CELL-MATERIALS INTERACTION: MORPHO-FUNCTIONAL BEHAVIOUR

M. MATTIOLI BELMONTE,[1] G. BIAGINI,[1] F. GRECO,[2] N. SPECCHIA,[3] M.G. PONZI BOSSI,[4] L. GOBBI,[4] F. RUSTICHELLI,[4] C. RIZZOLI[5]
[1] Inst. of Normal Human Morphology, University of Ancona, Italy.
[2] Orthopaedic Clinic, University of Ancona, Italy.
[3] Inst. of Physical Science, University of Ancona, Italy.
[4] Orthopaedic Clinic, Catholic University, Rome, Italy.
[5] Inst. of Histology and Gen Embriology, University of Bologna, Italy.

To date, the capacity of cells to bond with biomaterials appears to be based on the ability of these materials to form a dynamic interfacial zone. So several investigators are carrying out "in vivo" studies on the formation of interfacial structures between the implant surface and the bone, mainly in orthopaedics and dentistry. Also cell culture models are important instruments for investigating interface reactions, and they may be used as a first screening for biomaterials. In particular the "in vitro" systems are suitable models for investigating bone-biomaterial interactions.

With regards to our experience, we have carried out "in vitro" studies on the biological behaviour of dental materials for implants and prosthesis applications. Furthermore we investigated the "in vitro" characteristics of ceramics and composites, focusing our attention on the biocompatibility and toxicity of these biomaterials in relation to various cell types.

Much work has been carried out on the cyto-toxicity and bio-compatibility of materials used in dentistry but there has been relatively little investigation correlating the actual composition of these materials and their interaction with "in vitro" cells. Our intention was to evaluate the components and the bio-compatibility of some of the materials commonly used for dental implants and prostheses. Microanalysis and scanning electron microscopy were used to study ultrastructural aspects and the chemical structure of the following materials: HLA3, SUSTAIN, DECOL IM, MI, BO, CH, RHEIN, HERA BOND, HERA-SG, PAGALINOR, TITACROM, STELLITE, DA, DEI e VALIANT. Gum tissue fibroblasts were also cultivated on samples of each of these to study their bio-compatibility. Our observations confirm the bio-compatibility of some Titanium alloys and also demonstrate how noble alloys such as HERA-BOND and HERA SG are able to encourage cell growth. STELLITE and PAGALINOR also turned out to be bio-compatible while the lack of cell growth on TITACROM could be ascribed almost in part to the relative instability of the mate-

rial and consequent liberation of toxic ions. As far as the material composition analysis is concerned, in some cases the actual composition did not agree with that provided by the manufacturer. This could have been due to a problem in the sampling and/or the product not having been stored properly.

Ceramics and composites are also attracting interest as supports for bioreactors to enhance cell proliferation and to develop efficient tools for large scale production of biomedical molecules [1-3].

The aim of our work was to evaluate "in vitro" the biocompatibility of ceramics and composites (glass-ceramics) which bind themselves to the cells by means of chemycal bonds, mainly corderite and hydroxyapatite and the possibility of using ceramics supports for bioreactors to guest mammalian cells, specialized too. Ceramic materials were assayed "in vitro" with fibroblasts, melanoma cells A2058. Lewis lung carcinoma cells BC215 and CHO, in order to evaluate biocompatibility and proliferative induction, and to obtain information about their suitability as model supports for the production of specific therapeutic compounds. Ceramics were sterilized at 140° C for 90 min, inoculated with the cells and incubated at 37° C for 5 days. Colture assays were followed by SEM analysis: specimens were fixed in 2% glutaradehyde in 0.1 M cacodilate buffer, postfixed in osmium tetraoxide, deydrated and gold coated.

Morpho-structural results that arose from experiments were deduced through microscopic observation with phase contrast microscope and with scanning electron microscope. A satisfactory cell growth was observed on the rough walled corderite (Fig. 1); microporosity was found to be important for growth. Cell growth was found to be satisfactory also on hydroxyapatite, whilst it was scarce on some other ceramics and glass composites. On the surface of corderite, magnesium, alluminium and silicon cations may interact with hydrophilic parts of the cell membrane. Adhesion of cells to the support surfaces takes place via chemical bond with the membrane proteins and is modulated by the combination of the Z potential of the ceramic with the membrane potential. Hydrophilicity of the inorganic surface is important for good adhesion and proliferation [4]. The hydrophilicity may be altered by the serum proteins present in the culture medium wich also tend to be abdsorbed. Cell-coated hydroxyapatite may undergo corrosion, as revealed by the SEM analysys (Fig. 2), in agreement with literature data [5, 6]. The resorption of inorganic material by cell elements, particularly osteoclasts, takes place spontaneously with no addition of particular factors (such as osteocalcin) to the growth medium. Hydroxyapatite appears therefore to be less suitable than corderite for setting up machinery structurally stable with the passing of time, as a bioreactor should be. Corderite in fact is more inert as far as corrosion is concerned, and its permanence in the culture media with consequent cell adhesion does not seem to modify submicroscopic structural organization.

Ceramics manufactured at high reaction temperatures are very stable mate-

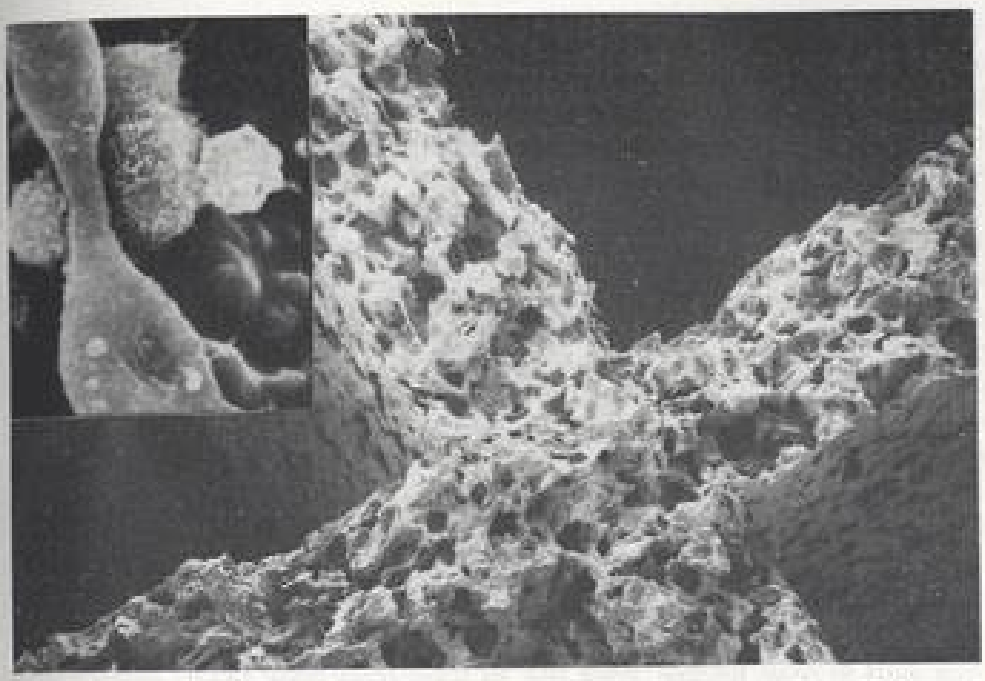

Fig. 1 - Low magnification of fibroblasts cultured on corderite (SEMx50). Inset: High magnification of BC213 cells growth on corderite (SEMx2096).

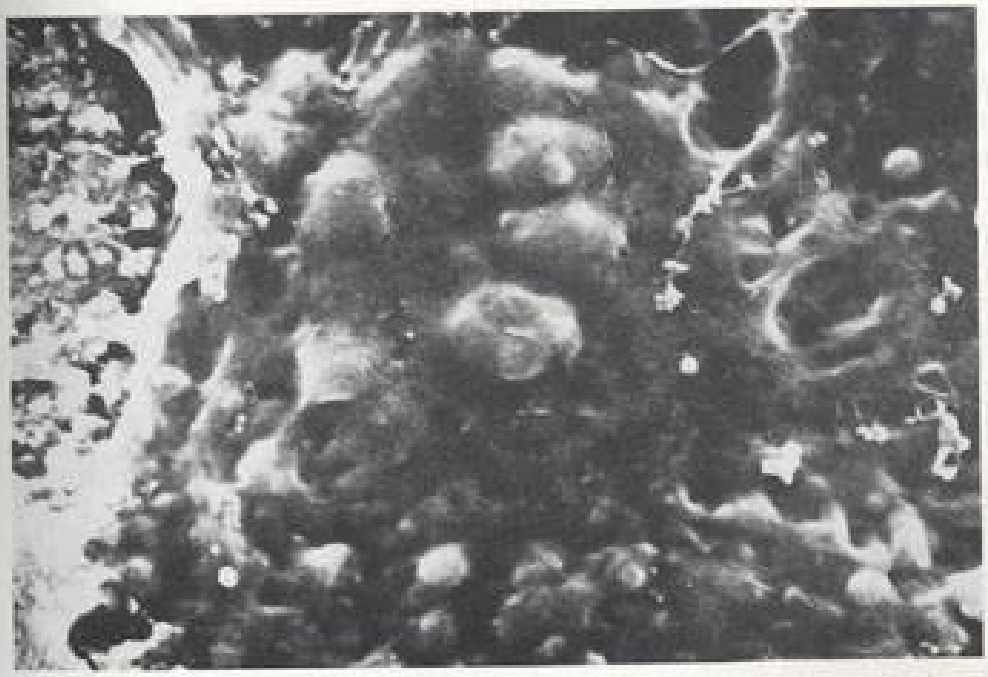

Fig. 2 - Fibroblasts cultured on hydroxyapatite: cell seems to swallow little residual of ceramic material (SEMx4000).

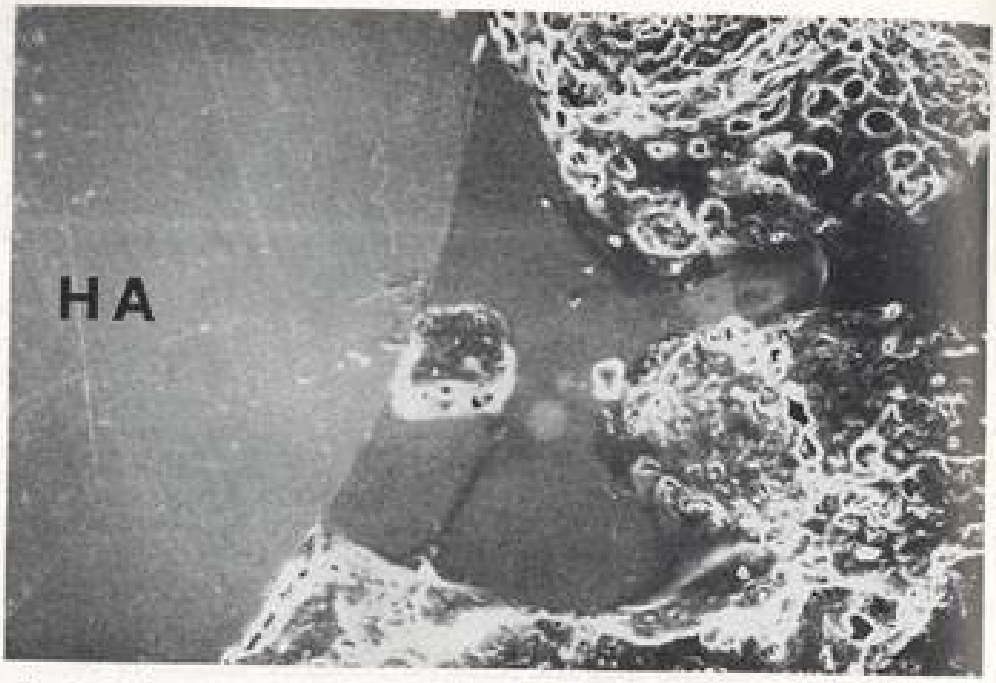

Fig. 3 - Hydroxyapatite implant: high magnification of boneceramic interface (SEMx960).

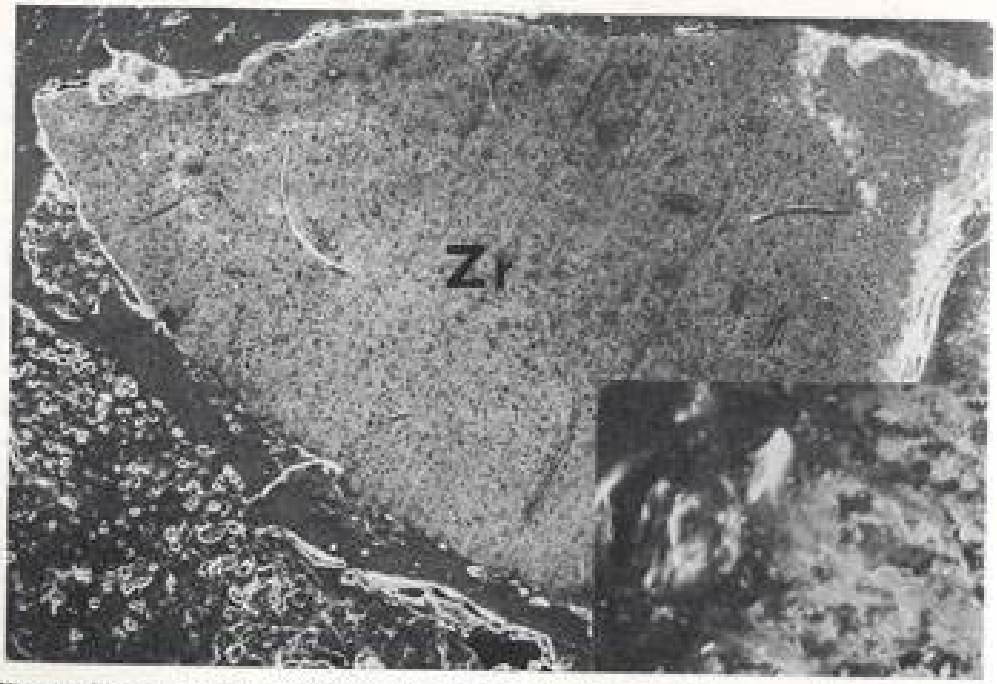

Fig. 4 - Zirconia implant (SEMx30). Inset: high magnification of bone-ceramic interface with aspects of osteo-integration (SEMx2400).

rial and considering ceramics as a potential biomaterial it is important to underline that these materials remain stable and inhert when placed in the body. Application of such materials are described for cardiovascular orthopaedic and dental purposes. Bio-inert ceramics such as Allumina-oxide are materials that do not provoke foreign body reaction when they are in close contact with the implant. Zirconia is another example of inert ceramics.

Our "in vivo" studies on ceramics such as Hydroxyapatite on animal models show very positive bio-physicochemical behaviour of the implanted prototypes, with aspects of osteointegration (Fig. 3). Prototypes of plaques for osteosynthesys made with hydroxyapatite were manufactured and subsequently tested. The utilized powders based on hydroxyapatite were prepared according to a new method of mechano-chemical synthesis [7]. This method showed to be decisive in obtaining ceramics products more mechanically resistant in respect to the ones prepared by traditional methods.

Finally, we have carried out studies in order to evaluate the biological behaviour of sintered Zirconia. As far as bone implantation is concerned sintered block of Calcium Partially Stabilized Zirconium Oxide (Ca-PSZ) were implanted in the proximal tibial metaphysis in adult NZW male rabbits. 1 month after surgery zirconia blocks were surrounded by soft tissue without signs of focal acute toxicity and inflammatory processes. The implant were completely surrounded by hard tissue at 6 and 12 months. Flourescence microscopy of undecalcified tetracycline-labelled specimens demonstrated at 3 months the osteogenetic activity around the implant, while polarising microscopy revealed the presence of no lamellar bone. At 1 year bone growth achieved the integration of the ceramic as also evidenced by X-ray microanalysis. At this stage no fibrous intervention was observed at the bone implant interface, but bone directly bonding zirconia was present (Fig. 4).

These results will allow us to increase the possibility of accelerating technological transfers from research laboratories to industry.

REFERENCES

[1] Int. Rev. Cyt. 1992; 142: 145.
[2] Blood. 1993; 82: 378.
[3] J. Cell. Biochem. 1993; 51: 274.
[4] Biomaterials 1993; 1/2: 29.
[5] Biomaterials 1991; 12: 253.
[6] Clin. Orthop. 1981; 157: 259.
[7] J. Mat. Sci. 1994; In press.