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Biocomposites with submicrocrystalline sintered corundum and bioglass system as substrates and their structural and physical properties. Short- and long-term cultures of the fibroblast human skin on these substrates

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The results of studies of the effects of the process for obtaining biocomposites containing alumina submicrocrystalline sintered corundum grains as a matrix and a bioglass $CaO–SiO_2–P_2O_5–Na_2O$ system as a bound phase on its microstructure and biocompatibility are presented. Microscopic observation was carried out on samples (SEM), the real, an apparent, helium density, total porosity, surface area, S_{BET}, volume and percentage of macro- and mesopores, the biocomposites surface roughness parameters (R_a, R_z, R_t, profile material ratio R_{mr}, linear bearing part, amplitude distribution of the ordinate) were determined. It has been found that with the increase in glass system content, the density of biocomposite is decreasing. Biocompatibility was determined by the degree of growth of a human skin fibroblast cell line CCL 110 cultured on these substrates for 96 and 360 hours. The best results for maintaining short-term (96 h) culture were obtained on the substrate without densification with 10 wt% admixture of a bioglass system.

Keywords: submicrocrystalline sintered corundum, bioglass $CaO–SiO_2–P_2O_5–Na_2O$ system, substrates, SEM, surface roughness S_{BET}, human skin fibroblast, short- and long-term culture.

1. Introduction

Biocompatible corundum-glass system composites are a new generation of ceramic--glass materials used in tissue engineering, which is a regenerative medicine area [1–4]. Biomaterials substrates (inorganic, polymeric, hybrid) are two-dimensional or special scaffolds, which are settled with the cells (*e.g.*, fibroblasts) by growing them *in vitro*,

and then the resulting product material is implanted in place of the defect [5]. The main task of such scaffolds is the physical support for cells and control their proliferation, differentiation and morphogenesis [1, 9].

The basic criteria that should fulfil the substrate [5–8] are formulated as follows:

– the substrates should contain open pores that connect to each other about the right size to integrate the cells and then tissues as well as to promote their tissue vascularity;

– they should have appropriate chemical properties (bioactivity, non-toxic) to promote attachment of cells to substrates, their differentiation and multiplication, and mechanical properties (tensile strength, torsion, hardness, Young's modulus) close to the natural materials;

– they should be made of materials with controlled biodegradability (biosorption), so that tissue could be replaced after a specified time basis;

– they should not cause adverse reactions (including allergic);

– they should be easily manufactured in various shapes and sizes.

Taking all these requirements into consideration, the substrates, which are biocompatible corundum-glass system composites were synthesized. Biocomposites, by combining the characteristics of these materials (Al_2O_3, glass), allow to achieve unique properties such as high mechanical strength, crack resistance, high biocompatibility and bioactivity [10, 11].

The aim of our work is to obtain corundum glass biocomposites and to meet the above criteria, both obtained in a simple, inexpensive and energy efficient way. This phase of work is devoted to the influence of the process of the preparation of substrates on their microstructure and biocompatibility.

2. Subject matter and methodology of research

The object of this study is a composite containing a matrix from submicrocrystalline sintered corundum and bioglass of $CaO–SiO_2–P_2O_5–Na_2O$ system (FB3) in 10, 20 and 30 wt%, obtained by the powder metallurgy technique, in the process of free sintering in air atmosphere, in an electric furnace. Submicrocrystalline sintered corundum is a variation of α-Al_2O_3, a new generation of corundum with an ultradispersive microstructure formed by transformation in the sol–gel process. A grain of F150 (from 125 to 150 μm) granulation was milled for 20 hours in order to achieve the specific surface area on the level 10.3 m^2/g [12].

Bioglass of the $CaO–SiO_2–P_2O_5–Na_2O$ system was obtained by fritting at 1350 °C using an electric furnace. The glass was milled for 20 hours to obtain a specific surface area (about 1.92 m^2/g).

The composites were obtained by two techniques:

– cold pressing and sintering,

– cold pressing, sintering and isostatic densification.

Samples of small (∅10×2 mm) and large (∅16×5 mm) size were pressed on a screw press, and afterwards some of them they were isostatically densificated or not. The heat treatment was performed without the mould in an electric furnace in air,

according to the established characteristics of isothermal soak, at maximum temperature for 2 hours.

Phase composition of biocomposite samples was identified using X-ray diffractometer August Siemens Type D 500 Cristal Reflex with a copper lamp with monochromatic radiation.

The physical properties (microscopic observations, porosity, water absorption, apparent density) were investigated on tablets ∅16×5 mm.

Microscopic observations conducted with scanning electron microscopes Joel JSM 6460 LV type were performed in high vacuum ($\sim 1.3 \times 10^{-3}$ Pa) at 20 kV accelerating voltage, magnification 20×, 100× and 1000× using of BEC image.

The measurements of the real density (d_{real}) of powder samples and helium density (on tablets ∅16×5 mm) were performed using helium pycnometer AccuPyc1330, Micrometrics company. Before the relevant measurements, the samples were initially desorbed by 10-fold pure helium flushing. Five parallel measurements were conducted for each sample. The results were used to calculate the closed porosity. The measurements of apparent density (d_{ap}) and total porosity (P_c) were carried out using GeoPyc density analyzer, model 1360 manufactured by Micrometrics. Ten simultaneous measurements were made for each sample. The apparent density (g/cm^3), the volume of pores in the material V_c (cm^3/g) and total porosity (%) were determined.

The measurements of the surface area (S_{BET}) and porosity were performed using a multifunctional apparatus (ASAP 2010, Micromeritics American company). The specific surface area was determined by physical S_{BET} nitrogen adsorption at liquid nitrogen temperature (77 K) from the Brunauer–Emmet–Teller equation (the theory of multilayer adsorption). Before the measurement, the surfaces of the test samples were subjected to desorption at temperature 1050 °C, in a vacuum and by flushing with pure helium. The sample degassing time was about 8 hours. The surface degassing state was controlled in an automatic mode.

For the surface area calculations based on the data from the adsorption isotherms, the relative pressure range p/p_0 was from about 0.06 to about 0.20%, and the volume and dimensions of the mesopores were calculated using p/p_0 of 0.97%.

The geometric structures of the biocomposites sample surface were determined, using a TOPO 01vP profilometer, by measuring the surface topography parameters (R_a, R_z, R_t), the image of 2D and 3D spatial, the profile material rate and amplitude distributions of the ordinates.

The biocompatibility of substrates was determined by observing the growth of human skin fibroblasts (line CCL 110 LGProchem company), cultured on tablets ∅10×2 mm in the Department of Applied Spectroscopy, Institute of Nuclear Physics PAS (Poland), using small tablets because of the procedural requirements.

The surfaces of substrates for cell cultures were prepared (FB3 *w*1*ssc*, *w*2*ssc*, *w*3*ssc*) by performing a quasi-metallographic section, 12-hour bath in a 70% alcohol solution and the UV lamp (λ = 254 nm) irradiation on each side of the sample. On such treated surfaces, the fibroblasts were cultured for 96 and 360 hours in a DMEM medium (Dulbecco's Modified Eagle Medium, Sigma) supplemented with 10% fetal

calf serum (FCS, Sigma) and 1% mixture of antibiotics (streptomycin, neomycin, penicillin). After 96 and 360 hours, the actin cytoskeleton was stained using phalloidine fluorescently labelled with Alexa-Fluor 488. Fluorescent images were recorded using the Olympus IX71 microscope equipped with a mercury lamp 100 W, the MWIG2 filter, and the digital camera XC 10 (working under Cell® program).

3. Results and discussion

The phase composition was determined for the samples doped with 10 wt% bioglass admixture, isostatically densificated or without densification (*w*1*ssc*) – Fig. 1. The identity of the spectra was found in both cases, suggesting the identity of the phase

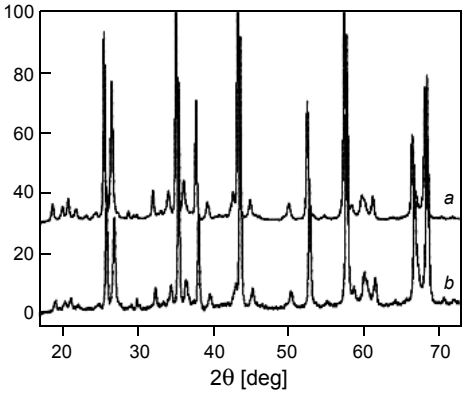

Fig. 1. The XRD spectra for *w*1*ssc* samples: isostatically densificated (*a*), without densification (*b*).

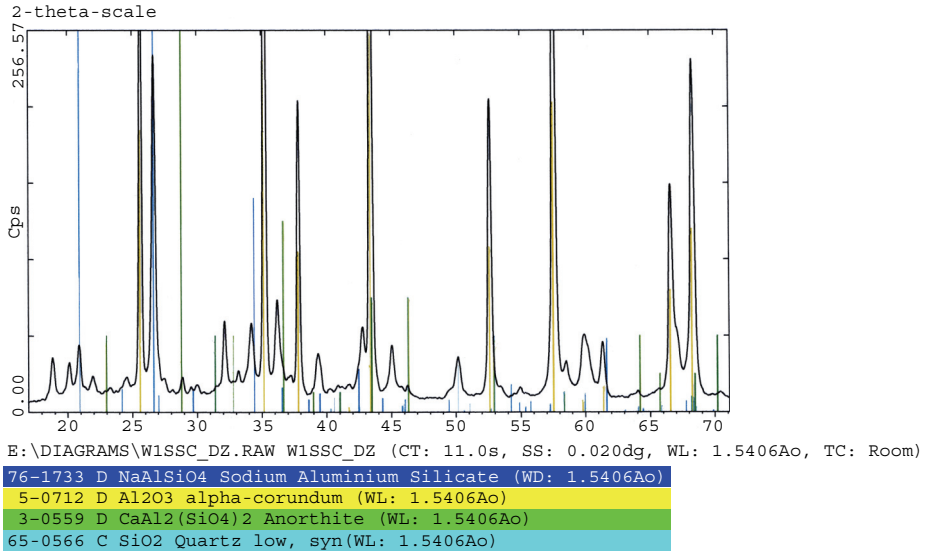

Fig. 2. Identification of XRD spectra for *w*1*ssc* isostatically densificated sample.

composition of the samples, regardless of the method used. It was identified in both samples of α- and κ-Al_2O_3 (κ in the trace amounts), anorthite – calcium aluminium silicate – $CaAl_2(SiO_4)_2$, sodium aluminium silicate $NaAlSiO_4$ and quartz SiO_2 (Fig. 2).

Microscopic observations carried out with the scanning electron microscope revealed the differences in the microstructure of isostatically densificated samples and of those without densification. The microstructures of composites contained grains of an irregular shape and of varying dimensions and pores. The dimensions of grains were of the order of less than 1 μm to several μm. Leeks (pores) had a varied shape and dimensions of the order of several micrometers. The samples obtained without densification were more porous; having more smaller pores (mesopores). The isostatically densificated samples had dense microstructure with a small amount of larger pores. The photos of the samples formed by various techniques are presented in Figs. 3 and 4, under magnification 1000×.

The studies of real density of the sample d_{real} (Table 1) showed that, regardless of the method of preparation, with an increasing glass content in the composite, the real density decreases (from 3.615 to 3.474 g/cm^3). It resulted from different densities of submicrocrystalline sintered corundum (3.887 g/cm^3) and glass FB3 system (2.564 g/cm^3).

From the results of determining the apparent density and the skeleton of both open and closed pores it became evident that the apparent density decreases with

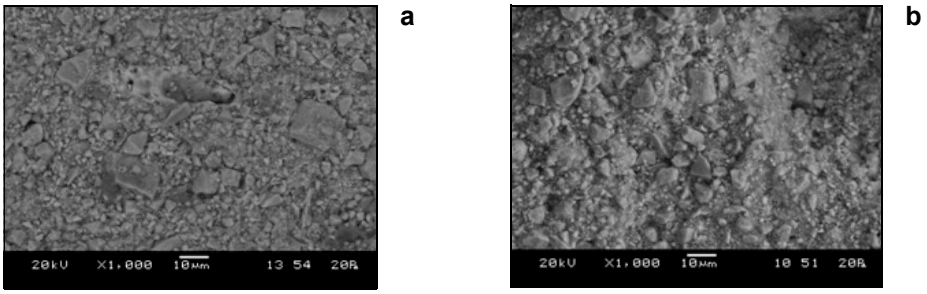

Fig. 3. The SEM observations of the sample *w*1*ssc*, magn. 1000×; isostatically densificated (**a**), without densification (**b**).

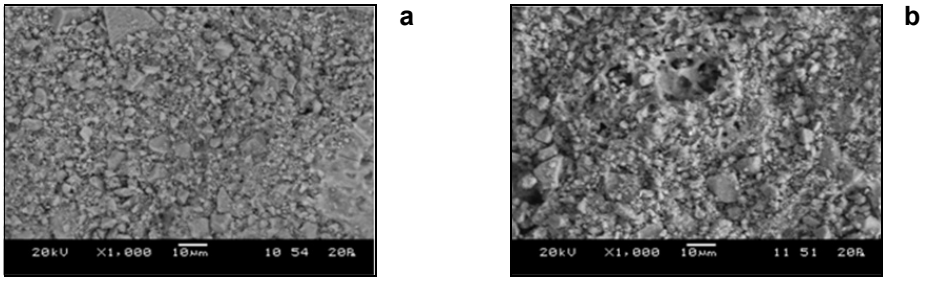

Fig. 4. The SEM observations of the sample *w*2*ssc*, magn. 1000×; isostatically densificated (**a**), without densification (**b**).

T a b l e 1. Density and porosity of biocomposite samples.

	Sample					
	*w*1*ssc* without densification	*w*1*ssc* isostatic densification	*w*2*ssc* without densification	*w*2*ssc* isostatic densification	*w*3*ssc* without densification	*w*3*ssc* isostatic densification
d_{real} [g/cm^3]	3.6150 ±0.0050	3.6150 ±0.0050	3.5296 ±0.0193	3.5296 ±0.0193	3.4743 ±0.0252	3.4743 ±0.0252
d_{hel} [g/cm^3]	3.6684 ±0.0089	3.5457 ±0.0109	3.5010 ±0.0167	3.5237 ±0.0030	3.3942 ±0.0249	3.4319 ±0.0402
$d_{apperend}$ [g/cm^3]	2.2821 ±0.0054	2.3179 ±0.0052	2.1564 ±0.0070	2.2516 ±0.0097	1.9637 ±0.0044	2.0442 ±0.0052
V_{pores} [cm^3/g]	0.166	0.149	0.178	0.160	0.215	0.198
V_{macro} [cm^3/g]	0.161	0.146	0.176	0.159	0.214	0.197
V_{mezo} [cm^3/g]	0.005	0.003	0.002	0.001	0.001	0.001
P [%]	36.9	35.9	38.9	36.2	43.5	41.2
S_{BET} [m^2/g]	1.99	1.45	1.02	0.88	0.89	0.50

an increasing glass content in both cases: the isostatic densification and without densification. However, the value of the apparent density of isostatically densificated samples was higher than of the samples without densification, which resulted from the process of obtaining. Based on the density measurements of helium and the actual porosity of the closed set, closed porosity (Pc) results showed that in the test samples the opened pores were present, while the closed pores were in negligible volume. The specific surface samples with an increasing S_{BET} bioglass content decreased in both cases (samples isostatically densificated and samples without densification). The values of S_{BET} in 0.5 to approximately 2.0 m^2/g testified about the low content of mesopores whose volume ranged from 0.001 to 0.005 cm^3/g.

It is visible that the total porosity increased with an increasing bioglass content for both isostatically densificated samples (from 0.146 to 0.197 cm^3/g) and those without densification (from 0.161 to 0.214 cm^3/g), containing mainly the macropores with dimensions greater than 0.1 μm, which resulted from increased quantities of bioglass mainly having opened pores.

The geometric structure of the surfaces isostatically densificated or without densification (*w*1*ssc*) was evaluated by measuring the surface topography performed using a TOPO 01vP profilometer developed and produced in IAMT (see Figs. 5 and 6). There were defined the basic parameters of roughness (R_a, R_z, R_t). In the isostatically densificated sample, the porosity visible in profile showed few large cavities (inequality). This was confirmed by the image analysis of 2D and 3D. The participation

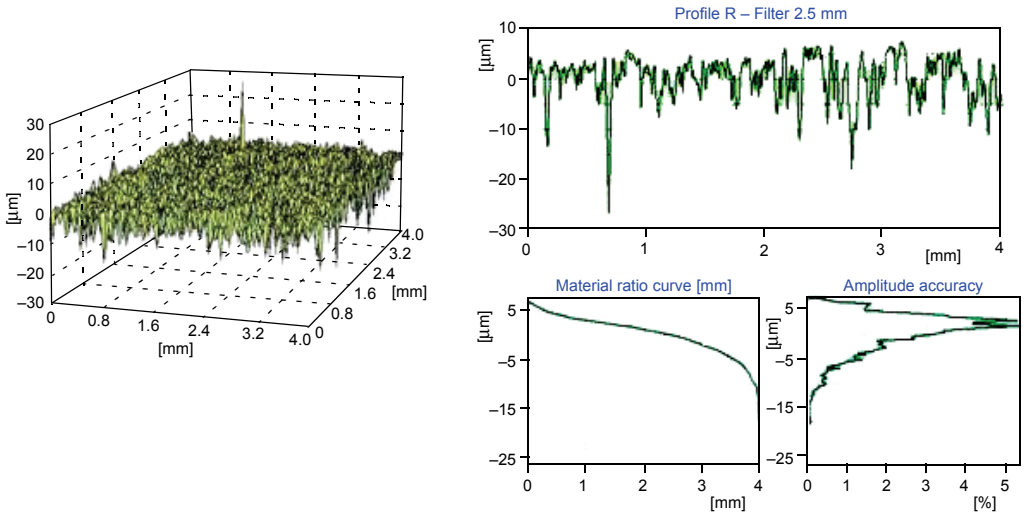

Fig. 5. The image of 2D and 3D, along with profile material rates and amplitude distribution of the ordinates for isostically densificated *w*1*ssc* samples.

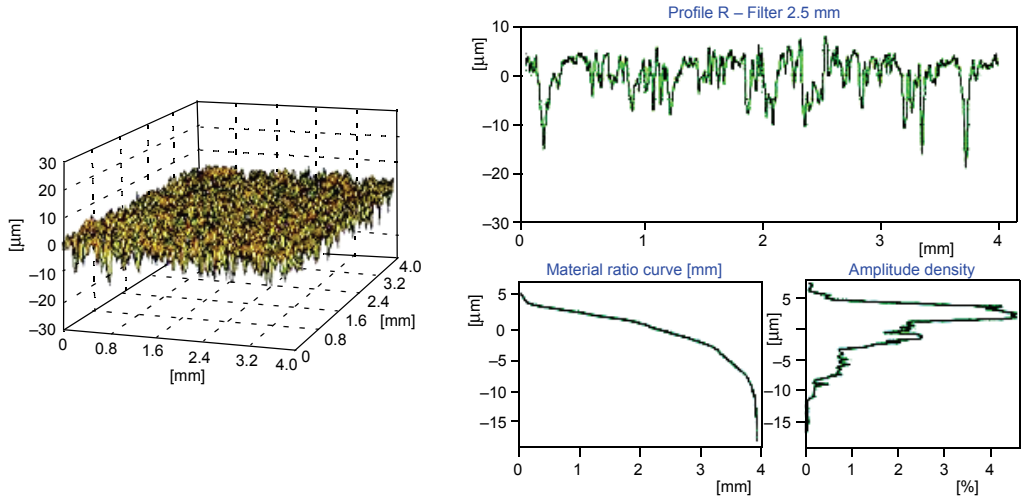

Fig. 6. The image of 2D and 3D, along with profile material rates and amplitude distribution of the ordinates without densification *w*1*ssc* samples.

of the profile material rates and amplitude distribution of the ordinate was shifted towards negative values.

The profile roughness of the sample without densification has a more blurred shape, with more small pits and a few large inequalities compared with the profile of the isostatically densificated sample (Figs. 5 and 6). It was testified by the image analysis of 2D and 3D. The amplitude distribution of the ordinate showed the presence of inequality-type cavities.

The microscopic observation of fibroblasts cultured on three types of substrates showed that the largest number of them grew on the surfaces containing the least amount of reinforcing phase (10 wt% bioglass admixture). Cultured fibroblasts showed better growing capability for substrates without densification, manifested in a larger number of living cells. Figure 7 shows the comparison of the amounts of fibroblasts cultured on two types of pills. The fewer the cells grew, the worse were surface properties required for the normal growth of cells (more cells died). Percentages represent the number of cells per pellet, as compared to the number of cells in the Petri dish. In the case of isostatically densificated substrates, for all samples, the number of cells was smaller. The number of cells did not depend linearly on the content of admixture of bioglass system in the substrate composite, assuming a V-shape.

The larger number of fibroblast present on the surfaces without densification was accompanied by the elongated shape of single cells (Figs. 8–10). For the surfaces after isostatic densification, the spindle-like shape was lost and a more round one was observed, indicating less favorable conditions for cell growth.

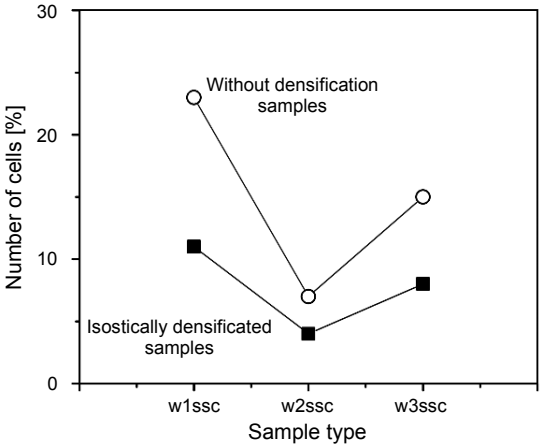

Fig. 7. Comparative amounts of the fibroblasts cultured on the biocomposite pellets of different composition.

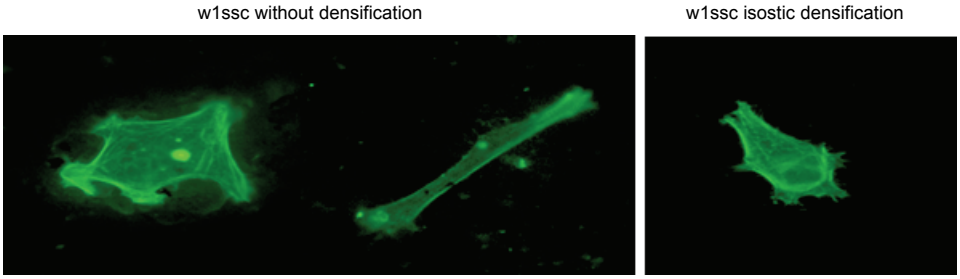

Fig. 8. Short-term culture (96 hours) on the *w*1*ssc* substrate.

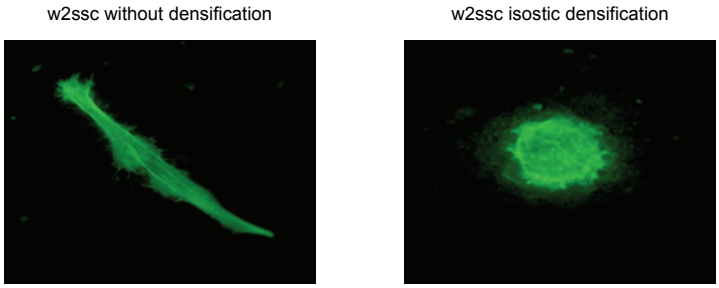

Fig. 9. Short-term culture (96 hours) on the *w*2*ssc* substrate.

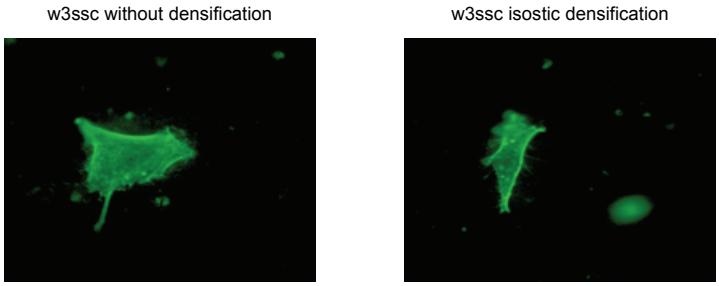

Fig. 10. Short-term culture (96 hours) on the *w*3*ssc* substrate.

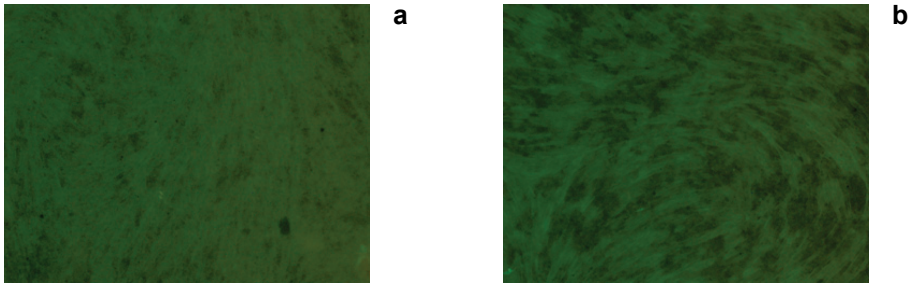

Fig. 11. Long-term culture (360 hours) on the substrate with (**a**) or without (**b**) densification *w*1*ssc.*

In the case of the long-term culture of fibroblasts (360 hours) there were not significant differences in the number of cells cultured on the surface of substrates with or without densification at the type *w*1*ssc* (Fig. 11).

4. Conclusions

Basing on the studies conducted so far, it can be stated that:

– the phase composition of *w*1*ssc* samples (with or without isostatic densification) was identical, regardless of the method of manufacturing. It was identified for *w*1, *w*2, *w*3*ssc* samples, α- and κ-aluminium oxide and at *w*1*ssc* anorthite $CaAl_2(SiO_4)_2$, at *w*2*ssc*, *w*3*ssc* $NaAlSi_2O_6$, additionally;

– observations of biocomposites substrates with a scanning electron microscope showed increased porosity in the samples without densification and with a large number of small pores. Isostatically densificated samples had a more compact structure, with a small amount of larger pores (see Figs. 1–3);

– evident is the difference in the total porosity between isostatically densificated and without densification samples. The samples without densification have a higher porosity with an increasing bioglass system (Table 1). A similar trend is maintained at isostatically densificated composites; with an increasing glass content the total porosity increases, while the apparent and real density decreases. It may be found that most pores in all the substrates are far greater than 0.1 μm;

– the analysis of parameters, figures and distributions showed significant asymmetry of the distribution in the negative value direction of unevenness for the both cases. It was bigger for not densificated samples;

– there is a lack of a clear simple relationship between the number of living fibroblasts and the chemical composition of substrates. All substrates without densification seemed to provide better growing conditions for fibroblasts, while isostatic densification induced worse surface properties for cell growth.

– the different dependence on the process of the preparation of substrates (isostatically densificated and without densification) was observed in the long-term culture (360 hours). On the substrates without densification, the same amount of the cells grew in the elongated shape as on isostatically densificated substrates.

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