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Ceramic support for cell cultures

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Research was carried out on the use of ceramics as supports to host mammalian cells. The research was part of a programme whose priority was to study the possibility of using ceramics in the non-traditional sector of the biomedical field. The aim of the study was also to verify the suitability of particular types of ceramics dealt with in the literature for these applications. Among the different samples tested the cordieritic one proved to be very interesting, at least in relation to the cellular cultures considered. The chemical composition of the material is not, however, the only important aspect, since other parameters concur to make the hosting of cells highly acceptable. Of particular importance is the rugosity and porosity of the surface and its flaking, not only externally, but also in the pores.

1. Introduction

A wide number of different experiments have demonstrated the ability of ceramic materials to host cell colonies. The targets connected with this use are many: growth of specific cells lines, diagnostic checking procedures, production of chemicals, production of pharmaceutical principles with bioreactors [1–3], production of filtering devices including biochemical dispensers [4] temporarily or permanently implanted in the human body.

A comparative study was carried out on different ceramics in order to look for and select those with the best hosting performances. The study dealt with the physico-chemical micromilieu after cell insemination. The substances examined in this first study and used to prepare suitable ceramic materials were alumina, hydroxyapatite, bioglasses and cordierites.

A further step consisted in studying ceramics prepared in different ways (different treatments of powders, different shaping methods, different thermal treatments, etc.) once the most suitable substance was singled out.

For all materials, particular care was devoted to examining the influence of porosity, its texture, and the intrinsic nature of the ceramic in relation to cytopathologic results. Further physico-chemical measurements, e.g. concerning the fluid motion inside the porous body of the ceramic, will be carried out on the ceramic material showing the best biological performance in terms of growth of cell colonies. The ceramic matrix provides a large surface in a small volume, particularly for bioreactors, thereby minimizing the flexural strength on cells so that a serum-free medium can be utilized. A non-porous ceramic is utilized also in the case of cellular harvesting, when either tripsinization or cold shock and pH variations are necessary. Cultures in ceramic supports ensure the best cellular increase, higher metabolic activity, and increased general productivity in the case of a reactor [5–7].

Part of this research was devoted to a systematic study of *in vitro* cultures to compare different kinds of ceramic materials (biological glasses included) in order to locate ceramic substrates with good cell-hosting capabilities. To this aim [1], evaluations were made of the physico-chemistry of the ceramic [2], the best suitable texture [3], the possibility of ion exchange with the walls of the ceramic pores [5], and the cell types to be used for these preliminary cultures.

2. Materials and methods

The ceramics used to test cellular biocompatibility *in vitro* were sterilized in a dry atmosphere in an oven at 140 °C for 90 min and were subsequently put into small wells of 1.2 cm diameter on slabs of Greiner's plastic. Each ceramic sample was tested four times with the three cell types indicated in Table I, together with the culture medium.

50 000 cells ml^{-1} were seeded for each wall containing the material to be tested. The cultures were subsequently kept in a thermostatic device at 37 °C under

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TABLE I Utilized cells lines and culture media

Cell line	Culture medium
Fibroblasts from human skin esplantation	IMDM + 10% FCS + AM
Tumoral cells BC215 from Lewis lung carcinoma	Waymouth medium + 10% FCS + 0.1% *l*-glutamina + AM
Melanoma cells A 2058	IMDM + 10% FCS + AM

IMDM = Iscove's Modified Dulbecco Medium; FCS = Fetal Calf Serum; AM = Antibiotic Mixture constituted with 200 U/ml penicylline and 200 μM/ml streptomicyne.

a CO_2 5% atmosphere for 5 days. Before any seeding, cells were tripsinized in a trypsin-EDTA 0.25 M solution in PBS and, after centrifugation at 9000 rpm for 10 min to remove the trypsin, they were tested with Tripan Blue to evaluate their vitality and were counted in Nauebauer's camera. Before being prepared for scanning examination (washings with PBS, fixation with glutheraldheyde 2% in a cacodilate buffer at pH = 7.4), the seeded cells on the material to be analysed for biocompatibility were observed by phase contrast microscopy. The cell lines used were cultivated ones (cutaneous fibroblasts, A 2058 melanocytes tumoral cell line and BC 215 Lewis lung carcinoma cell line). Compatibility evaluations were normally carried out after 5 days of culture. For submicroscopic evaluation, specimen were fixed with 2% glutaraldheyde in 0.1 M cacodylate buffer at pH = 7.4, postfixed in OsO_4 1% always in cacodylate buffer, dehydrated in increasing ethanol concentration and critical point dried in a CO_2 solid environment, and examined by scanning electron microscope (SEM).

3. Results and discussion

The morpho-structural results of the experiments obtained by microscopic observations using phase contrast microscopy and SEM, are collected in Table II. Cell adhesion of a material is influenced by a series of parameters such as the mutual combination of the electrostatic load of the ceramic surface (Z-potential) with that of the cell membrane (membrane potential), or the weak chemical interaction between the components of the ceramic materials occurring at the surface and functional groups of the proteic molecules of the cellular membrane. Microporosity has been shown to be a fundamental factor for cell proliferation, although to a different extent for each cell line, particularly for the cordierite coded DF (Fig. 1). A common parameter is the hydrophilic degree of the material surface on which cells must achieve good adhesion and proliferation [8].

The hydrophilic behaviour may be influenced by serum occurring in the culture medium, because adsorption of the serum protein may condition subsequent cell adhesion and proliferation. A moderately hydrophilic substratum appears generally to be the best [9, 10] because serious proteins are adsorbed with weak bonds and are therefore more easily moved by adhesive proteins (e.g. fibronectin) produced by the same cells. Cells reply to physical stimuli with alterations in their biology and biochemistry, and the degree of adhesion of a cell to a substrate will condition the shape as well as structural and functional organization of the cell itself [11]. This takes place through generation of intracellular second messengers (cAMP, phosphoinositides, etc., also influenced by Ca^{2+}). Adhesion takes place by formation of specific specialized membranes which, anchoring themselves to the substrate, develop a structure called "focal contact" [12]. Among the principal cellular translucers, the ionic non-selective channels and the selective ones for specific ions such as Ca^{2+}, Na^+ and Cl^- are mentioned. Strong interactions with extracellular supports may gives rise to a phenotypic transition [13]. The frequency of cell proliferation and division may increase with the degree of adhesion to a substrate. A wide anchoring of a cell to a substrate with remarkable cellular spreading may activate a Na^+/H^+ exchange with alkalinizations of the cytoplasm and increased rates of DNA, even if the ratios among extracellular matrix, proteic bonds, cell deformation, alkalinizations and DNA synthesis do not have an absolute valence because they have not yet been clearly defined [2, 3].

The possibility that the nature of the substrate may induce different phenotypical modulations is highly important for a planned target, not only on a quantitative cell-ingrowth level, but also in order to create conditions favouring an up-regulation of the cell

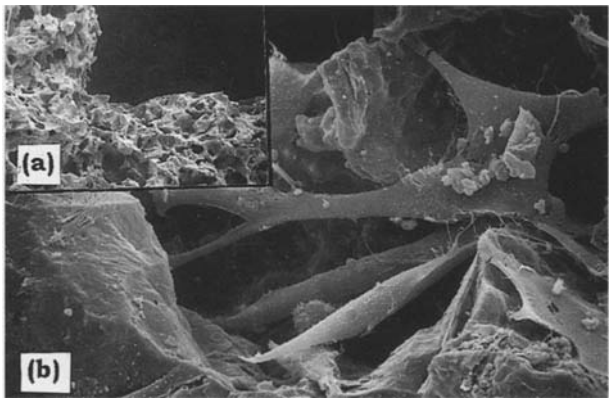

Figure 1 SEM micrograph of fibroblasts cultured on cordierite DF: (a) low magnification (×31); (b) elongated cells tend to go into a hollow of the ceramic (×775).

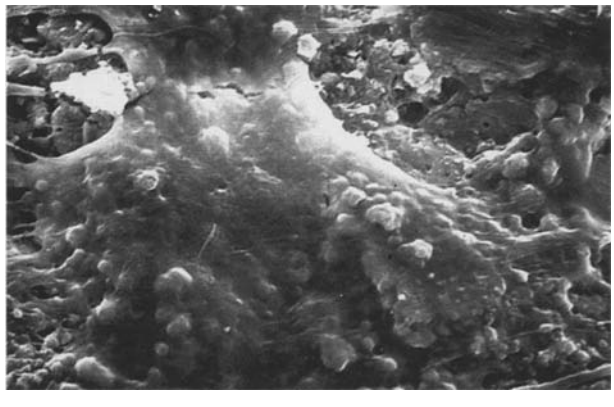

Figure 2 High SEM magnification of fibroblasts cultured on hydroxyapatite. Note that cells seem to swallow little residual ceramic material (×2500).

TABLE II Morpho-structural results (FC = cutaneous fibroblastic cells; M = melanocite tumoral cells: line 2058; BC = Lewis lung carcinoma cells: line 215)

Material	Physical variations after sterilization	Type of cell culture	Observation by phase contrast microscopy after 5 days	Observations by SEM	General classification
Biological glass AP40		FC	Adhering cells confluent all around the sample, no debris		FC +
Biological glass RKKP		FC	Both cells types are confluent all around the sample, no debris		FC + ±
		M A2058			
Hydroxyapatite granules		FC	Adhering cells confluent all around the sample, no debris		FC +
HA 7	turned azure	FC	Both cells types are confluent all around the sample, no ceramic debris		FC+ + + ±
		M A2058			
HA 8	turned azure	FC	Both cells types are confluent all around the sample, no ceramic debris		FC + + +
		M A2058			
Cordierite ST/EKB	more brittle	FC	No adhering fibroblasts, many dead cells, much ceramic debris	Few not adhering globose	BC +
		BC 215	Few surviving and suffering cells	cells	FC ± −
		M A2058	Formation of cells agglomerations, many dead cells	−	M ± −
Cordierite EX22		FC	Not confluent living fibroblasts but suffering	−	FC + ±
		BC 215	Many dead cells	Not completely adhering	BC + +
		M A2058	Agglomerates of dead melanocite cells	globose BC and M cells	M ± −
Cordierite DF with smooth surfaces	more brittle	FC	Not adhering scattered cells, much ceramic debris		FC + + +
Cordierite DF treated with HF 2%	more brittle	FC	Both cell types are confluent all around the sample, no ceramic debris		FC + ±
		M A2058			M ±
Cordierite DF with rough surfaces		FC	Adhering confluent fibroblasts, a few suffering	−	FC + +
		BC 215	Well over the confluency	BC and M cells are strongly	BC + + +
		M A2058	Well over the confluency	adherent to ceramic	M + + ±

+ + + good; + + fair; + scarce; ± insufficient (or half step of addition); − negative.

metabolism, determining an eventual higher secretion of cell-synthesis products of interest. From the results it was ascertained that cell ingrowth on substrates of bioactive ceramics, such as hydroxyapatite and biological glasses, which is in itself spontaneously possible with no need to add particular factors to the culture liquid [14], might be impaired by the microerosion processes that reduces the stability of the substrate. In the case of the hydroxyapatite substrate (Fig. 2) a characteristic behaviour is observed as a result of corrosion due to exposure to the simulating body fluid (SBF), a behaviour which, even if negligible from the point of view of cracking interaction, is nonetheless deleterious for cell adhesion because the substrate crumbles giving rise to non-adhering "slurry". In the case of bioactive glasses a similar event takes place even if the nature of the substrate at the interface changes in time, becoming slippery. Many not properly well sintetized cordierites appear to crumble because of cell adhesion, with the consequence that cells find it difficult to adhere. A similar behaviour is characteristic of cordierite too if treated with HF to make it rougher, for in this case the toxicity of F^- ions may be involved. Cordierites sintered at higher temperatures give the best results.

4. Conclusions

Highest-purity cordierite sintered at high temperature proves to be a ceramic material fit to host cell cultures. Cells appear to adhere and work very well on such supports, establishing good linking in the process. This kind of ceramic material is therefore expected to be useful for many applications, particularly for the production and/or treatment of biological and/or pharmacological fluid substances and it could also find wide use in the manufacture of filters and reactors. The ceramics known to be biocompatible (e.g. hydroxyapatite, biological glasses) appear less suitable, because they are liable to interact with cells that have a tendency to dissolve the surface layer on which they adhere. Moreover, the material must be properly sintered so as not to give rise to debris which will prevent cells from achieving firm adhesion on the structure of the ceramic surface. Although a number of well-sintered hydroxyapatite samples exhibit good cell adhesion, hydroxyapatite in itself is too sensitive to the acidic pH values within which it is chemically unstable. Cordierite instead is able to guarantee chemical stability even under the acidic pH conditions in which many cell culture procedures are carried out.

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