THE SEARCH FOR A BIO-SUPPORTIVE GLASS SUBSTITUTE:
USE OF RAMAN SPECTROSCOPY FOR THE CORRELATION OF THE TEXTURE OF POROUS SILICA GELS AND THEIR CAPACITY TO MAINTAIN CELL GROWTH

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Abstract: Raman spectroscopic and textural data are presented for a series of bio-supportive glasses of varying porosities. Correlations with micro-Raman spectra of living tissue implanted on the xerogel glasses show the importance of the glass porosity for the design of bio-supportive systems.

Present research on bio-supportive materials is no longer centered on the idea of simply replacing a tissue. It is now felt that the material should stimulate a biochemical response from living tissue. Such a stimulation of tissue regeneration will then favour the production of effective fixation between the prosthesis and the tissue [1]. One of the most characterized of the bio-active materials is 45S5 Bioglass [2] whose structure is based on SiO₂, Na₂O, CaO and P₂O₅. When the glass is placed in physiological fluids, it elicits a biochemical response, the mechanism of which involves an ion exchange between the fluid and the glass [2,3]. This ion exchange leads to the formation of an apatite layer on the implant surface, on which the bone cells attach and proliferate [4]. Many studies have been devoted to the role of the individual chemical components which favour the ion exchange in these glasses. But there is also firm evidence that the pore structure itself plays an active role in the bio-activity[1]. The mechanism concerns first the fact that the nanometer-scale porosity in sol-gel glasses offers better control of the dissolution rate after implantation. But this porosity also ensures a higher area-to-volume ratio to which the cells can adhere [5].

The present paper addresses the question of the relative importance of these two factors. Since it has been shown that pure silica xerogels can also have a certain level of bio-supportive nature, even though inferior to that of the “bio-glasses”, it was decided to undertake a study on such a simplified sol-gel derived glass, so as to consider only the effect of porosity. In this work, the molecular structure and textural properties of a series of porous silica xerogels have been studied using Raman spectroscopic techniques and pore-size-distribution measurements. This data is correlated with micro Raman and micro infrared spectra of glass-supported carcinoma human lung cells. The results have then been analysed to understand the effect of the mean pore size on observed biological viability.

The class VI silica samples used in this study were prepared following a procedure which yields optically transparent nanoporous xerogels which are partially densified and then stabilized at temperatures between 800 and 1200°C [6]. The resultant materials possess high surface areas (about 800 m²g⁻¹), large pore volumes (greater than 1 cm³g⁻¹) and a narrow distribution of
nanopores. By simply changing the time of subsequent heat treatments at the temperature of 1050 °C, the pore size can be tuned as desired from 5 to 80 nm. In the present work, the cylindrical samples, typically about 3 to 4 mm in thickness and about 5 mm in diameter were heat treated in an open-air furnace for periods of time ranging from 0 to 16 hours. The corresponding mean pore dimensions were measured using BJH techniques for the smaller pore sizes and mercury porosimetry for the larger ones. The measurements yielded mean pore dimensions ranging from 740 Å for the untreated samples to 50 Å for the sample treated for 16 hours. A typical BJH graph is presented in Figure 1 for a silica xerogel heated for 16 hours. One can observe indications of good interconnectivity, as well as a relatively narrow distribution of the pore diameters centered at around 50Å.

![Figure 1: BJH pore-size distributions obtained from N\textsubscript{2} desorption for a silica xerogel heat-treated for 16 hours at 1050°C.](image)

Representative Raman spectra for four silica xerogels heat-treated for periods of 0 to 16 hours are shown in Figure 2. (Textural studies allowed an estimation of the corresponding mean pore size for each sample.) In the internal-mode region of the spectra, four main bands are of interest: the sharp peaks at around 490 cm\textsuperscript{-1} and 606 cm\textsuperscript{-1} assigned to symmetric breathing modes of 4- and 3-membered silica rings, respectively, the SiOH stretching mode near 980 cm\textsuperscript{-1} and the broad network Si-O-Si bending vibrations around 420 cm\textsuperscript{-1} [7-10]. After baseline correction, thermal reduction of the intensities and curve-fitting the bands with Gaussian functions, band-area ratios were determined [11]. The variations of the area ratios $A_{D1}/A_T$, $A_{D2}/A_T$ and $A_{SiOSi}/A_T$ (where $A_T$ is the total area) were followed as a function of pore dimension. Results for the variations of these ratios have been correlated to changes in the molecular arrangement in the sol-gel matrix/pore structure as a function of heat treatment.
The carcinoma human lung cells were obtained from a commercial source in frozen form. After resuscitation, the cells were transferred to a growth medium, placed in an incubator and allowed to multiply in a CO\textsubscript{2} atmosphere at 37\textdegree C until they reached a certain confluent level. At that time they were seeded onto silica discs which had been heat treated so as to have different pore sizes. The cells were then allowed to develop on their glass substrates in the incubator environment. After seeding and incubation, an immunostaining study was carried out whereby some proteins such as vinculin that are present in adhesion plaque of the cell-substrum were made to fluoresce (See Figure 3.). Hence, the observation of the vinculin attachment protein was used as proof of the cell attachment onto the nanoporous silica substrate [12]. For Raman microspectroscopic measurements, the xerogel glass samples with their implanted cells were immersed in a petri dish filled with PBS buffer and examined under the immersion microscope objective of a LABRAM (Dilor) micro-Raman spectrometer.

The Raman spectra of Figure 4 are characteristic of those obtained of cells on the glass substrates. For clarity, examples for two glasses of two extreme porosity values are presented (pore sizes of 75\AA{} and 740\AA{}, respectively). One can note that in both cases peaks corresponding to molecular vibrations of all major cellular components, nucleic acids, proteins, lipids and carbohydrates are observed. However, the proteins (1660 cm\textsuperscript{-1} amide I, 1200-1300 cm\textsuperscript{-1} amide III, 1005 cm\textsuperscript{-1} phenylalanine, 938 cm\textsuperscript{-1} backbone C=C stretching) and the surface lipid groups (1450 cm\textsuperscript{-1} and 1736 cm\textsuperscript{-1} ester) [13] have the strongest contributions to the Raman spectra. Consideration was given to three different intensity ratios. The first two of these, amide I (1660 cm\textsuperscript{-1}) / lipid (1450 cm\textsuperscript{-1}) and amide I (1660 cm\textsuperscript{-1}) / lipid (1750 cm\textsuperscript{-1}) are used as indications of the metabolic rate; thus the higher these ratios, the better the bio-support of the substrate [14]. The third ratio, phenylalanine (1005 cm\textsuperscript{-1}) / amide I (1664 cm\textsuperscript{-1}), characterizes the viability of the cells and hence, once again, the higher this value, the better can be judged the bio-support of the glass [14].

Figure 2: Raman spectra of silica xerogels at various times of heat treatment.
Figure 3. A549 lung cell stained to show viniculun attachment protein on nanoporous silica substrate

Figure 4. Average micro-Raman spectra of living carcinoma human lung cells seeded onto heat-treated xerogel glass samples. (Upper spectrum: xerogel with porosity of 75A; Lower spectrum: xerogel with porosity of 740A)

The results obtained for these studies of lung cells on xerogels of different pore sizes show that all three ratios, those due to I_{amide} / I_{lipid} and that for I_{phenylalanine} / I_{amide} vary in the following order: 740 < 400 = 200 < 50 < 75. Comparison of these results with information supplied by viniculin
fluorescence measurements indicate that these band-intensity ratios do, indeed, reflect the bio-supportive nature of the xerogels and that the extent of bio-support is dependant on the pore size of the support material. The relation between the molecular structure and the measured textures (pore volumes, pore diameters and surface) of the xerogel glasses and the apparent bio-supportive nature of these materials will be discussed.

References: