11 shows the results about cell growth and cell migration. Dense α-TCP and dense β-TCP lead to similar results. T(−) and β-TCPd give rather similar results, whereas β-TCPp leads to a slightly smaller migration area but a much higher cell density, which

Fig. 10. Microstructure of a composite made with TCP granules presintered at 500°C but subsequently impregnated with *Vaseline* to make them unwettable.

Table 1. Biomaterials used for the cytocompatibility study

Materials	*Sintering*		*Porosity (%)*	*Notation*
	Temperature	*Time(h)*		
β-TCP	1100°C	3	25	β-TCPp
β-TCP	1100°C	24	3	β-TCPd
α-TCP	1400°C	48	2	α-TCP
TiO_2	1200°C	3	15	TiO_2p
TiO_2	1400°C	3	4	TiO_2d
Composite : 38% TCP/TiO_2	1300°C	2	40 (TCP) 0 (TiO_2)	Composite

Table 2. Cell viability

Materials	*Viability (%)*
T(−)	65
β-TCPp	91
β-TCPd	86
α-TCP	89
TiO_2p	80
TiO_2d	75
Composite	92

confirms that porous TCP stimulates cell multiplication. For titania, the cell density is slightly lower than that observed with T(−) but the cell migration is favored. For both materials, porosity seems to decrease cell migration. However, it must be pointed out that the cell migration area may be underestimated in very porous materials, whose surface is irregular. Finally, the TCP–TiO_2 biocomposite leads to values rather close to those observed in β-TCPp.

For cell viability, Table 2 shows the percentage of viable cells harvested by a coloration treatment of cultures. Comparison with reference material T(−) shows that all the TCP and TiO_2 materials give excellent cell viability, although the best results are brought when the reactive TCP phase is present. The cell viability obtained with the TCP–TiO_2 biocomposite is equal to that obtained with the β-TCPp. In all materials, we can note that cell viability increases with porosity.

Figure 12 shows the results of adhesion tests. It is usual for such tests to consider that an area superior to 4500 (arbitrary unit) means weak cell adhesion, an area between 4500 and 3000 means medium adhesion, and an area inferior to 3000 means strong adhesion. Using this criterion, one can see that TCP and TiO_2 materials lead to medium adhesion, with porous materials exhibiting slightly better properties than dense material, and TCP–TiO_2 biocomposites lead to strong adhesion. Besides the chemistry and morphology of the duplex (TCP + TiO_2) structure, one can think that the surface rugosity of the composites also plays a role.[31,32]

This study confirms that porosity is a key parameter when cell functional characteristics (viability, proliferation, or attachment) are concerned. Porosity favors cell growth and cell density but reduces cell migration. The increase in cell growth and cell adhesion observed in TCP–TiO_2 composite, in comparison with titania, shows the beneficial influence of TCP. In fact, the TCP–TiO_2 composites exhibit a cell response that is similar to that of the TCP ceramics.

4 Conclusion

A cytocompatibility study[33,34] has shown that cell density, cell viability, and cell adhesion are similar for TCP ceramics and TCP–TiO_2 composites having a core of dense titania and a surface of porous tricalcium phosphate granules.

The goal of the study was not of investigating mechanical properties. However, the fact that dense titania, which is used for making high-performance thread guide,[35] exhibits reasonable

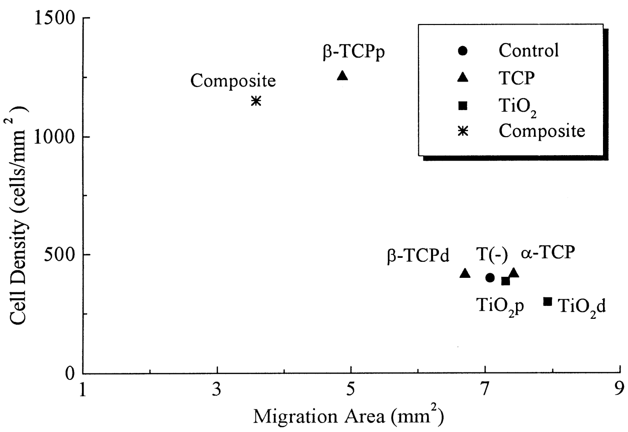


Fig. 11. Growth and migration of bone cells grown for 14 days on the ceramic substrate.

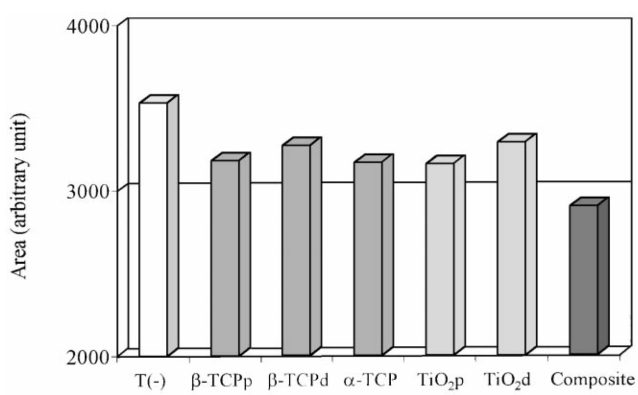


Fig. 12. Adhesion of bone cells grown for 14 days on the ceramic substrate.

toughness (>3 MPa $m^{1/2}$) and strength (>150 MPa) and good fatigue properties, implies that the mechanical properties of the composites must be sensibly higher than those of porous TCP. Moreover, the compatibility of titania with titanium suggests that the composites might offer potential for making ceramic-metal parts.

Acknowledgement

K.-S. O. expresses his warm gratitude for the financial support of RETCAM in relation to his post-doctoral stage in France.

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