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SYNTHESIS AND CHARACTERIZATION OF Ag EMBEDDED $CaO-P_2O_5-ZrO_2$ GLASSES, AS A BIOCOMPATIBLE DENTAL MATERIAL WITH LOWER RISK FOR HUMAN HEALTH

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The present article describes the synthesis and characterization of a new dental biomaterial on the type SiO2 -CaO-P2O5-ZrO2 with 0, 1, and 3, % mol Ag in order to obtain homogeneous glass composition with antibacterial activity and enhanced biocompatibility, thus reducing the risks for the patient and the dental practitioner. The structure and surface morphology of samples were characterized by X-ray diffraction (XRD), infrared (FTIR) and Raman spectroscopy, Scanning Electron Microscopy (SEM). X-ray photoelectron spectroscopy (XPS) was used to determine the nature of chemical bonding and the surface composition of the samples. The behavior of obtained samples was investigated in vitro on a human fibroblast cell line. Optical microscopy images and cell viability tests showed that our obtained samples had no negative effect on the cells morphology and behavior.

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1. Introduction

The biocompatibility of dental materials is an actual theme and there already is a consensus around the cytotoxicity of some of the most used materials for fillings in dentistry the composites, the adhesives and the resin modified glass-ionomers. They are used for their superior aesthetic and resistance in time relative to others materials but studies shown that are cytotoxic on pulpal and gingival cells [1-3].

There are results suggesting that the cytotoxic potencies demonstrated by these materials might be of clinical relevance, since all dental adhesives disturbed the cellular redox state of pulp cells in monolayer cultures. So they can modify pulp cell metabolism when the materials are used directly contact pulp tissue or in deep cavities [4]. Previous studies on dental composite materials suggest that mutagenic components of biologically active composite resins should be replaced by more biocompatible substances to avoid risk factors for the health of patients and dental personnel [5, 6].

Zirconium dioxide (ZrO2) known as zirconia, is a ceramic material with a wide range of applications as advanced ceramics, optical devices, sensors, electrochemical capacitor electrodes, fuel cells, catalysts, synthetic gem stone [7]. To achieve mechanical demands, it is doped with different oxides that stabilize its structural phases [8].

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In dentistry zirconia is used for high aesthetic bridges and crowns and a new family of dental implants [9]. An in vivo study on yttrium and zirconia based material implanted in a hip of sheep shows that this material may present osseointegration capacity without signs of inflammation or adverse body reaction [10].

Due to zirconia inert character and superior mechanical properties it inspired us the aim of this study, to push the borders of actual use in dentistry in order to obtain three nanostructured biomaterials with healing and antibacterial properties to use as a long term resistant dental filling, material for direct pulp capping and a material for sustain the repair processes in accidental perforations of pulp chamber floor. The added value of our material is given by the presence of silver (Ag) which is well known for its antibacterial properties. For this we synthetize and test on a human fibroblast cell line a new type of zirconium - containing calcium phosphate material by melt quenching method.

2. Experimental

The studied glasses have the composition expressed by the formula (1-x) [$50P_2O_5 48CaO_2ZrO_2$] with x = 0; 1 and 3 mol %Ag. They were prepared using the conventional melt quenching method and as raw materials appropriate amounts of reagent grade NH3·H2PO4, CaCO3, H3BO3 and ZrO2. The obtained melts were quickly cooled at room temperature by pouring and stamped between two copper plates at the first cooled with liquid nitrogen.

The obtained samples in the form of transparent plates were milled in a mortar to obtain a fine powder consisting of micrometric particles. The structure of prepared samples was investigated by X-ray diffraction using a standard Bruker X D8 Advance diffractometer with a monochromator of graphite for CuKα. The diffractograms were recorded in 2θ range from 10º to 90º with a speed of 1˚/minute.

To investigate the samples morphology a scanning electronic microscope (SEM) was used on the type FEI QUANTA 3D FEG dual beam – in high vacuum mode using EDT (Everhart Thornley Detector) and an accelerating voltage of 30kV. To amplify the secondary electrons signal a layer of 5 nm thickness was deposited with Pt-Pd into Agar Automatic Sputter Coater, in Ar atmosphere.

Fourier transform infrared (FTIR) measurements were performed at room temperature in the 350-4000 cm^{-1} range with a 6100 Jasco spectrometer with a maximum resolution of 0.5 cm^{-1} and signal/noise ratio 42,000:1. For these measurements identical amounts of glasses were powdered and mixed with KBr in order to obtain thin pellets (thickness was about 3 mm) containing approximately 1.05 wt% glass powders.

X-ray photoelectron spectroscopy (XPS) analysis was performed using a monochromated Al Kα radiation, operated at 280 W. The base pressure in the analysis chamber spectrometer was less than 5 X 10^{-9} mbar. All spectra have been corrected for the charging effect. Surveys were measured in steps of 1 eV and high resolution spectra with 0.05eV steps.

Cell culture

The human fibroblast cell line (HFL1, ATCC® CCL-153™) was cultured on membrane in Dulbecco's Modified Eagle's Medium supplemented with fetal bovine serum to a final concentration of 10%. The cells were incubated with 10 μg/mL Ag embedded SiO_2-CaO-P_2O_5-ZrO_2 glasses for 48h. The glasses were UV irradiated just before they were placed on the cells to reduce the contamination risk. Control cells without Zr glasses were grown in parallel under the same conditions. For SEM analyses the membrane with attached cells was dried with CO2 using a Polaron CPD (Critical Point Dryer). Then the membrane was fixed onto Al stubs using double side carbon sticky tabs and Pt/Pd coated with an Agar Automatic Sputter Coater. SEM images were recorded with a Quanta 3D FEG SEM (FEI).

3. Results

X-ray diffraction spectra of samples of (1-x)[$50P_2O_5 48CaO_2ZrO_2$] with x = 0; 1 and 3 mol %Ag are shown in Figure 1. Broad shape diffractograms indicate a vitreous character of all analyzed samples.

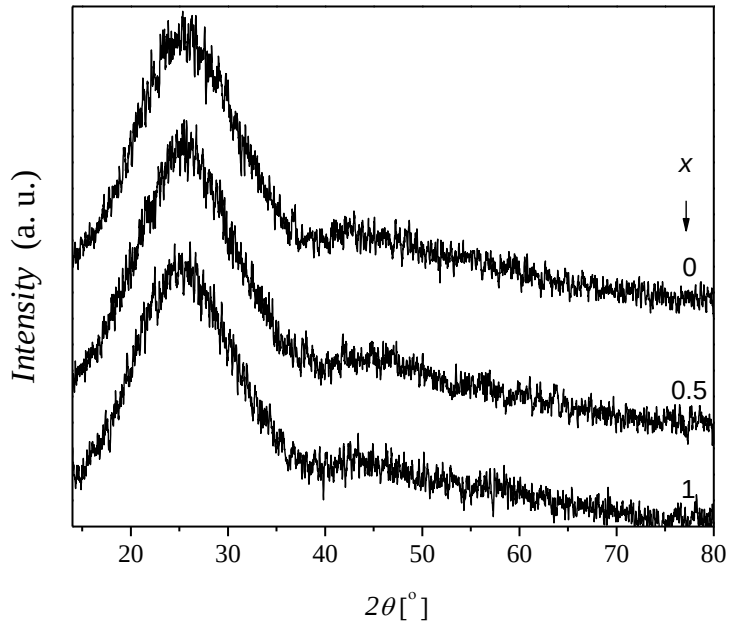

Fig. 1- X- Ray difractograms of the system (1-x)($50P_2O_5 48CaO_2ZrO_2$) with x = 0; 1 and 3 mol %Ag.

FT-IR absorption spectra of the examined vitreous samples are shown in Fig. 2. Spectra presents in the low range wave numbers (400-800 cm^{-1}) two broad absorption bands assigned as follows: a strong band, positioned at ~ 510 cm^{-1} assigned to bending vibrations of bonds O = P - O [11-12] and an average band centered at app. 745 cm^{-1} generated by symmetric stretching vibrations of the P-O-P bonds of structural ring units associated to Q2 units [13].

In the 800-1400 cm^{-1} spectral range three well defined absorption bands appear placed at ~ 916, 1116, 1270 cm^{-1} and a low-intensity shoulder positioned at 1005 cm^{-1}.

The band at 916 cm^{-1} originates from the asymmetric stretching vibration of short chains P-O-P linkages and pyrophosphate groups (Q1); the band at ~ 1005 cm^{-1} is generated by vibration characteristic of tetrahedra 'P' isolated (Q0 units orthophosphate) related to various cations [14]; the band at ~ 1115 cm^{-1} is given by symmetrical and asymmetrical vibrations associated to PO3 tetrahedra groups Q1 and the band at ~ 1270 cm^{-1} originates from the asymmetric stretching vibration of the P = O link from Q2 units [15].

In view of the above assignments one can say that the network of the matrix glass is composed mainly of structural units Q1, Q2 Q0 respectively. Since the structure of P_2O_5 glass consists of Q3 units in which three oxygen atoms are bridged (P-O-P) and an oxygen atom is double linked with the P atom (P = O), it is obvious that the appearance in the glassy network structure of the Q2 and Q1 units is given by Ca^{2+} cations that are able to break three-dimensional continuous network of P2O5.

The presence of ZrO_2 in the composition of the vitreous matrix gives rise to a new band in FT-IR spectra registered at ~ 1400 cm^{-1}.

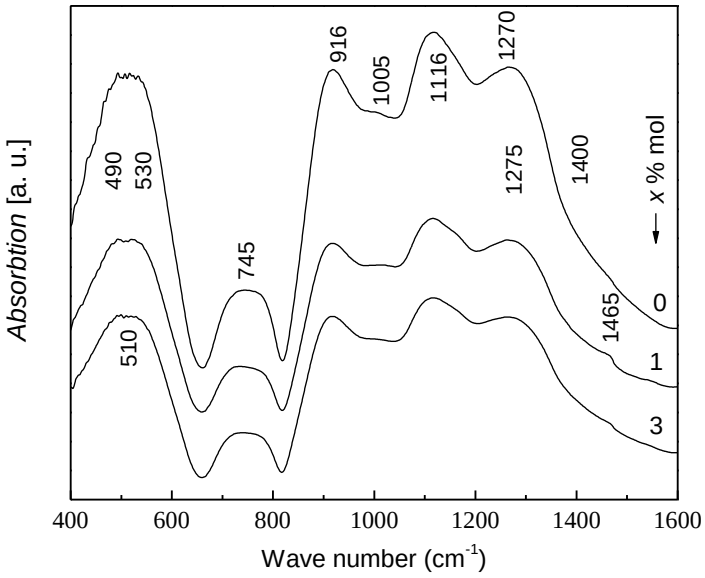

Fig. 2- FT-IR spectra characteristic of vitreous (1-x)(50$P_2O_5$48CaO_2ZrO_2)with x = 0; 1 and 3 mol %Ag.

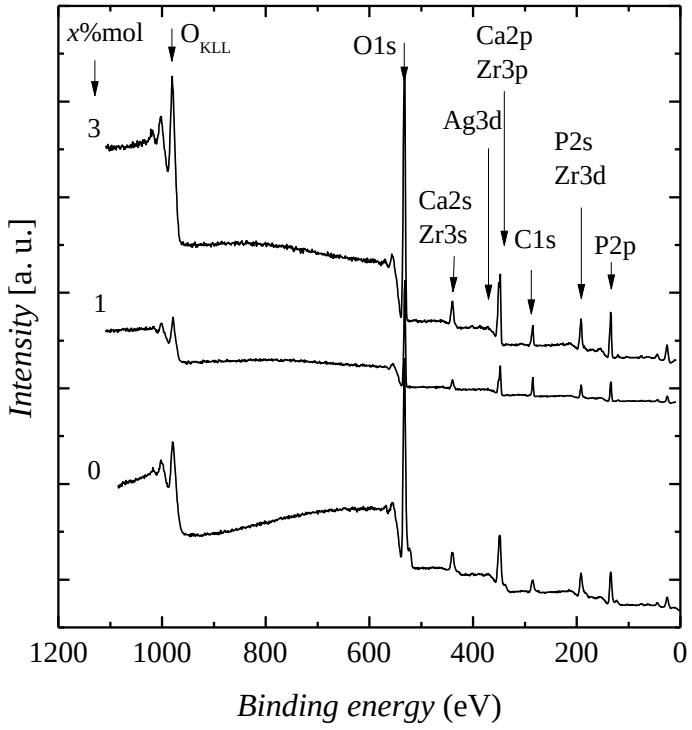

Fig. 3-XPS survey spectra for the investigated samples system.

Survey scans X-ray photoelectron spectra (Fig. 3) where recorded to determine the chemical species developed on the samples surface of (1-*x*)[50$P_2O_5$48CaO_2ZrO_2] system with *x* = 0; 1 and 3 mol %Ag. All XPS and Auger peaks from the constituent elements of the obtained glasses were clearly identified and marked on the spectra (see Fig. 3). The obtained survey spectra for the studied glass compositions are quantified in terms of peak intensities and peak positions. The C1s transition has been used as an energy reference for the spectra. The comparison of the obtained XPS intensities of the investigated samples was done via so called percentage atomic

concentrations, based on the ratio of the intensity to the total intensity of electrons in the measurement. The results obtained are listed in the Table 1 which shows the concentration of the elements present at the investigated samples surface in at %.

Table 1-Compositions of the obtained glasses at the surface as determined from XPS survey spectra.

Sample	Elemental concentration (at%)						
	Ca	P	O	C	Zr	Ag	Ca/P
$x = 3$	10	19.8	58.8	11	0.2	0.1	0.5
$x = 1$	8.8	19.5	50.1	21.5	0.2	<0.1	0.4
$x = 0$	8.7	18	63.1	11.1	0.2	----	0.5

The XPS survey spectra confirm once more the elemental composition of the experimental glasses.

Unlike SEM, XPS is a surface sensitive technique; the depth from where the signal is coming is approx. 0.005µm, with a detection limit of 1% in atomic concentration for chemical elements [16-17]. After 48 h from the addition of the Zr glasses, HFL1 cells continued to be viable. In the presence of $(1\text{-}x)[50P_2O_5 48CaO_2ZrO_2]$ with x = 0, 1 and 3 mol %Ag glass system the cells proliferated similar with cells in the control samples.

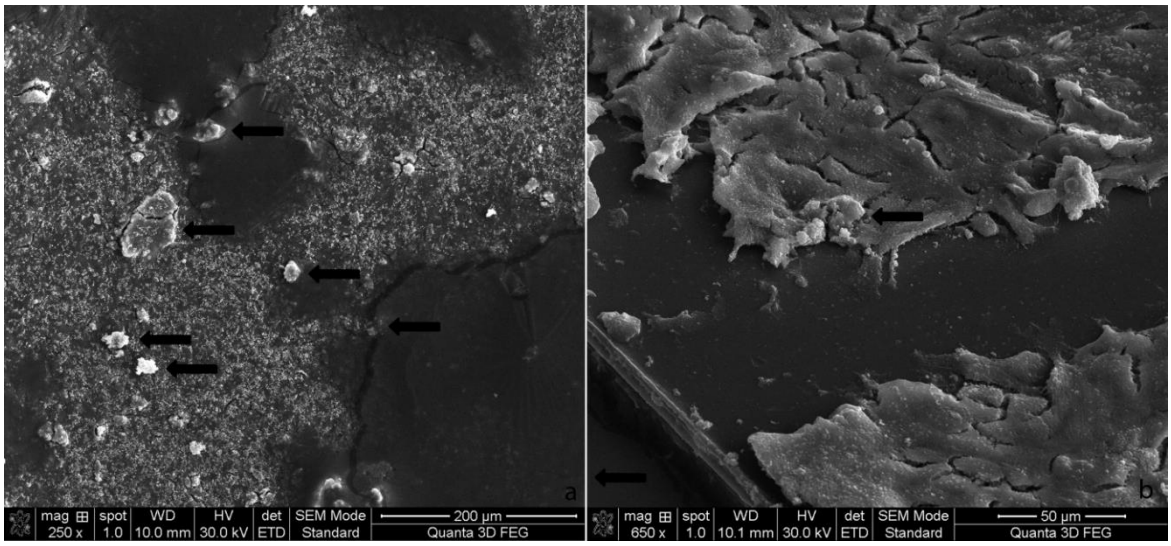

Fig. 4- SEM image at scale bar, 200µm (fig. 4a) and 50µm (fig. 4b,) recorded 48 h after glasses addition to cell culture.

Figure 4 shows at a 250x magnification(fig.4a) and at a 650x magnification(fig.4b) the aspect of prepared glasses, of diverse shapes and sizes, in contact with HFL1 cells. At higher magnifications, 2000x (fig.5a,b,c,d), 4000x(fig 6a) and 7500x (fig.6b) small parts of the experimental material seem to be partially covered by cells and the HFL1 cells remain viable in the presence of Zn glasses. The glasses have generally anfractuous shapes with one or more sharp edges.

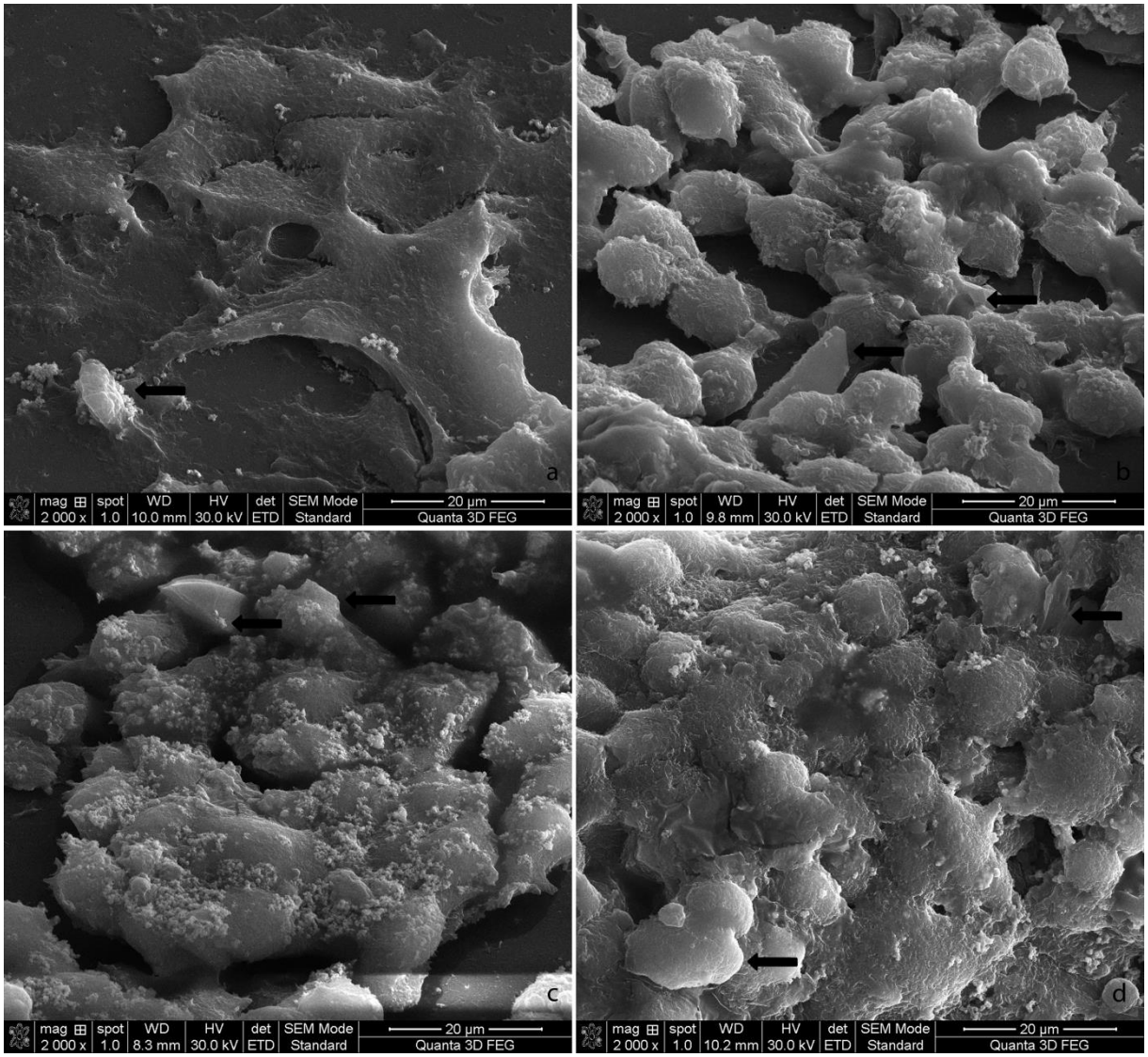

Fig. 5a,b,c,d- SEM image of cells at 20µm scale bar after 48h interaction with the material.

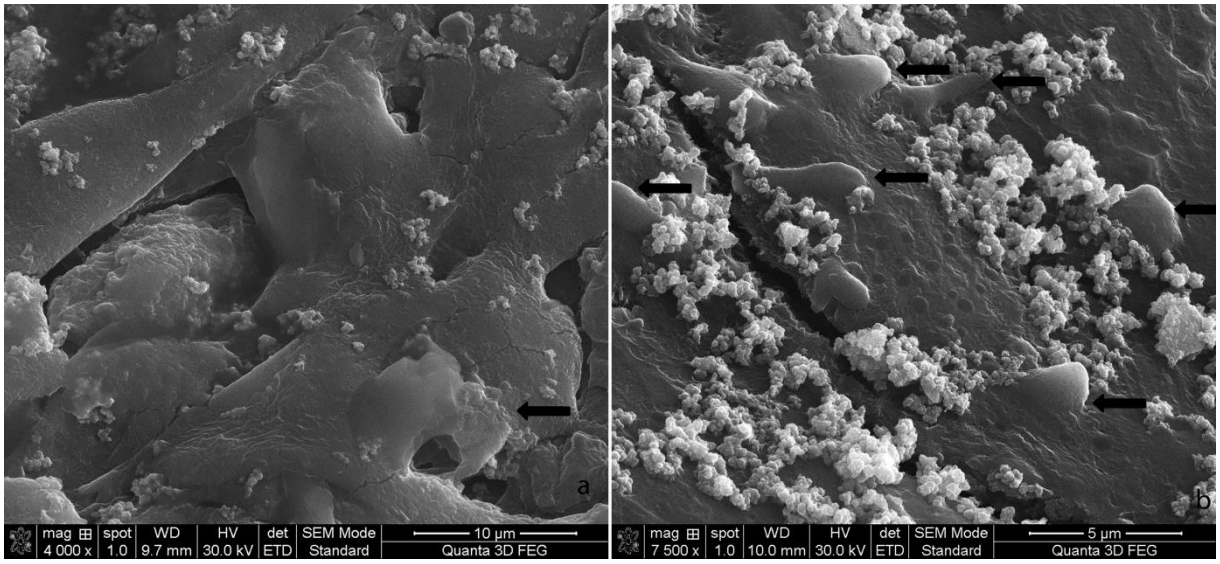

Fig. 6-SEM images at 10µm scale bar (fig.6a) and 5µm scale bar (fig.6b), show glass particles surrounded by the fibroblasts.

The experiments were run in duplicate and three independent experiments generated similar results.

4. Discussion

The biomaterials field is a very dynamic one and the evidence based medicine tries to apply and keep the rhythm with the new studies. As the technology develops we are able to find more dates about already used materials and this can lead us to change protocols or adapt doses or search new materials less toxic and more resistant. An example is what happened in the case of mercury that is use in dental silver amalgam, where the initial dose considered safe in 1978, 0.5μg/kg body weight /day, was lowered by the Environmental Protection Agency in 2001 at 0.1μg/kg body weight /day. [18]

The materials present in our oral cavity are exposed to a permanent corrosion, exacerbated in the zones where they are under the pressure of masticatory forces. For instance Vimy et al. [19] when they analysed the air from the oral cavity of patients with amalgam fillings found that it contains Hg vapours and their concentration increases after chewing so these patients chronic exposed to Hg. The solution it was thought to be the dental composite materials that have many advantages but scientific studies sustain that they release monomers that present different degrees of cytotoxicity, so is indicated that the manufactures reduce the water soluble components [20]. Even the light-curing glass-ionomer cements and compomers release monomer or additives hat was demonstrated to be cytotoxic on 3T3 fibroblasts [21].

In this context of searching for new materials with a better biocompatibility we intended approach the $(1-x)[50P_2O_5 48CaO_2 ZrO_2]$ with x = 0; 1 and 3 mol %Ag like a more stable material for the use in the oral cavity.

The obtained results sustain our hypothesis that the vitreous obtained system can be used as a base for future biocompatible materials with low risks for the human body.

5. Conclusions

The main results obtained from the investigation of the vitreous obtained $(1-x)(50P_2O_5 48CaO_2 ZrO_2)$ with x = 0; 1 and 3 mol %Ag system are:

1. the investigated system was first reported in the literature; 2. the vitreous character of the samples was proved by X-ray diffraction; 3. on the basis provided by IR absorption spectroscopy data, it has been shown that the local structure of these vitreous materials is dominated by phosphatic units Q^1, Q^2 and Q^0; 4. XPS techniques prove that the Ca/P ratio remains roughly the same with increasing Ag content.

SEM images showed that some of the Zr glasses were covered by human fibroblast cells. Evaluation of the cells viability showed that, 48 h after the addition of the Zr glasses HFL1 cells continued to be viable. The cells proliferated similar with cells in the control wells. The obtain results encourage us on continuing the tests of this new material with *in vivo* laboratory studies. Our next project is to test on laboratory animal direct dental effects, using these glasses for pulp capping, dental fillings or to facilitate the repair processes in accidental perforations of pulp chamber floor.

Future works is to be done on the obtained samples in order to further investigate the possibility of doping them with various trace elements and establish a solid basis for the use of these materials in dental applications and bone tissue engineering.

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