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Biocompatibility tests with fibroblasts of CaO rich calcium silicate glasses

M. CATAURO, M. G. RAUCCI
Department of Materials and Production Engineering, Piazzale Tecchio, 80125 Naples, Italy
E-mail: catauro@cds.unina.it

M. A. CONTINENZA
Surgical Sciences Department, University of L'Aquila, Italy

A. MAROTTA
Department of Materials and Production Engineering, Piazzale Tecchio, 80125 Naples, Italy

It is known that $CaO\text{-}SiO_2$ glasses are bioactive and able to bond to living bone [1]. As reported in the literature [1–4], the essential condition for glasses and glass-ceramics to bond to living bone is the formation of a bone-like apatite layer on their surfaces. *In vitro* studies are performed [1, 2] by soaking the glasses in a simulated body fluid (SBF) to study the hydroxyapatite formation on the surface. Biocompatible materials are those materials that can bound *in vitro*, or better *in vivo*, to living cell or tissue [5–7].

The biocompatibility of biomaterials is very closely related to cell behavior on contact with them and

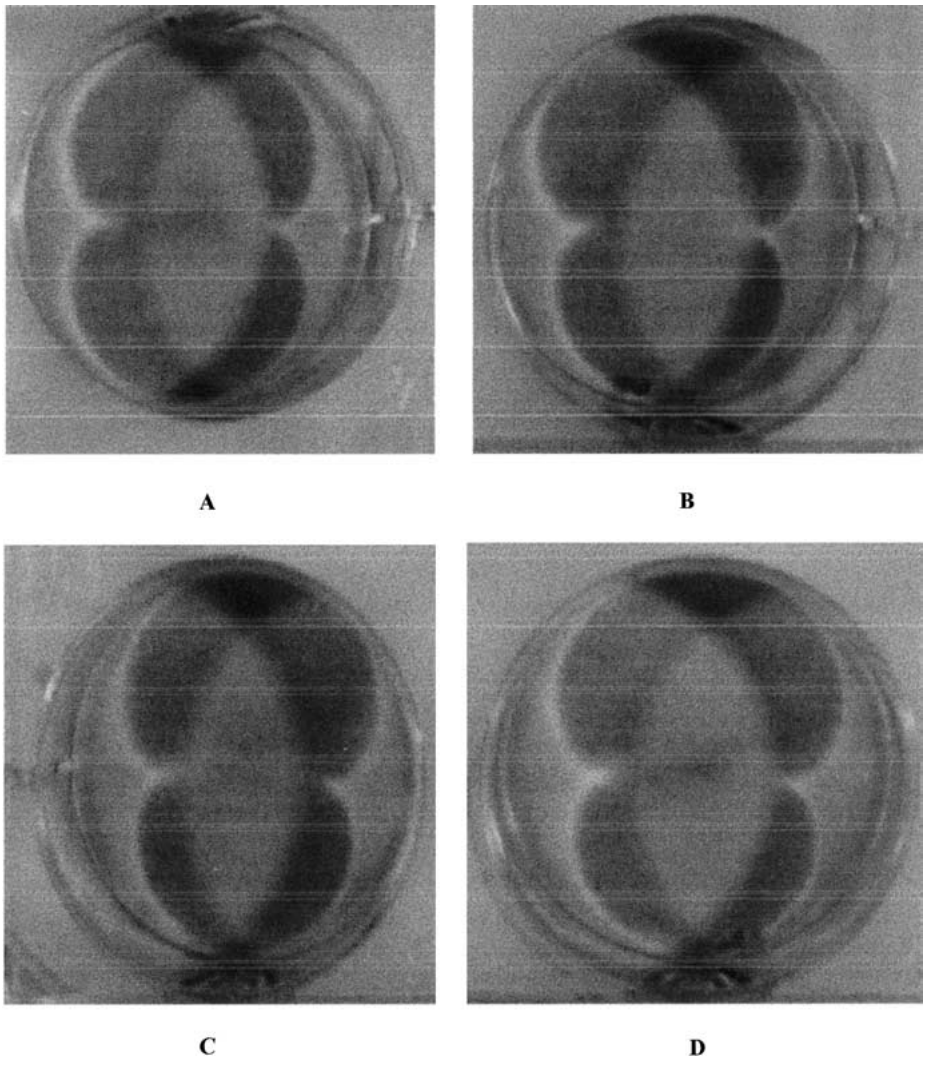

Figure 1 Macrophotography of cultures at the 14th day from seeding. A: control dish; B: dish containing $x = 0.40$ glass fragment; C: dish containing $x = 0.50$ glass fragment; D: dish containing $x = 0.60$ glass fragment. In all dishes cell monolayer was continuous and well stratified.

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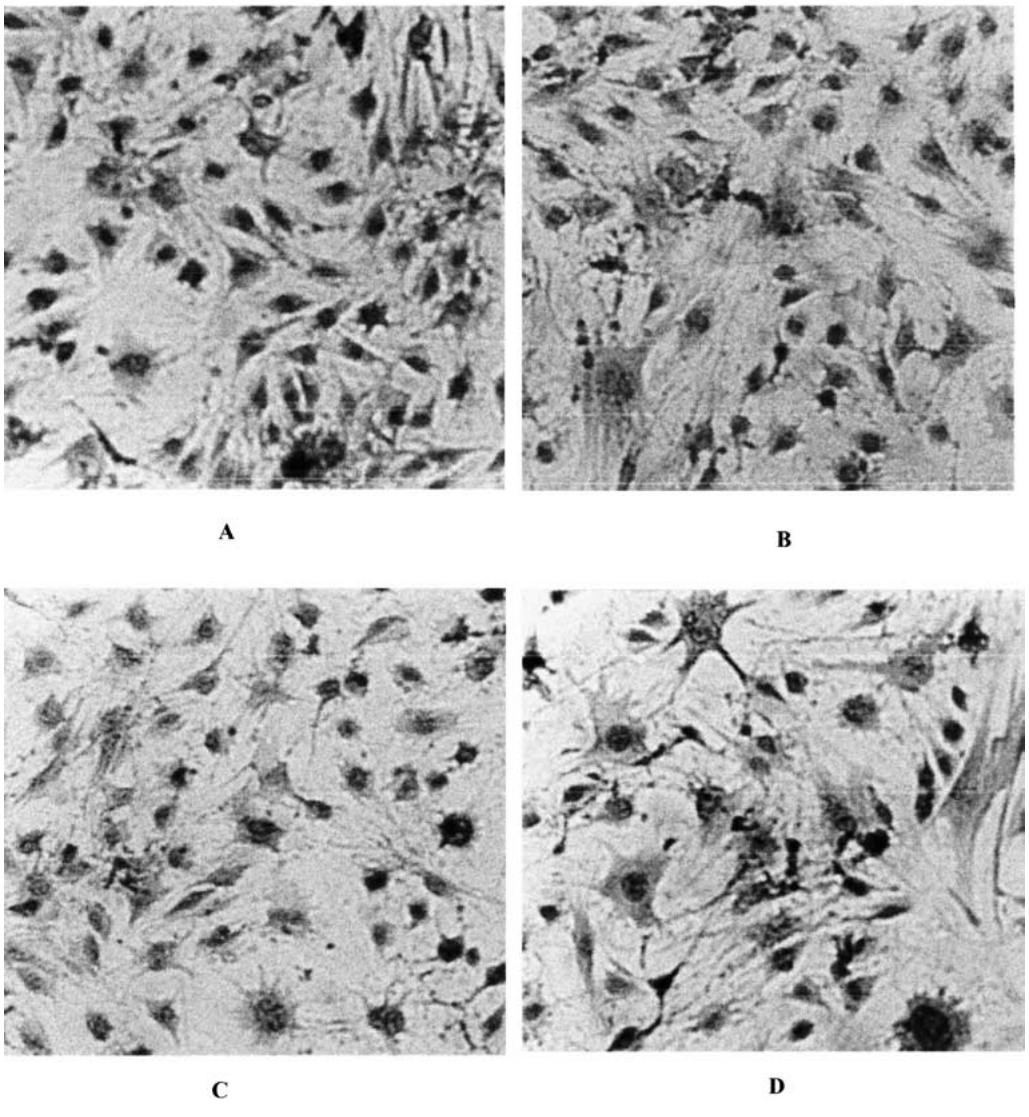

Figure 2 Light microphotography of fibroblast monolayer 7 days after seeding (100× magnification). A: control dish; B: dish containing $x = 0.40$ glass fragment; C: dish containing $x = 0.50$ glass fragment; D: dish containing $x = 0.60$ glass fragment. In all dishes cell growth was regular and fibroblast morphology was well preserved.

particularly to cell adhesion to their surface. Surface characteristics of materials, whether their topography, chemistry or surface energy, play an essential part in fibroblast adhesion on biomaterials.

In the present preliminary work a series of *in vitro* biocompatibility test on CaO rich calcium silicate glasses, was performed, to study the cell behavior when seeded on 1 cm^2 material fragments, introduced inside an *in vitro* culture system. A strain of physiologically adherent cells, like fibroblasts, was deliberately selected to facilitate cell attachment to the materials.

The glass compositions are expressed by this general formula:

$$x\mathrm{CaO} \cdot (1 - x)\mathrm{SiO_2}$$

with $x = 0.40$; 0.50; 0.60. In the course of the experiments, each glass will be named by the corresponding x value. The glasses were prepared by mixing appropriate quantities of ultra pure calcium carbonate and silica in a batch sized to yield 3 g of glass. The glasses were melted at 1500 °C for 15 min in an uncovered Pt crucible in an electric oven. The crucible containing the glass was weighed both before and after the glass removal and the weight of the glass agreed with that anticipated from the batch calculation. This result indicates that the actual glass composition is close to that based on the glass batch. The melts were quenched by plunging the bottom of the crucible into cold water. Although this resulted in fracture of the obtained glass, for all the compositions many pieces of transparent glass, sufficient in size for the experimental measurements, were obtained by this technique.

Physiologically adherent cells, like fibroblasts, were extracted from calvaria periosteum of newborn Wistar rats. Tissue fragments were softly digested by trypsin in a soft shaking water-bath and, after a double washing, the obtained cell pellets were seeded and incubated in DME medium supplemented with 10% Foetal Calf Serum and Antibiotic-Antimicotic solution. Cells were stored in a 95%air/5%CO_2 incubator at 37 °C in a humidified atmosphere, until confluence. After three passages for clone expansion, cells were seeded onto the different glass fragments, directly introduced inside the culture dishes (NUNC) after their sterilization in autoclave with a program of 20 min at 120 °C. All the experiments were performed in triplicate, as prescribed

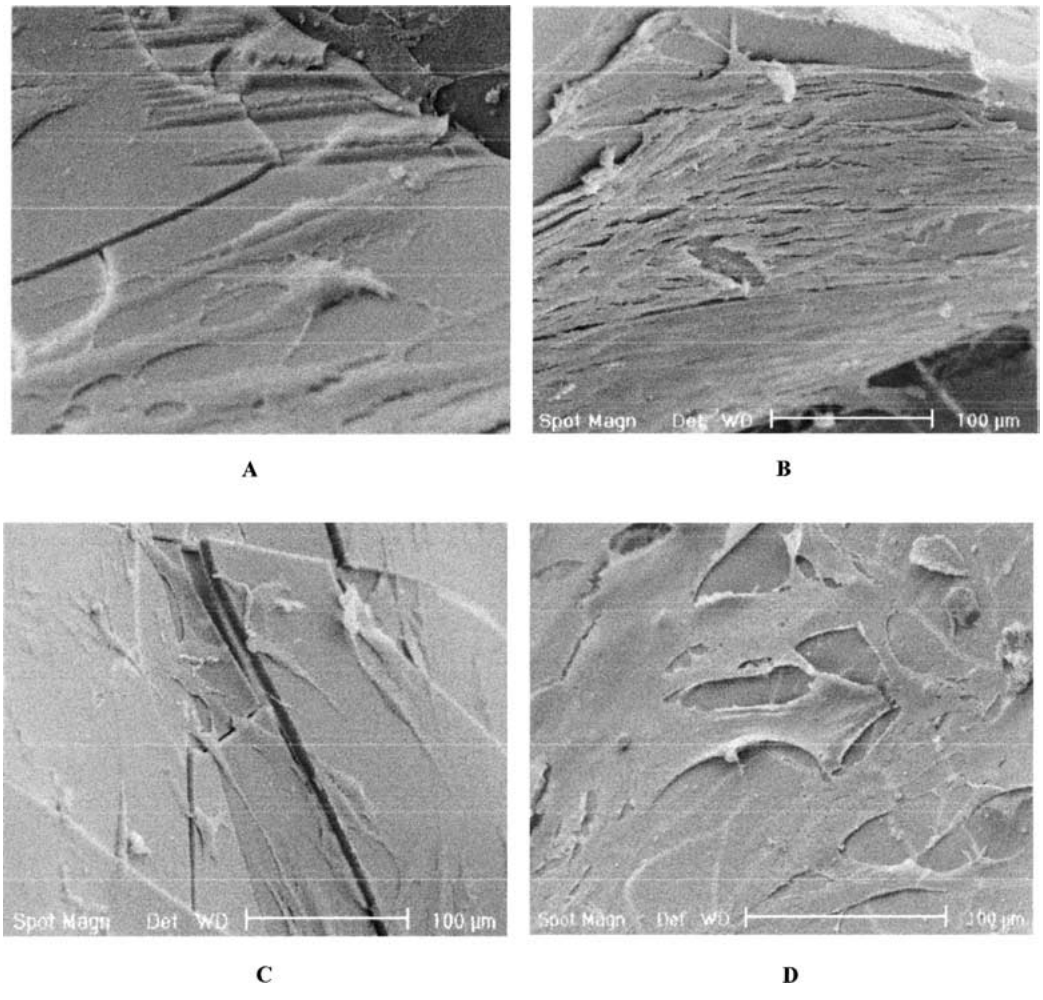

Figure 3 SEM microphotographs of glass fragments recovered from cultures. A: after 7 and B, C, D after 14 days from seeding. A and B: $x = 0.40$; C: $x = 0.50$; D: $x = 0.60$ glass fragments. Fibroblast grew on the glass surfaces as well as in the dish floor, maintaining their flat and star-like shape until the end of the protocol (B, C and D).

by international ISO protocols. The biomaterials containing cultures were monitored daily by inverted light microscope (LM) observations, and the selected incubation times were 3, 7, and 14 days after seeding. At the end of each experimental set, the cells attached to the dish bottom were fixed and processed separately from that adhering to the biomaterial surfaces. For the light microscopy, the cell monolayers were MayGrunwald-Giemsa colored and observed by Leitz Laborlux microscope at 10×, 20× and 40× magnifications. For SEM observations, glass fragments recovered from cultures were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, glued onto stubs, critical point dried, gold sputter coated, and finally observed by a Philips XL30CP microscope.

The Fig. 1A–D illustrate the results obtained by the morphological analyses. Cells showed a very good compliance toward all tested biomaterial fragments, growing in the glass containing dishes as well as in the controls. Six hours after seeding, fibroblasts normally attached to dish bottom, showing their normal star-like shape, spreading and growing very well. At the 3rd and 7th day, in spite the presence of glass fragments, the LM analysis (Fig. 2) showed that cells maintained their conventional shape, forming a well stratified monolayer with minimal disruptions at the biomaterial-frayed points. This behavior remained until the 14th day of incubation (Fig. 1), without any other morphological alteration or irregularity. The SEM study of the glass fragments recovered from cultures at each expiry date of the protocol, showed that fibroblasts could easily attach to the glass specimens and had spread over their surfaces (Fig. 3A–D).

This study has shown that the *in vitro* biocompatibility of all tested biomaterials is very good because the glass is well tolerated inside the dishes and it does not influence cellular attachment and spreading.

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