

## Research Article

# Characterization of Sucrose Thin Films for Biomedical Applications

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Sucrose is a natural osmolyte accumulated in the cells of organisms as they adapt to environmental stress. In vitro sucrose increases protein stability and forces partially unfolded structures to refold. Thin films of sucrose ( $C_{12}H_{22}O_{11}$ ) were deposited on thin cut glass substrates by the thermal evaporation technique ( $P \sim 10^{-5}$  torr). Characteristics of thin films were put into evidence by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Photoelectron Spectroscopy (XPS), scanning electron microscopy (SEM), and differential thermal analysis and thermal gravimetric analysis (TG/DTA). The experimental results confirm a uniform deposition of an adherent layer. In this paper we present a part of the characteristics of sucrose thin films deposited on glass in medium vacuum conditions, as a part of a culture medium for osteoblast cells. Osteoblast cells were used to determine proliferation, viability, and cytotoxicity interactions with sucrose powder and sucrose thin films. The osteoblast cells have been provided from the American Type Culture Collection (ATCC) Centre. The outcome of this study demonstrated the effectiveness of sucrose thin films as a possible nontoxic agent for biomedical applications.

## 1. Introduction

The obtaining of uniform adherent thin films, deposited on different substrates and having a good biological activity, now represents an important aim in the field of biochemical research. The difference between biomaterials and passive materials (dielectrics) is mainly related to their specific biochemical function. The biomaterial transfer requires the preserve of molecular function [1–3].

Sucrose is a compatible osmolyte, belonging to a class of low molecular weight compounds ( $C_{12}H_{22}O_{11}$ ) produced by both prokaryotic and eukaryotic cells to protect proteins against the deleterious effects of harsh environmental conditions of water, cold, and heat stress [4]. In particular, the study of sucrose-protein interactions has attracted considerable attention for the importance of sucrose in biochemical science and in the pharmaceutical industry, where it is commonly used as an additive to protein formulations, to protect labile proteins from the harmful effects of high temperature, freezing, and drying [4]. In cell

metabolism, membrane resistance under sucrose is very low because of a large negative surface potential [5]. It has been suggested that certain carbohydrates may stabilize membranes at low-water activities [6]. The organization of water at a lipid/membrane interface is a matter of interest, because it determines important functional properties of biomembranes such as the excluded volume, hydration forces, and the dipole potential. Sucrose has been reported [7] to be an efficient cryoprotectant, a property attributed to the replacement of water at the membrane level. Different techniques such as the sucrose gap technique for assessing membrane potentials of nerve axons with extracellular electrodes have been developed; in particular, the gap technique has proved useful for measuring pharmacological modifications of channel properties that are irreversible [5]. The sucrose gap technique has also seen widespread application at the level of the multicellular muscle tissue. As it was stated [6] the effects of sugar on the physical properties of phospholipids have been examined using a variety of physical techniques. It has been reported that, in the presence of

carbohydrates (monosaccharides) as pentoses and hexoses and disaccharides as well as trisaccharides, the temperature ( $T_m$ ) of maximal excess apparent specific heat ( $C_{max}$ ) of 1,2-DPPC (1,2-dipalmitoyl-3-sn-phosphatidylcholine) is “essentially unchanged” and the calorimetric enthalpy ( $\Delta H_{cal}$ ) is decreased. Surface pressure-area and surface potential-area measurements obtained from monolayers of 1,2-DPPC indicate that at low-surface pressures ( $10\text{--}30\text{ mNm}^{-1}$ ) sucrose ( $\geq 1.5\text{ M}$ ) causes the monolayer to become more liquid-expanded, that is, increases the molecular area, which makes the film more liquid in character [6]. As presented in [6], during the phase transition process there is a melting of “ice-like” water that reflects the difference in the amount of ice-like water around the hydrocarbon tails between gel and liquid crystalline phases attributed to a smaller difference in ice-like water in the two phases in the presence of carbohydrate [6]. Mechanisms proposed for the effect of sucrose on protein [8] may help to explain the effect of sucrose on aqueous suspension of 1,2-DPPC liposomes. We remark the propensity of sugar solutions, for example, to harden into amorphous glassy solids thus forming the basis for the worldwide sugar confection industry as well as the ability of organisms to endure severe desiccation [9]. Knowledge of details regarding the structure and dynamics of the membrane when influenced by the surrounding medium gives an insight into the mechanisms of the protective action of the different saccharides for membranes under stress and into the number of water molecules at the hydration layer around the chemical group of phospholipids [9]. For its protective properties sucrose as well as iron oxide-doped dextran is used as biological probes in Magnetic Resonance Imaging (MRI).

In this paper we present a part of the characteristics of sucrose thin films deposited on glass in medium vacuum conditions. Fourier Transform Infrared Spectroscopy (FTIR) results reveal that the obtained complex films preserve the structure and composition of the initial material. The morphology of the thin films was characterized by scanning electron microscopy (SEM). The biocompatibility of the iron oxide-dextran thin films was demonstrated by the MTT test, with the aid of osteoblasts cells. The biological tests confirm the interest regarding the culture medium to be studied in tooth decay, due to the fact that the increasing frequency of sugar application alters dental plaque by reducing its mineral protection capacity [10].

## 2. Experimental Methods

**2.1. Sample Preparation.** Powder of sucrose was supplied by Merck Company at 99.99% purity. The powder was in a specific quantity for vacuum deposition. The sucrose thin films were deposited by thermal evaporation using a HOCH VAKUUM Dresden system. The thin films were deposited on glass substrates. For evaporation in medium vacuum ( $P \sim 8 \times 10^{-6}$  torr), it was used a wolfram boat, and the intensity of the maximum current through boat was  $I^{max} \sim 40\text{ A}$  for  $t \sim 5\text{ sec}$ . The “as deposited” sucrose thin films were characterized by different techniques, namely, SEM, XPS, FTIR, and biological tests.

## 2.2. Sample Characterization

**2.2.1. FT-IR Spectroscopy.** The functional groups present in the powder and in the thin films were identified by FTIR Spectrum BX spectrometer ( $7000\text{--}350\text{ cm}^{-1}$ ) in transmission mode with the resolution  $8\text{ cm}^{-1}$ . IR spectroscopic studies were performed in the range  $1800\text{--}800\text{ cm}^{-1}$ .

**2.2.2. Scanning Electron Microscopy (SEM-EDS).** The surface morphology and growth mode of the deposited sucrose thin films were investigated by scanning electron microscopy (SEM) in an XL-30-ESEM TMP system. For the elemental analysis, the electron microscope was equipped with an energy dispersive X-ray (EDS) attachment.

**2.2.3. X-Ray Photoelectron Spectroscopy (XPS).** Soft X-ray Photoelectron Spectroscopy (XPS) is one of the most important techniques for the study of the elemental ratios in the surface region of a deposited thin film. The surface sensitivity (typically  $40\text{--}100\text{ \AA}$ ) makes this technique ideal for measurements as oxidation states or biomaterials powder as well as biomaterials thin films. In this analysis we have used a VG ESCA 3 MK II XPS installation ( $E_{K\alpha} = 1486.7\text{ eV}$ ). The vacuum analysis chamber pressure was  $P \sim 3 \times 10^{-8}$  torr. The XPS recorded spectrum involved an energy window  $w = 20\text{ eV}$  with the resolution  $R = 50\text{ eV}$  with 256 recording channels. The XPS spectra were processed using Spectral Data Processor v 2.3 (SDP) software.

**2.2.4. Thermal Analysis.** On the powder, differential thermal analysis and thermal gravimetric analysis were performed using the Shimadzu DTG-TA-50 and DTA 50 analyzer in the  $25\text{--}800^\circ\text{C}$  temperature range, air environment.

**2.2.5. Cell Culture.** The hFOB 1.19 osteoblasts cells line was purchased from ATCC (American Type Culture Collection) and maintained in DMEM, containing 3,7 g/L sodium bicarbonate, 4,5 g/L D-glucose, 4,7 g/L HEPES, 4 mM L-glutamine, 0,1 mM sodium pyruvate, 100 U/mL penicillin, 100 U/mL streptomycin, and 10% (v/v) fetal bovine serum. Cells were grown in 5%  $\text{CO}_2$  at  $37^\circ\text{C}$  and plated at  $5 \times 10^4\text{ cells/cm}^2$  in  $\varnothing 100\text{ mm}$  culture dishes with a medium change twice a week. When 70–80% confluence was reached, cells were passaged, and hFOB 1.19 cells prior to passage 4 were used in this study.

Cells cultured in dishes prior to passage 3 were detached by treatment with trypsinEDTA (0.25% and 0.03%, resp.) and loaded on sucrose thin films at a seeding density of  $5 \times 10^4\text{ cells/cm}^2$  in 24-well plates. Cells cultured in 24-well plates at the same seeding density were used as control.

**2.2.6. Cell Viability.** Cells were seeded at the same density as previously ( $10^4\text{ cells/cm}^2$ ) in Petri dish and incubated on sucrose thin films for 6, 12, and 24 hours. The cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction test [18]. The cells were incubated at  $34^\circ\text{C}$  and 5%  $\text{CO}_2$  atmosphere for 6, 12, and 24 hours. The medium from each well was removed

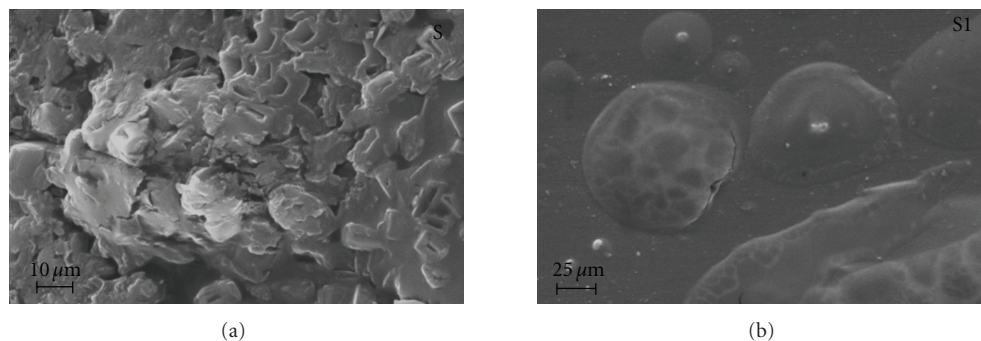


FIGURE 1: The SEM images of sucrose powder (sample S) and thin films (sample S1).

by aspiration, the cells were washed with 200  $\mu\text{L}$  phosphate buffer solution (PBS)/well, and then 50  $\mu\text{L}$  (1 mg/mL) of MTT solution was added on each well. After 2 hours of incubation the MTT solution from each well was removed by aspiration. A volume of 50  $\mu\text{L}$  isopropanol was added, and the plate was shaken to dissolve the formazan crystals. The optical density at 595 nm, for each well, was then determined using a Tecan multiplate reader (Tecan GENios, Grödic, Germany). The absorbance from the wells of cells cultured in the absence of sucrose thin films was used as the 100% viability value.

**2.2.7. Cell Morphology.** The cells were plated at day one at a density of  $5 \times 10^4$  cells/cm<sup>2</sup> on the sucrose thin films. At specific time points cells were imaged by a bright field inverted microscope (Olympus IX7). Images were acquired by specific software Cell F using a CCD video camera COLORVIEW.

**2.2.8. Analysis of the Actin Cytoskeleton.** The hFOB 1.19 cells were incubated on sucrose thin films for 24 h, and the culture medium was removed. Then, they were washed twice by phosphate saline buffer, and one mL of 4% paraformaldehyde was added for 20 minutes at room temperature. After three washes with phosphate saline buffer and one with 2% bovine serum albumin in 0.1% Triton X-100, a solution of 2  $\mu\text{g}/\text{mL}$  phalloidin coupled with FITC in 1.2 bovine serum albumin was added. After one hour of incubation in dark, this reagent was removed. The cells were analyzed at an Olympus IX71 microscope using an excitation wavelength of 495 nm and an emission one of 513 nm.

### 3. Results and Discussion

In Figure 1 (sample S and sample S1), we present the SEM micrographs for sucrose powder (sample S) and sucrose thin film deposited on glass (sample S1). In order to record a SEM image, the sucrose powder was deposited on a double adhesive carbon band, and afterwards a fine layer of gold was deposited (the sample S showed a disordered aspect with scratches and valleys on a uniform background specific for

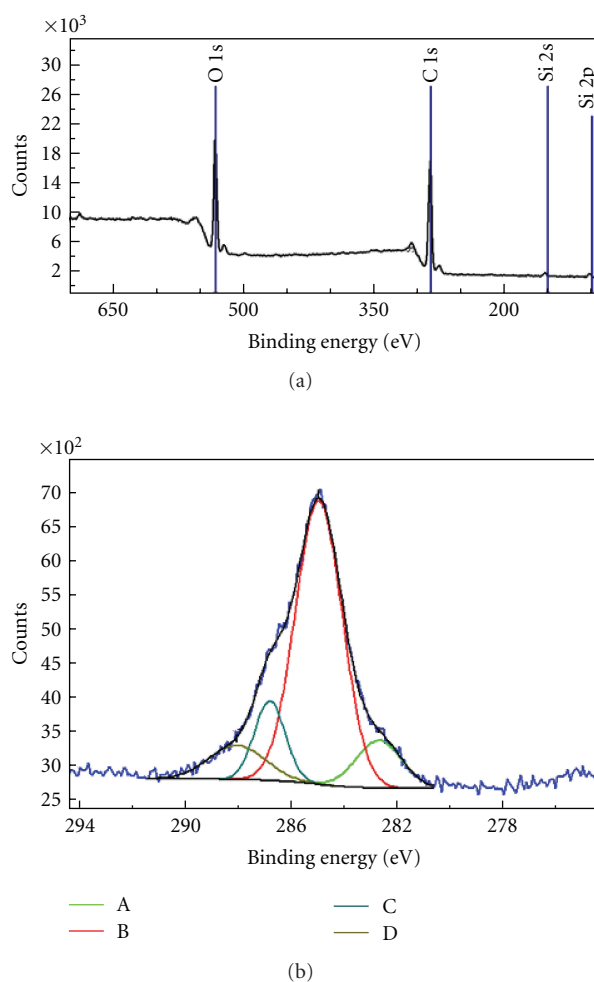


FIGURE 2: The XPS spectrum of sucrose thin film deposited on glass (a) and Carbon (C 1s) deconvolution analysis (b).

noncrystalline samples). For the sucrose thin film (sample S1), we remark an ordered aspect of droplets.

In Figure 2 we present the data related to the XPS analysis of sucrose thin films.

XPS technique has been tested as a powerful tool for qualitative determination of surface composition of one

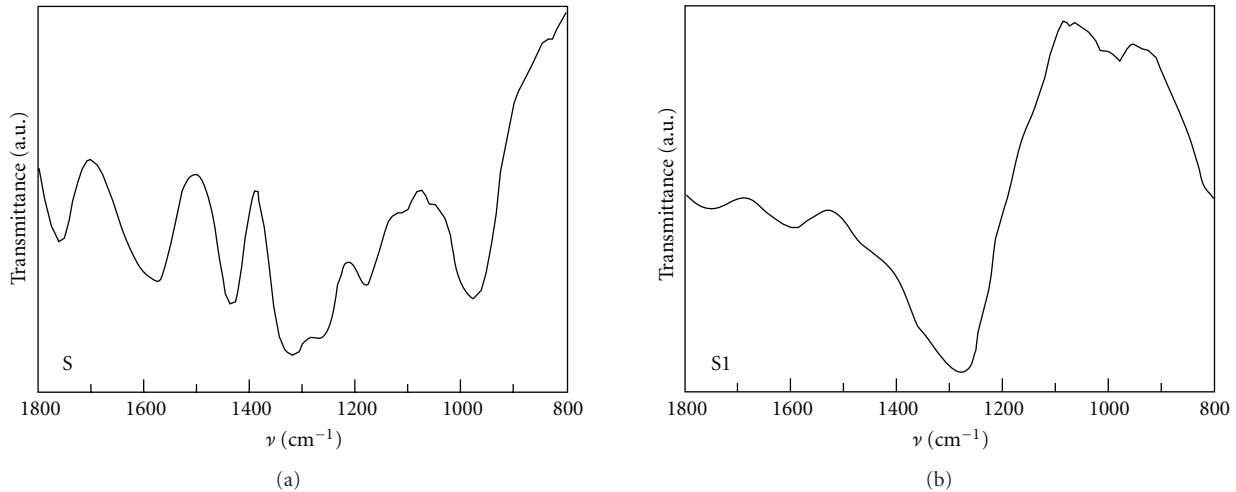


FIGURE 3: The FT-IR spectra of sucrose powder (sample S) and thin films (sample S1).

material. The XPS spectrum of sucrose thin film deposited on glass (a) shows the binding energy of C (1s, 284.99 eV), O (1s, 532.45 eV), and Si (2p, 103.22 eV), respectively. As it can be observed, Carbon (C 1s) has four lines in SDP (b) deconvolution analysis, namely,

- (i) A-peak that is raised from the sample holder;
- (ii) B-peak (BE: 285 eV) related to C–C bond, and it represents a part of adsorbed carbon on sample surface together with the C–C bond in sucrose (as a dominant bond);
- (iii) C and D represent Carbon related to a C–O (BE: 286 eV) and C=O (BE: 288 eV) or free radicals of different substances adsorbed at sample surface.

The deposited thin films of sucrose were investigated by FTIR spectrometry with the aim of obtaining first information about their molecular structure as compared to the powder material (sample S) used for the targets preparation (Figure 3). The IR spectra for S and S1 samples show the vibration modes of sucrose. In these spectra the characteristic bands of sucrose are observed in the 1250–800 cm<sup>-1</sup> range both for S and S1. From this point of view the exposed spectra are similar [11]. As presented in the literature [12], the most suitable region for the IR measurements of sucrose has been found to be the 1250–800 cm<sup>-1</sup> region; namely, the shoulder at 800 cm<sup>-1</sup> of sample S can be related to CH<sub>2</sub> group, and the range 950–1300 cm<sup>-1</sup> is related to vibration mode C–O–C group [13, 14]. The vibration range related to hydrogen bonded water molecules adsorbed on the surface is present in the region 1600 cm<sup>-1</sup> for the sample S (as can be observed in Figure 3). The band at 1750–1850 cm<sup>-1</sup> is related to a C=O bond [14]. The IR spectra of the sample S and sample S1 were recorded in (attenuated total reflection) ATR regime. The difference in intensity of different transmission peaks is related to the thickness of sucrose thin films as the measured exposed volume decreases.

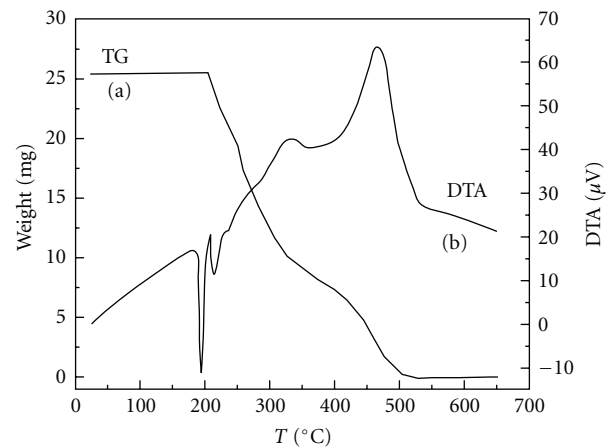
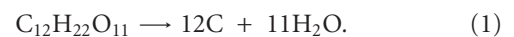


FIGURE 4: The DTA/TGA evolution curves for sucrose powder.

The thermal profiles of the sucrose powder material are shown in Figure 4. A slow weight loss can be observed during the heating process until around 300°C. The DTA curve (curve b) has three endothermic peaks, at around 195°C, 226°C, and at around 400°C, respectively, resulting from heat-induced decomposition. In previous thermogravimetry studies, correlated with mass spectral analyses, the slow weight loss until about 100°C was attributed to detachment of adsorbed water molecules from the sucrose surface [15, 16]. The thermal decomposition of sucrose was considered to start at temperature higher than 195°C. Sucrose decomposes as



Osteoblasts cells were monitored in order to detect any possible influence due to sucrose that might modify the cell growth and viability. No change in osteoblasts morphology after 24 h exposure to thin films was observed under phase contrast microscopy (Figure 5). According to Figure 5(b),

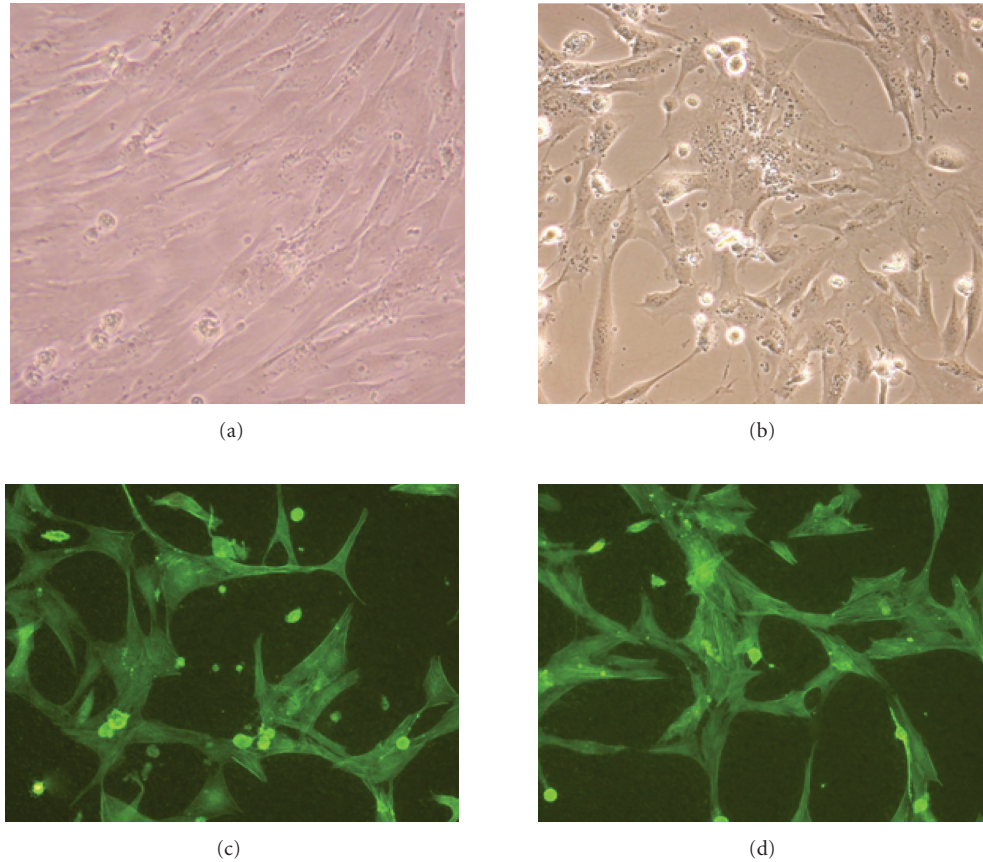


FIGURE 5: Micrographs in phase contrasts for hFOB 1.19 osteoblasts: (a) control; (b) S1. Fluorescence micrograph for hFOB 1.19 osteoblasts: (c) control; (d) S1.

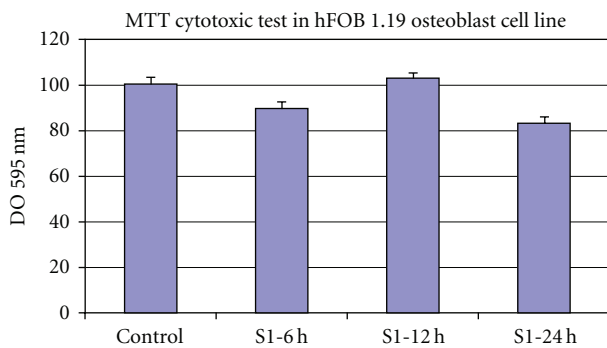


FIGURE 6: The viability of hFOB 1.19 cells grown S1 sucrose thin films. The values are calculated as means  $\pm$  SE ( $n = 10$ ) and expressed as % from controls.

it can be seen that a layer of fusiform osteoblast cells has been coated on the surface of the sucrose thin film samples.

In order to prove the physiological growth on the supports hFOB 1.19, the cell actin was labelled with the phalloidin-FITC conjugate.

Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate

constant for the dissociation of actin subunits from filament ends, which essentially stabilizes actin filaments through the prevention of filament depolymerization [13]. Overall, phalloidin is found to react stoichiometrically with actin, strongly promoting actin polymerization and stabilizing actin polymers [14, 17, 18].

In order to evaluate the degree of compatibility of sucrose thin films with hFOB 1.19 osteoblasts, the MTT test for short term (6, 12, and 24 hours) was performed for exploring the possible cellular toxicity at interaction with these surfaces (Figure 6). Our data have shown that the viability of cells did not decrease significantly compared to control over 24 hours (89.6% after 6, 102.3% after 12, and 83.1% after 24 hours), suggesting that osteoblasts were not damaged at the contact with the sucrose thin films.

#### 4. Conclusions

Sucrose biomolecular thin films were grown by vacuum deposition techniques. The sucrose thin films vacuum deposited on glass presented a good adhesion.

This method resulted in the deposition of uniform thin films, with chemical composition and molecular structure identical to those of the starting biomaterial used for the target preparation.

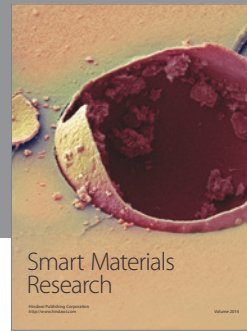
The substrates made sucrose thin films could be suitable supports for osteoblasts adhesion and proliferation without any modification of their structure and function. The osteoblasts cells are adherent cells; that is, they are not suspended in the culture medium. In the fact these cells cover the sugar films. It is important to conclude that S1 sucrose thin films have microcells configurations that allow them to be used for obtaining medical biocompatible supports.

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