JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 1125– 1130

Surface characterization of completely degradable composite scaffolds

M. CHARLES-HARRIS, M. NAVARRO, E. ENGEL, C. APARICIO, M. P. GINEBRA, J. A. PLANELL*
Reference Centre for Bioengineering of Catalonia. Department of Materials Science, Universitat Politècnica de Catalunya, Av. Diagonal 647, 08028 Barcelona, Spain
E-mail: josep.a.planell@upc.edu

The goal of this study was to characterise the surface properties of completely degradable composite, polylactic acid and calcium phosphate glass, scaffolds. The composite scaffolds are made by solvent casting or phase-separation, using chloroform and dioxane as a solvent respectively. The surface properties were measured on composite films which were made using the same procedure as for the three-dimensional (3D) scaffolds without the pore-creating step. The surface morphology, roughness, wettability and protein adsorption capacity of the films was measured before and after sterilisation with ethylene oxide. The results reveal the influence of solvent type, glass weight content and sterilisation on the wettability, surface energy and protein adsorption capacity of the materials. The addition of glass particles increase the hydrophylicity, roughness and protein adsorption capacity of the surface. This effect, however, depends on the extent of the coating of the glass particles by the polymer film, which is much higher for dioxane films than for chloroform films. This information can be used to interpret and understand the biological behaviour of the 3D scaffolds made of this composite materials.

1. Introduction

Three-dimensional scaffolds for Tissue Engineering applications must fulfil many mechanical, structural, chemical, degradation and surface properties in order to be functional [1, 2]. The use of a composite material, which could synergically combine the properties of each of its constituents, may be the winning approach to attain these challenging requirements.

Surface properties such as wettability, surface energy and roughness are known to play a key role in the success of biomaterials in general [3, 4]. The characterisation of composite materials is very complex, however, due to the multiple effects each of its constituent may have on the surface. It is important to understand the surface characteristics of the composite material in order to both interpret and optimise the biological behaviour of the material.

A composite material for biomedical applications has been obtained by combining polylactic acid (PLA) with a soluble calcium phosphate glass [5]. The composite material has been used to make highly porous three-dimensional (3D) scaffolds made by solvent casting and particulate leaching, or phase-separation methods. An important step in the characterisation of these scaffolds is to assess their surface properties. To do so, composite films have been made using the same procedure as for the 3D scaffolds without the pore-creating step.

The goal of this study is to characterise the surface properties of the composite films. Their surface morphology, roughness, wettability and protein adsorption capacity has been measured. This thorough characterisation is necessary in order to analyse the relationship between surface properties and the biological behaviour of a material, as well as offering valuable understanding of the material itself.

2. Materials and methods

2.1. Materials preparation

The surface properties of composite films were measured. The parameters studied were: (a) solvent type: chloroform (C) or dioxane (D) (corresponding to three-dimensional fabrication by solvent casting and phase separation respectively), (b) glass weight percent (wt.%): 0, 20 and 50%, (c) sterilisation with ethylene oxide: sterilised/unsterilised.

Composite films were made of Purasorb (Purac Biochem.) poly-95L/5DL-lactic acid and a titania-stabilised calcium phosphate glass [6]. The soluble glass is of the P_2O_5-CaO-Na_2O-TiO_2 system. In the case of chloroform, 5% w/v of PLA was dissolved in chloroform on an orbital mixer during 48 h. After complete dissolution, sieved glass particles ($<40\ \mu$m) were added at 20 or 50 wt.% and the paste was spread onto

*Author to whom all correspondence should be addressed.

a Teflon sheet and air-dried for 3 days. In the case of dioxane, 5% w/v of PLA was dissolved in a mixture of 95% dioxane and 5% water, under magnetic stirring at 50 °C overnight. Sieved glass particles were added and the paste was spread as with the chloroform. In all cases, special care was taken to identify the face exposed to air, which was then used for the surface characterisation.

Though films are the closest approximation to the pore wall material within three-dimensional scaffolds, their surface irregularity could distort the wettability measurements. In order to assess this effect, a "polishable" material was made by hot pressing films with 0, 20, and 50 glass wt% at 160 °C. The material was then polished finishing with 1 μm diamond paste, and its surface properties were characterised.

Samples were sterilised with ethylene oxide.

2.2. Surface characterisation

For each measurement, 1×1 cm samples were mounted onto glass cover slips using double-faced scotch tape, allowing for easy handling. The unsterilised samples were cleaned by sonication in distilled water for 10 min before each test.

The qualitative morphology of the surface was characterised by Environmental Scanning Microscopy (ESEM), which allows viewing the samples without applying high vacuum or a metallic coating, which could alter surface characteristics. Both the superior and inferior faces of the films were imaged before and after sterilisation.

The surface roughness of the materials was measured with white light interferometry on a WYKO NT1100. The field of view used for the measurements was 604.4 $\times$ 459.9 μm. Three samples of each composition were tested; five measurements were taken per sample. Both the superior and inferior faces of the films were measured before and after sterilisation. The roughness of the polished hot pressed material was measured, for sake of comparison, for compositions with 0 and 50 wt% of glass. The recorded parameters were: the mean spacing between adjacent local peaks over an evaluation length (Sa), the kurtosis, or "peakedness" of the surface about the mean plane (Sku), the skewness, or the asymmetry of the surface about the mean plane (Ssk,), and the Surface Area Index (SAI), or the ratio between the surface area of the sample and the area of the field of view. These parameters were chosen in order to have a complete roughness characterisation including amplitude (Sa,Ssk), spatial (Sku) and hybrid (SAI) roughness parameters.

The wettability and surface energy of the samples were measured using the sessile drop technique on a Dataphysics Contact Angle System OCA15Plus. 3 μl droplets of the measuring liquid were used in an atmospherically controlled chamber at room temperature. Three samples of each composition were tested, with three to eight droplets per sample. The contact angles were measured with ultrapure distilled water, GYBCO's Dulbecco Modified Eagle medium (DMEM) with 10% Fetal Calf Serum (FCS), and diodomethane. Water and diodomethane are a polar and apolar liquid respectively. A polar and an apolar liquid are needed in order to compute the surface energy of the materials by means of their wettability results. The DMEM +10% FCS is a polar liquid; it contains water, proteins, sugars and other organic components. It was used in order to assess the influence of these organic components on the contact angle of the surface. Furthermore, it is the medium the materials will be immersed in during cell culture. The contact angle of the hot pressed and polished materials was measured with ultrapure distilled water.

The results for the contact angles measured with water (polar) and diodomethane (apolar) were used to calculate the surface energy of the films using the following equation:

$$\gamma_{\mathrm{sv}} = \gamma_{\mathrm{sl}} + \gamma_{\mathrm{lv}} \cos\theta$$

Where γ_{sv} stands for the energy of the surface, γ_{sl}, stands for the interfacial tension between the solid and the drop, γ_{lv}, stands for the liquid-vapour surface tension, and $\cos\theta$ is the contact angle of the drop with the surface.

2.3. Protein adsorption

Protein adsorption was measured after 1 h incubation in DMEM with 10% FCS. 6mm diameter discs were cut out of each sample and immersed in 400 μl of DMEM + 10%FCS for 1 h at 37 °C. After incubation, samples were rinsed in phosphate buffered saline (PBS) in order to remove loosely adsorbed proteins, and transferred to clean tubes. The adsorbed proteins were desorbed by adding 200 μl of 5% sodium dodecyl sulphate (SDS) into each tube, the tubes were left overnight at 37 °C. The amount of adsorbed protein was measured with a Bicinchoninic acid (BCA) Protein Assay Reagent Kit (PIERCE). The absorbance data was referenced to a bovine albumin serum standard. The absorbance measurements were made on a KCJunior Spectophotometer at 562 nm.

3. Results

3.1. ESEM

ESEM images of the upper face of the films showed a homogeneous distribution of the glass particles throughout their surface. The inferior faces, in contact with the Teflon sheet, seemed, qualitatively, less rough for the composition with glass particles. The glass particles on the surface of the composite films dissolved in chloroform seemed to be exposed due to the polymer film peeling off the glass particle (Fig. 1(a)). For dioxane-dissolved films, the polymer seemed to wrap around the glass particles better (Fig. 1(b)).

3.2. Roughness

Various roughness parameters of the films and hot pressed materials were measured using white light interferometry. This technique also allows for a morphological evaluation of the surface as can be seen in Fig. 2.

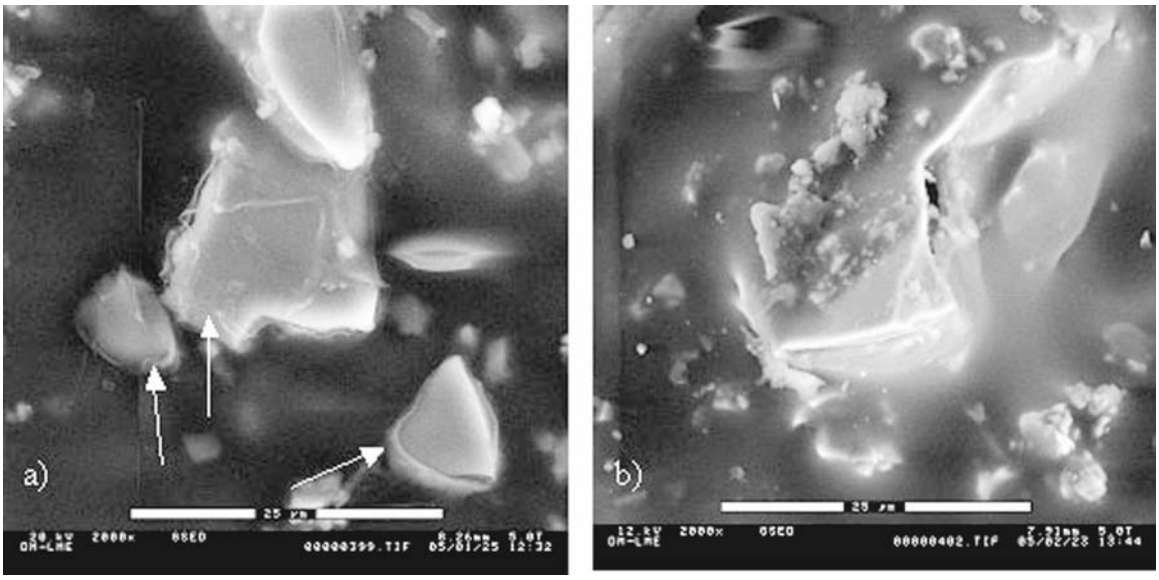

Figure 1 ESEM images of the superior face of composite films dissolved in (a) chloroform and (b) dioxane. White arrows point at glass particles which are not well covered by the polymer film.

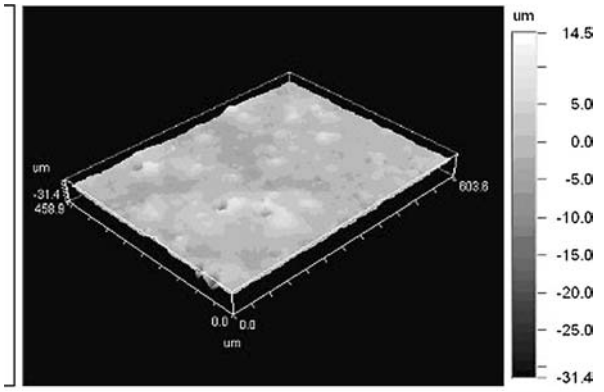

Figure 2 Three-dimensional display of the surface of a film with 20 wt.% of glass dissolved in dioxane obtained by white light interferometry.

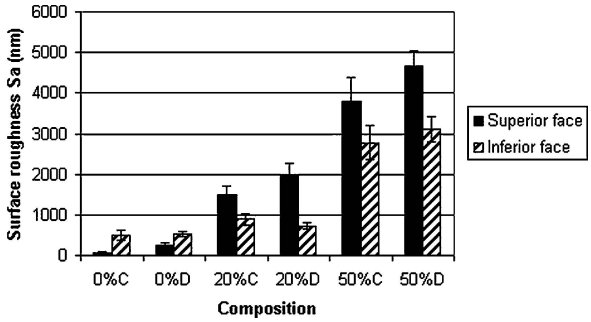

Figure 3 Comparison between the roughness (Sa) of the superior and inferior (in contact with Teflon sheet) of the composite films. The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50% glass weight percent.

The addition of glass particles increased the roughness of all materials (Table I). The films made using dioxane as a solvent had higher Sa than those made with chloroform. The superior and inferior faces of the films had different roughness. For composite films (with glass particles), the face in contact with the Teflon sheet gave lower roughness (Sa) values, whereas for films without glass the inferior face was slightly rougher (Fig. 3), probably due to the roughness of the Teflon sheet. Sterilisation did not have a significant effect on the surface roughness of the films.

3.3. Contact angle

The contact angle results for the films showed different trends for films made using chloroform as a solvent and those dissolved in dioxane. The measurements made using either water or DMEM + 10% FCS, which are both polar liquids, followed similar trends. Contact angles measured with diodomethane, an apolar liquid, showed a different trend.

3.3.1. Results using water or DMEM + 10% FCS as contact liquids

For films made with chloroform, the glass particles increased the hydrophylicity of the films (decreased the contact angle), whereas for dioxane films the contrary occurred, i.e. contact angles increased with increasing glass wt% (Fig. 4). The contact angles measured with DMEM + 10% FCS were lower than those measured with water. Sterilisation decreased the contact angle of

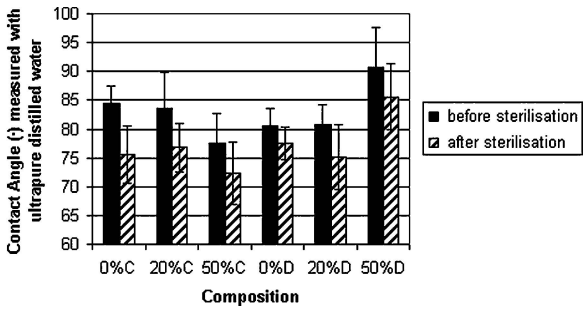

Figure 4 Contact angle values measured with ultrapure distilled water on composite films before and after sterilisation with ethylene oxide. The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50% glass weight percent.

TABLE I Roughness parameters for the composite materials[a]

Composition	Sa (nm)	Sku	Ssk	SAI
0%C polished hot pressed	54 ± 0,01	11.2 ± 8	−0.99 ± 0,7	1.01 ± 0,00
PLA/G5 polished hot pressed	238 ± 0,11	53.1 ± 27	−5.25 ± 2,7	1.09 ± 0,02
0%C film	74.41 ± 32,64	189.16 ± 365,74	−3.36 ± 8,24	1.01 ± 0,01
20%C film	1491.81 ± 217,59	12.36 ± 4,86	0.156 ± 0,564	1.09 ± 0,02
50%C film	3806.71 ± 587,28	5.01 ± 1,10	−0.407 ± 0,45	1.53 ± 0,16
0%D film	253.10 ± 70,77	36.33 ± 54,95	0.28 ± 2,96	1.02 ± 0,03
20%D film	1963.22 ± 318,53	8.91 ± 2,89	0.418 ± 0,56	1.13 ± 0,03
50%D film	4659.56 ± 388,11	3.57 ± 0,51	−0.067 ± 0,30	1.63 ± 0,11

[a]Sa = spacing between local peaks, Sku = kurtosis of the surface, Ssk = skewdness surface plane, and SAI = surface area index.

the films when measured with either water or DMEM + 10% FCS (Fig. 3). This decrease was statistically significant in all cases except for compositions 20 and 50%D measured with DMEM + 10% FCS.

3.3.2. Results using diodomethane

The contact angle of the chloroform films decreased with glass wt%, although there were no significant differences between compositions 20 and 50%C. For dioxane films, the highest contact angle was measured on 20%D. Interestingly, sterilisation tended to increase the contact angle measured with diodomethane, although the difference was only significant for the films dissolved in dioxane.

3.3.3. Comparison between polished hot pressed materials and films

The polished hot pressed materials are much smoother than the films due to the polishing. The polished hot pressed material gave consistently lower contact angles than the films (Fig. 5). The addition of glass particles also increased the hydrophylicity of the hot pressed materials.

3.3.4. Surface energy

Fig. 6 shows the surface energy of the composite films after sterilisation. For the films dissolved using chloroform as a solvent, a higher glass content tends to increase the surface energy. For films dissolved using dioxane, the composition with 50 wt% glass tends to have a lower surface energy than the composition without glass or with 20 wt% of glass. The differences are not statistically significant however. The same tendencies were observed for the unsterilised materials. Sterilisation tended to increase the surface energy of the materials. If the surface energy is decomposed into its dispersive and polar components, the effect of sterilisation can be further analysed. In effect, sterilisation with ethylene oxide tends to increase the polar component of the surface energy of the composite materials substantially (Fig. 7) and tends to decrease the dispersive component of the surface energy slightly.

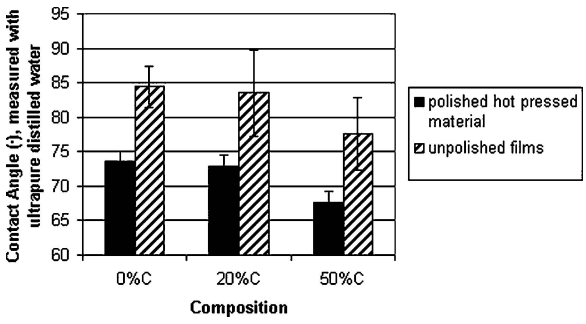

Figure 5 Comparison between the contact angle of the polished hot pressed material and the unpolished films (using chloroform as a solvent). The measuring liquid was ultrapure distilled water.

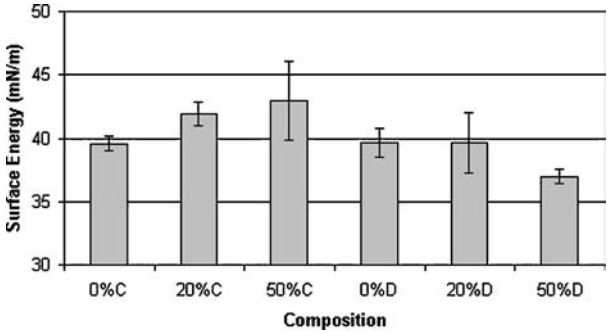

Figure 6 Surface energy of the composite films after sterilisation. The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50% glass weight percent.

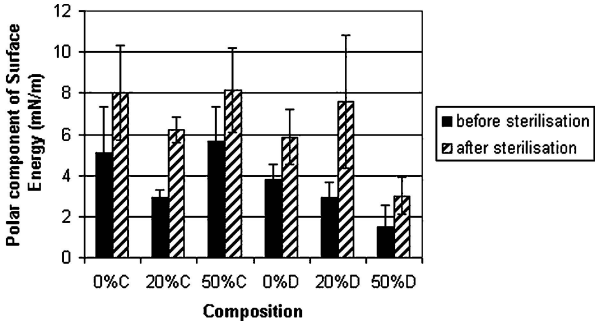

Figure 7 Polar component of the surface energy of the composite films before and after sterilisation with ethylene oxide. The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50 glass weight percent.

3.4. Protein adsorption

Fig. 8 shows the concentration of proteins adsorbed onto the material surfaces. For chloroform and dioxane films, the total amount of adsorbed protein increases with glass wt% significantly. Fig. 9 shows the protein concentration for each composition normalised with the SAI values of the superior and inferior faces of the

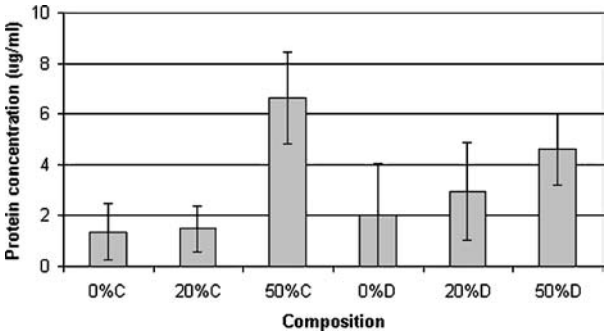

Figure 8 Protein adsorption of the composite materials (μg/ml). The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50% glass weight percent.

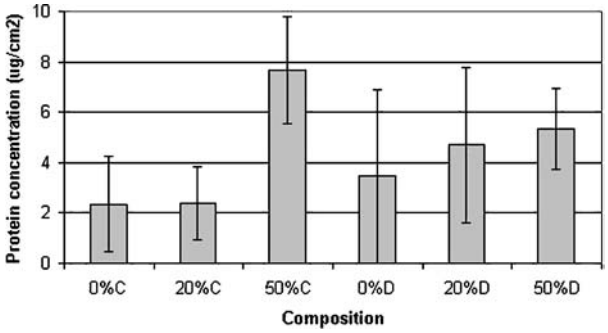

Figure 9 Protein adsorption of the composite materials normalised by their surface area (μg/cm^2). The compositions include films made using chloroform (C) as a solvent, or dioxane (D), and with 0, 20 or 50 glass weight percent.

films. For the chloroform films, composition 50% C has a larger protein concentration. For the dioxane films, however, the differences between the compositions are not longer statistically significant, though they follow the same trend.

4. Discussion

The field of surface characterisation is inherently complex. This complexity stems from sample requirements, the characterisation measurement itself, and the registered parameters. Contact angle measurements can be used as an example to illustrate this fact: (a) Contact angles should ideally be measured on smooth, rigid, chemically and physically inert surfaces, this is often difficult to achieve without altering the surface meaningfully, (b) The accuracy of the results depends on the quality of the surface, the skill of the experimenter, the purity of the measuring liquid and its interaction with the surface, and (c) The contact angle can be measured statically or dynamically, and its value changes with time [7, 8].

The same can be applied to roughness measurements, which require a large range of parameters for adequate characterisation [3, 9–11]. The results listed on Table I exemplify this fact. For a given composition, certain parameters such Sa and Sku, which are very sensitive to outliers in the surface data, have large scatterings, whereas SAI, a hybrid parameter, is very stable.

Materials with different compositions or morphologies further increase this complexity. A change in composition, for example, can affect both the chemistry and the morphology of a surface, and it may also influence the surface energy, heterogeneity or stiffness. Thus, it is important to establish a well-defined protocol in order to obtain reproducible results, and even so, one should expect high dispersion.

In addition to the challenges of surface characterisation, this study is also subject to the irregularity of the films used. The composite films are, however, the closest approximation to the pore-wall material of the 3D scaffold. Lück *et al.* [12] and Jee *et al.* [13] use a similar approach to characterise the surface of microspheres and tissue engineering constructs respectively. In this study, a hot pressed polished material was also characterised in order to compare these results.

The results indicate that the solvent used to make the films seems to determine the coating of the glass particles on the surface of the films (Fig. 1). Although this observation in qualitative, it could explain the different trends in wettability, surface energy and protein adsorption observed between chloroform and dioxane-dissolved films. The degree of coating could be due to the hydrophylicity of the materials involved. In effect, the soluble calcium phosphate glass used in this study is highly hydrophilic (28.9 °). The preparation of the dioxane films involves 5% of water in the solvent mixture. Water is infinitely soluble in dioxane, but only slightly soluble in chloroform (0.02 w/w). These differences could explain why the polymer film dissolved in the water and dioxane mixture is able to coat the superficial glass particles better.

Thus, in the case of the chloroform-dissolved films, most of the superficial glass particles are exposed and therefore influence both the chemistry and the roughness of the surface. The contact angle measurements for these films, as for the hot pressed materials, reflect the hydrophilic effect of the glass particles on the surface (Fig. 5). In the case of the dioxane films, however, most of the glass particles are coated with polymer, which means they contribute mainly to the roughness of the surface, increasing the material's hydrophobicity. This result can be related to Rupp *et al.*'s [14] conclusions on the relationship between roughness and hydrophobicity which states that roughness increases the hydrophobicity of hydrophobic materials, although its interpretation depends on the definition of hydrophobicity. For Rupp *et al.*, in reference to titanium, the limit between hydrophobicity and hydrophilicity lies at 90 °, whereas Vogler [4] defines a hydrophobic material was one with a water contact angle $\theta > 65$ °, in reference to biomaterials in general.

Sterilisation has an important effect on the surface characteristics of the materials (Fig. 4). Both the composite films and the hot pressed material become more hydrophilic by treatment with ethylene oxide. Interestingly, surface energy calculations indicate the sterilisation mainly affects the polar component of the surface energy (Fig. 7). Cell adhesion had been found to depend on different sterilisation treatments [15]

Protein adsorption is related to the surface composition, wettability, charge and roughness [13, 16, 17]. Furthermore, the correlation of surface energy with

biological interaction, including protein adsorption, is often stated in the literature [4, 7], and its polar component is thought to play a role in cell behaviour [18]. In competitive protein environments, such as was used in this study, the concentration and nature of the protein layer on the material is known to change with time in what is known as the Vroman effect [19].

The protein adsorption pattern on the materials can be related to the coating of the glass particles on their surface as well. Before normalisation with SAI (if the concentration is measured in μg/ml) an increase in glass wt% increases the protein adsorption for all film compositions (Fig. 8). For dioxane films, (on which most of the glass particles are coated with polymer) there are no longer statistical differences between the compositions after normalisation with SAI (Fig. 9). For chloroform films, however, the exposed glass particles influence protein adsorption significantly. Thus, protein adsorption seems to be sensitive to the effect of the exposed glass particles, but less sensitive to the effect of the roughness (or to this magnitude of roughness), than other surface characterisation parameters studied.

In any case, cell behaviour depends not only on the nature of the adsorbed protein layer, but also on the surface characteristics below the layer, which in turn affect the conformation and viability of the adsorbed proteins [20–22]. The complete characterisation of the surface properties of these composite materials will be a valuable tool to couple with specific protein and cell culture assays, in order to understand their biological behaviour.

5. Conclusion

A thorough characterisation of the surface properties of composite films has been performed in this study. The results reveal the influence of solvent type, glass weight content and sterilisation on the wettability and surface energy of the materials. The addition of the soluble calcium phosphate glass changes both the morphology and the physico-chemistry of the surface of the material and affects protein adsorption. The results include a degree of scattering inherent to the characterisation of rough and irregular surfaces. Despite this fact, this information can be used to interpret and understand the biological behaviour of the three-dimensional scaffolds made of this composite material.

Acknowledgments

The authors wish to thank the Catalan Government for their funding (Grant 2003FI 00422). Special thanks to M. Pegueroles for her helpful advice and guidance, and to Klockner implant system for the sterilisation treatments.

References

1. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
2. L. C. LU and A. G. MIKOS, *Mrs Bulletin* **21** (1996) 28.
3. A. F. VON RECUM *et al.*, in "Handbook of biomaterials evaluation. Scientific, Techincal and Clinical Testing of Implant Materials" (2005).
4. E. A. VOGLER, *Advances in Colloid and Interface Science* **74** (1998) 69.
5. M. NAVARRO *et al.*, "Development of a biodegradable composite scaffold for bone tissue engineering: Physico-chemical", (topographical, mechanical, degradation and biological properties, 2006)
6. M. NAVARRO *et al.*, *J. Am. Cera. Soc.* **86** (2003) 1345.
7. B. D. RATNER, in "Biomaterials Science" (Elsevier Academic Press, 2004) 40.
8. LAM C.N. *et al.*, in "Handbook of applied surface and colloid chemistry" (John Wiley and sons, Ltd., 2001) 251.
9. K. J. STOUT and L. BLUNT, in "Surface Topography" (Penton Press).
10. W. P. DONG *et al.*, *Wear* **159** (1992) 161.
11. K. J. STOUT and L. BLUNT, *Surface and Coatings Technology* **71** (1995) 69.
12. M. LUCK *et al.*, *J. Control Release* **55** (1998) 107.
13. K. S. JEE *et al.*, *Biomacromolecules*. **5** (2004) 1877.
14. F. RUPP *et al.*, *Biomaterials* **25** (2004) 1429.
15. K. ANSELME, *ibid.* **21** (2000) 667.
16. P. Q. YING *et al.*, *Colloids and Surfaces B-Biointerfaces* **32** (2003) 1.
17. C. D. MCFARLAND *et al.*, *J. Biomed. Mater. Res.* **49** (2000) 200.
18. L. PONSONNET *et al.*, *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* **23** (2003) 551.
19. C. D. MCFARLAND *et al.*, *J. Biomed. Mater. Res.* **44** (1999) 1.
20. J. W. LEE *et al.*, *Biomaterials* **25** (2004) 1901.
21. *idem.*, *J. Biomed. Mater. Res.* **41** (1998) 422.
22. R. F. LENZA *et al.*, *J. Biomed. Mater. Res.* **67A** (2003) 121.

Received 1 July
and accepted 26 July 2005