Biocompatibility and cytotoxicity of bioglass-ceramic composite with various P_2O_5 content in Li_2O-SiO_2-CaO-CaF_2-P_2O_5 system on fibroblast cell lines

Soňa Jantová[a], Marica Theiszová, Patrik Matejov, Dušan Bakoš[b]

[a]*Institute of Biochemistry, Nutrition and Health Protection, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-81237 Bratislava, Slovakia*
[b]*Institute of Polymer Materials, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-81237 Bratislava, Slovakia*

sona.jantova@stuba.sk

Abstract

Synthetically prepared biomaterials that are used in clinical practise as alternatives of damaged, diseased or undeveloped parts of skeleton as well as missing parts of body for correction of inherited or pathological deformities or for traumatic injuries are the most often used materials. To improve the surface properties of biomaterials for cell adhesion and colonization, the bioglass with various content of P_2O_5 in oxide system Li_2O-SiO_2-CaO-CaF_2-P_2O_5 was prepared. In this work the biocompatibility and cytotoxicity of bioglass with 0%, 10%, 12% and 14% content of P_2O_5 were evaluated on mouse fibroblast NIH-3T3 cells and human fibroblast cells VH10 and B-HNF-1. The dishes without bioglass presence were used as negative control. We found that all used cell lines were sensitive to bioglass and all used methods have indicated slight cytotoxicity. The inhibition of cell proliferation was concentration- and time- dependent. The difference was observed among bioglass with various content of P_2O_5. The microscopic observations shown that control cells grew on the surface of the cultivation flask. The vast majority of them were scattered and exhibited a typical fibroblast morphology with an elongated and polygonal shape. All fibroblast cells growing on the surface of bioglass were homogeneously distributed on the substrate with good colonization. On the basis of the obtained results it can be concluded that bioglass with various content of P_2O_5 in oxide system Li_2O-SiO_2-CaO-CaF_2-P_2O_5 showed a slight cytotoxicity and very good biocompatibility.

Introduction

Bone is a dynamic tissue that can self-regenerate and self-model under normal physiological conditions. Bones play an important role in our lives, supporting our bodies and enabling us to perform various motions. Bone disease is a serious health condition that directly impacts on the quality of life of sufferers, particularly among the aged (Ohtsuki et al. 2009). Under some circumstances that result in large bone defects, such as trauma or tumour removal, bone cannot completely heal the defect site. Hence, bone grafting procedures have been developed to provide mechanical or structural support to the bone and to improve bone tissue formation.

The techniques used to repair damaged bones also are important and when the area of damaged bone is too large for self-repair, the damaged bones must be repaired by using of alternative materials such as autografts, allografts and artificial materials grafts. Artificial materials implanted into bone defects are generally encapsulated by a fibrous tissue and do not bond to living bone. To solve the problem of the foreign body reaction, bioactive ceramics have received much attention, and some bioactive ceramics are now clinically used as bone substitutes. Bioactive ceramics are generally regarded as ceramics that are designed to induce specific biological activity for repairing damage organs. For repairing bone tissue, the bioactivity is regarded as the capability to make direct contact with living bone after implantation in bone defects. The phenomenon of a new bone formation on the surfaces of bioactive ceramics is called osteoconductivity. Some bioactive ceramics have already been used to repair bone defects because their bioactivity allows them to achieve tight fixation resulting from direct bonding to living bone (Ohtsuki et al. 2009).

Bioactive glasses are special systems which are generally composed of SiO_2, CaO and P_2O_5. They can be synthesized by traditional melt quenching or by the versatile sol-gel process. The bioactive behavior of these glasses is defined as the ability to bond to soft and hard tissues by means of a series of reactions, which produces a strong, compliant interface between the glass and the tissue. Due to its superior quality in tissue integration and regeneration, bioactive glass has been used clinically in a variety of situations. Bioactive glass devices are now available to treat bone loss due to periodontal disease, conductive deafness,

alveolar ridge resorption and to fill cystic and surgically created defects, particularly in craniomaxillofacial sites.

Bioactive glasses in the system of SiO_2-CaO-P_2O_5-Na_2O have been shown to form a mechanically strong bone and soft tissue; bonding occurs by the rapid formation of a thin layer of hydroxycarbonate apatite (similar to biological apatite) on the glass surface when implanted or in contact with biological fluids. It has also been shown that bioactive glasses provide an ideal environment for colonization, proliferation and differentiation of human osteoblasts to form a new bone. Some compositions of bioactive glasses also show strong interaction with soft tissues which makes these materials attractive for applications in tissue engineering scaffolds. Thus, since bioactive glasses are able to form bonds to soft tissues, the incorporation of bioactive glass particles either as coatings or fillers into resorbable ceramic/polymer scaffolds is seen as a convenient way to develop scaffolds for tissue engineering applications (Gross, 1993).

Many compositions containing SiO_2, CaO and P_2O_5 are found to be biologically active. They include Ceravital®, Cerabone® A/W glass ceramics, β-tricalcium phosphate, sintered hydroxy apatite, 58S bioactive gel-glasses, modified 45S5 glass compositions and bioactive composites (Saravanapavan et al. 2003).

Composite material of bioglass – ceramic is characterized by properties that are generated by combination of its components properties. It is impossible of achievement by using ceramic or glass itself. Crystalline phases dispergated in glass improve mechanical properties of materials. Glass – ceramic with lithium silicate as the main crystalline phase achieves higher toughness as the average rigidity of teeth that is around 3.6 GPa. Crystalline fluorapatite increases even rigidity of it. In stomatology bioglass- and glass- ceramics based on lithium silicate and fluorapatite have wide application. They are prepared using conventional melting or sol-gel method. Process of preparations facilitates cast components in glass phase directly into the required shape of implants. Colour and brightness of this glass – ceramic type conform to demands of dental medicine.

Following this, Kuzielová et al. (2006) prepared a new composite bioglass-ceramic based on Li_2O-SiO_2-CaO-CaF_2-P_2O_5 system. The composite contained different contents of P_2O_5 and bioactivity of all samples has been proved *in vitro* by the formation of new layers of apatite-like phases after their soaking in simulated body fluid (SBF).

The aim of this experimental investigation was to use the embryonal three fibroblast cell lines (NIH-3T3, VH10, B-HNF-1) for study of biocompatibility and cytotoxicity of bioglass-ceramic composite with 0%, 5%, 10%, 12% and 14% P_2O_5 content. Standard plastic Petri dishes without bioglass sample were used as a negative control. The appropriateness of the use of these cells and their sensitivity to tested bioglass-ceramic samples we evaluated on the basis of four cytotoxic end points: cell proliferation, LDH release, protein and DNA cell content.

Material and Methods

Materials

The samples of bioglass-ceramic composite were prepared in the laboratory of Assoc. prof. Martin Palou, PhD. from Institute of Inorganic Chemistry, Technology and Materials, Faculty of Chemical and Food Technology, Slovak University of Technology (Kuzielová et al. 2006). P_2O_5 content in bioglass samples was 0%, 5%, 10%, 12% and 14%. The bioglass were sliced for squares with size of 1 cm^2 and sterilized 4 h at 200 °C in hot-air kiln.

Dulbecco´s modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin and antibiotics were purchased from Biocom Company (Bratislava, Slovakia). Trypan blue, MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Hoechst 33258 and the all other used chemicals were from Sigma (St. Louis, MO, USA).

Cell lines

Human fibroblast cell lines VH10 and B-HNF-1 and mouse fibroblast NIH-3T3 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells (starting inoculum 1.0 x 10^5 VH10 cells/mL, 5 x 10^4 B-HNF-1 cells/mL and 5.9×10^4 NIH-3T3 cells/mL) were grown in completely Dulbecco´s Modified Eagle Medium DMEM supplemented with 10% (vol/vol) fetal bovine serum, penicillin G (100 mg/L), streptomycin (100 mg/L) and kanamycin (100 mg/L) at 37 °C in humidified 6% CO_2 and 94% air atmosphere. Before a confluent monolayer was formed, the cells were harvested from the culture surface by incubation with a 0.25% solution of trypsin. When a suitable cell concentration was reached, the suspension was used for the experiments. The cells were currently in the exponential phase of growth. All experiments were performed in Petri dishes (Ø 60 mm). Cell viability was determined by a Trypan blue exclusion test.

Biocompatibility assessment

The biocompatibility of bioglass-ceramic composite samples was assessed on the base of the cell adherence and the colonization of fibroblast cultures on biomaterial surface by light microscopy. In the first step, the cell suspension was applied directly on the surface of bioglass, then the cells adhered for 1h in incubator at 37 °C and next the remaining culture medium was added. The control cells were cultured directly on the surface of Petri dishes. Colonization and the morphology of cells adherented and growing on the surface of Petri dishes and bioglass samples were assessed by light microscopy and photographed by Panasonic DMC/FX3.

Direct contact test

The basal cytotoxicity of bioglass samples was determined using the method of direct cell counting (Theiszová et al. 2008, Jantová et al. 2009). VH10 and B-HNF-1 cells were re-suspended in culture medium at density of 5×10^5 VH10 cells/mL and 2.5×10^5 B-HNF-1 cells/mL and plated on the surface of bioglass samples (size 1 cm^2) which were installed on the bottom in the centre of plastic Petri dishes. After 1 h of cell adherention in thermostate at 37 °C, 4 mL of culture medium were added and then the dishes were incubated for 48, 96 and 144 h, at 37 °C in a humidified atmosphere of 6% CO_2 in air. The negative control was performed by seeding the cell suspension directly on the bottom of plastic Petri dishes without glass in the centre. Cell proliferation was evaluated after 144 h in the absence or with the presence of bioglass samples. At 48, 96 and 144 h of treatment, the medium was removed, cells in monolayer were trypsinized (0.25%) (Biocom, Slovakia) for 3 min at 37 °C and counted in a Bürker chamber. Cell viability was determined by 0.4% Trypan blue staining.

Cytotoxic effect of bioglasses was evaluated in terms of inhibition of cell proliferation. Relative cell growth was calculated using the formula

$$\% \text{ of viable cells} = (K–E)/(K–Ko) \times 100$$

where Ko is the cell number at the time of the addition of bioglass, K is the cell number after 48, 96 and 144 h of cultivation without the bioglasses and E is the cell number after 48, 96 and 144 h of cultivation with the tested bioglass samples.

The cellular morphology was observed by a light microscopy (Meopta, Slovakia) and photographed by Panasonic DMC/FX3.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test

After 48, 96 and 144 h of NIH-3T3 cells cultivation with the tested bioglass samples, MTT-dissolved in culture medium (5 mg/mL solution of MTT) was added to the cells. Then, the cells were incubated for 3 hours at 37 °C. After incubation, medium containing MTT was removed. The reduced MTT in viable cells was dissolved by ethanol and its absorbance was measured on ELISA HUMAN READER at tested wavelength 550 nm and reference wavelength 630 nm (Mossmann 1983).

DNA content

The DNA content of each sample was measured using the DNA Quantitation kit (Sigma, Slovakia) according to Labarca and Paigen (1980). It is a fluorimetric method based on the binding of the bis-benzimide Hoechst stain (Hoechst 33258) to DNA.

After appropriate incubation periods, the samples were washed twice with phosphate buffer saline solution and trypsinized using 0.25% solution of trypsin. The cells were re-suspended in 1 mL of phosphate buffer saline solution. The Hoechst stain was prepared at a concentration of 1 mg/mL in 1x TNE buffer (10 x TNE buffer: 100mM Tris; 2.0 M NaCl; 10mM EDTA; pH 7.4). Tested samples (25 μL) were pipetted into a minicell cuvette. To each of these cuvettes, 50 μL of the Hoechst stain and 25 μL of 1x TNE buffer were added. The samples were incubated for 2 – 5 minutes at room temperature and protected from light. The samples were read on the Fluorescent Module of GloMax^{TM} 20/20 Luminometer (Promega, USA) at $\lambda = 360$ nm excitation and $\lambda = 460$ nm emission. The concentrations of DNA in tested samples were calculated using a standard curve generated from the known concentrations of DNA (Calf Thymus DNA, Sigma).

Protein content

After 24 h of NIH-3T3 cell cultivation in Petri dishes (6.5 x 10^4 cells/mL) tested bioglass samples were added. After 48, 96 and 144 h incubation at 37 °C in a humidified atmosphere of 6% CO_2 in air, the content of total cellular proteins according to Lowry et al. (1951) was determined. Bovine serum albumin was used as standard.

LDH quantification

Measurement of LDH release is an important and frequently applied test for cellular membrane permeabilization and severe irreversible cell damage. The amount of released LDH was measured according to Bergmeier (1970).

After appropriate incubation periods (48, 96 and 144 h), the same volume of the medium with and without cells was aspirated for each bioglass sample and stored in ice until measurement. Then the standard solutions for samples with released LDH (100 mM Tris-HCl buffer, pH 7.1, 15 mM NADH, 1.0 M pyruvate sodium salt) and total LDH (the same composition as for released LDH plus 10% solution of Triton-X-100) were prepared. The standard solutions were incubated at 31 °C for 5-10 min before measurement. The enzymatic reaction started by addition of very gently shaken sample, into the standard solution. The oxidation of NADH was measured on PU 8750 UV/VIS scanning spectrophotometer PHILIPS at $\lambda = 340$ nm. The absorbance decreased linearly during 60 s of measurement.

Statistical analysis

Results are shown as the arithmetic means ± s.d. (standard deviation) of the mean of three separate experiments (for each type of bioglass samples three separate Petri dishes were used in each experiment). Statistical analysis was performed by the ANOVA test for nonparametric measurements ($P < 0.05$ was considered statistically significant). The collected data were analyzed using linear regression tools of the Excel 2007 statistical software package (Microsoft Corp.).

Results and Discussion

Synthetically prepared biomaterials that are used in clinical practise as alternatives of damaged, diseased or undeveloped parts of skeleton as well as missing parts of body for correction of inherited or pathological deformities or for traumatic injuries are the most often used materials. To improve the surface properties of biomaterials for cell adhesion and colonization, the bioglass-ceramic composite with 0%, 5%, 10%, 12% and 14% content of P_2O_5 in oxide system of Li_2O-SiO_2-CaO-CaF_2-P_2O_5 was prepared by Kuzielová et al. (2006). In our work the biocompatibility and cytotoxicity of bioglass samples were evaluated on fibroblast NIH-3T3, VH10 and B-HNF-1 cells.

Firstly, the cytotoxic potential of bioglass-ceramic composite with 10% P_2O_5 content on murine fibroblast NIH-3T3 cells was evaluated in the primary screening. The cell proliferation and viability of the cells growing on the surface of Petri dishes (negative control) and bioglass-ceramic sample was evaluated by MTT test. At the same time, the content of cell proteins and DNA in the control and treated cells were also assessed after 48, 96 and 144 h of incubation. Light microscopy was used for morphologic study. NIH-3T3 cells growing on the

surface of standard plastic Petri dishes were used as the negative control. Results are presented in Fig. 1-3.
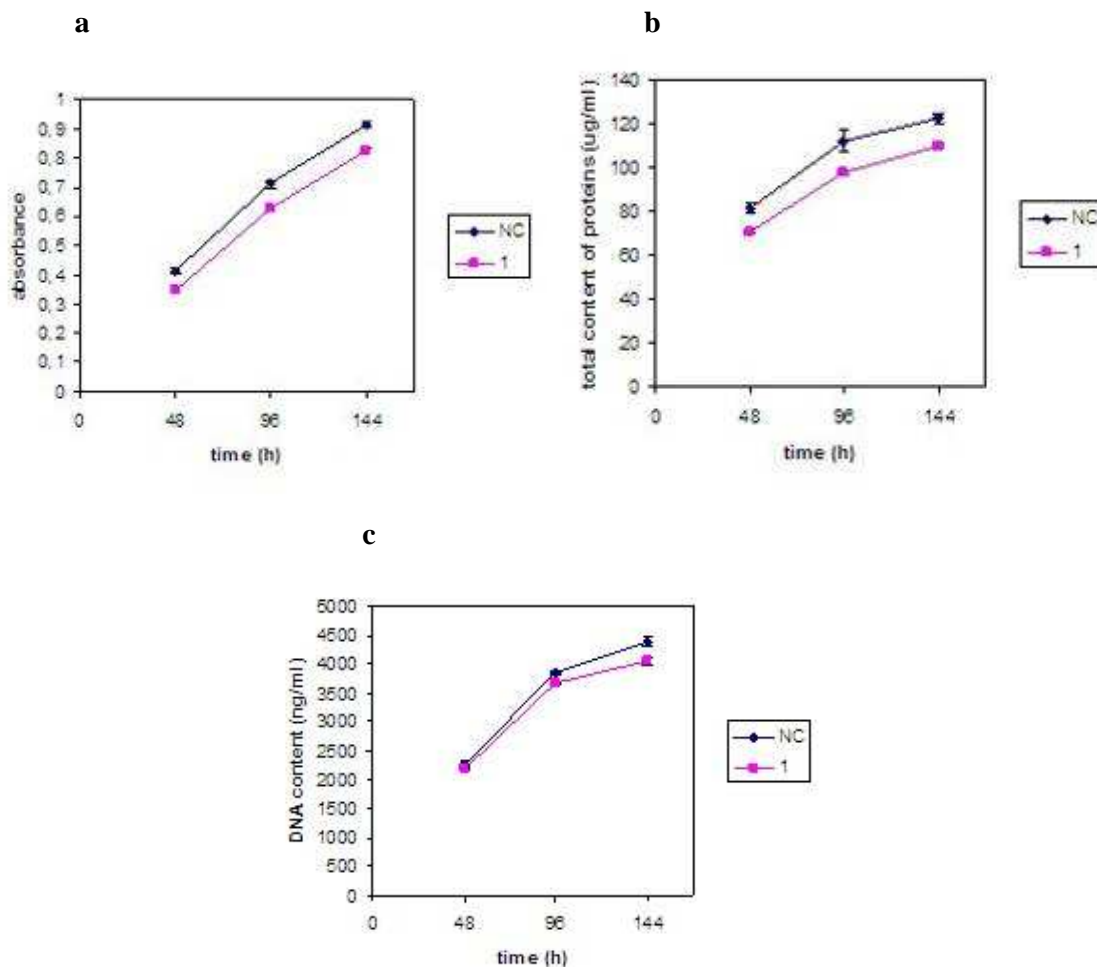
a

b

c

Fig. 1. The growth of NIH – 3T3 cells cultivated 48, 96 and 144 h on the surface of bioglass with 10% P_2O_5 content determined by MTT test (a), cell protein content (b) and DNA content (c). Control cells (NC) grew on the surface of plastic Petri dishes.

Fig. 1 shows the direct effect of negative control and tested bioglass-ceramic composite with 10% P_2O_5 on NIH 3T3 cell proliferation evaluated at 48, 96 and 144 h of culture by MTT test (a), protein content (b) and DNA content (c). During the whole time of cultivation, the absorbance obtained by MTT assay increased proportionally with the time of influence (Fig. 1a). As can be seen from Fig. 1b, the cell protein content proportionally grew with culture time in control cells (negative control) and in the affected cells (Fig. 1b), too.

Similarly, DNA content in control cells and affected cells increased with time of incubation with bioglasses (Fig. 1c).

The assessment of bioglass-ceramic sample cytotoxicity shown, that control NIH-3T3 cells (negative control) grew very well and after 144 h of incubation the monolayer was formed. On the other hand, the bioglass sample induced weak inhibition of NIH-3T3 cell proliferation. Negative control did not cause cytotoxicity during the all experiments. The cytotoxicity (inhibition percentage of cell proliferation) of bioglass-ceramic sample measured after 144 h of treatment by MTT test was 9.4%. Results obtained by cell protein and DNA content measurement shown, that cytotoxocity was 10% and 9%.

In the MTT test, to study the cell viability, murine fibroblast cell line NIH-3T3 growing on the Petri dish surface of negative control and bioglass-ceramic sample after 144 h were also photographed. The results are shown in Fig. 2. As can be seen in figure, after 144 h of treatment, the NIH-3T3 cells were blue stained (contained blue formazan crystals).

NC	10% P_2O_5

Fig. 2. Viability of NIH-3T3 cells cultivated 144 h on the surface of plastic Petri dishes (a), bioglass with 10 % P_2O_5 content (b) determined by MTT test.

The morphology of control and treated NIH-3T3 cells was assessed utilizing light microscopy (Fig. 3) .NIH-3T3 cells were platted on the surface of standard Petri dishes (negative control) and bioglass-ceramic composite with 10% P_2O_5 content. The NIH-3T3 cells treated with negative control and bioglass sample were homogeneously distributed on the substrate and produced a complete monolayer after 144 h of culture. The great majority of them were scattered and exhibited a typically fibroblast morphology with an elongated and polygonal shape. In some areas, cells in mitosis were observed. NIH-3T3 cells grown in direct contact with bioglass-ceramic sample (Fig. 3) did not show any morphological damage at 48 and 144 h of culture. Their morphology was completely similar to that of negative control cells.

Based on results obtained in primary screening, in the next experiments we studied the biocompatibility and cytotoxicity of bioglass-ceramic composite with another P_2O_5 content and other fibroblast cell lines. Samples with 0%, 5%, 12 % and 14% P_2O_5 content and the human fibroblast cell lines VH10 and B-HNF-1 were used for these studies.
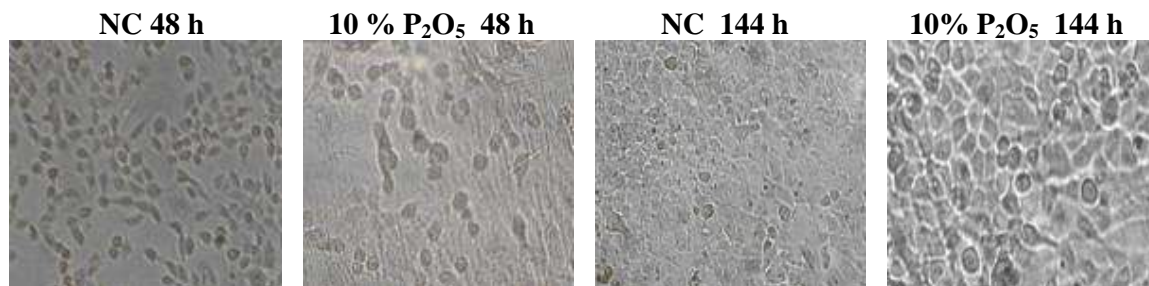
NC 48 h	10 % P_2O_5 48 h	NC 144 h	10% P_2O_5 144 h

Fig. 3. Morphology of NIH-3T3 cells cultivated 48 h (a) and 144 h (b) on the bioglass with 10% P_2O_5 content. Control cells (NC) grew on the surface of plastic Petri dishes. Magnification: 160×.

Biocompatibility of bioglass-ceramic composite with various P_2O_5 content was assessed after 144 h incubation of fibroblast cells VH10 and B-HNF-1 with bioglass samples by light microscopy. After application of cell suspension on plastic Petri dishes and bioglass samples surface the cell ability to colonize biomaterial was evaluated. The morphology and cell proliferation of adhered cells was further monitored. The standard plastic Petri dishes without bioglasses were used as the negative control. The results are shown in Fig. 4 and 5.

As based on Fig. 4, P_2O_5 content in bioglass-ceramic composite did not have the effect on the cell morphology and fibroblast adherence. VH10 and B-HNF-1 cells adherented in around of bioglass samples had the same morphology as the cells growing on the surface of Petri dishes (negative control). Increased number of necrotic cells was not observed.

The cytotoxic potential of bioglass-ceramic composite samples was evaluated together with the biocompatibility study. The glass-ceramics cytotoxicity was determined by the direct counting of cells growing on the bioglasses surface (Fig. 5). Viability and metabolic activity of cells were measured by determination of LDH release from treated cells. The results are shown in Fig. 6. The cells growing on the surface of standard plastic Petri dishes without bioglass-ceramic composite presence were used as negative control.

As seen from Fig. 5, the sensitivity of both human fibroblast cell lines on bioglass-ceramic composite samples was at the same level. Bioglass-ceramic composite with higher P_2O_5 content (12% and 14%) induced 14.9% and 17.0% (for VH10 cells) and 25.6% and

26.7% (for B-HNF-1) cell growth inhibition in comparison to the negative control. At the bioglass sample with 5% P_2O_5 content 5.7% (VH10 cells) and 16.3% (B-HNF-1 cells) cell growth inhibition was found. Bioglasses without P_2O_5 content caused lowest inhibition of cell growth, the cytotoxicity was 1.4% (VH10 cells) and 2.2% (B-HNF-1 cells).
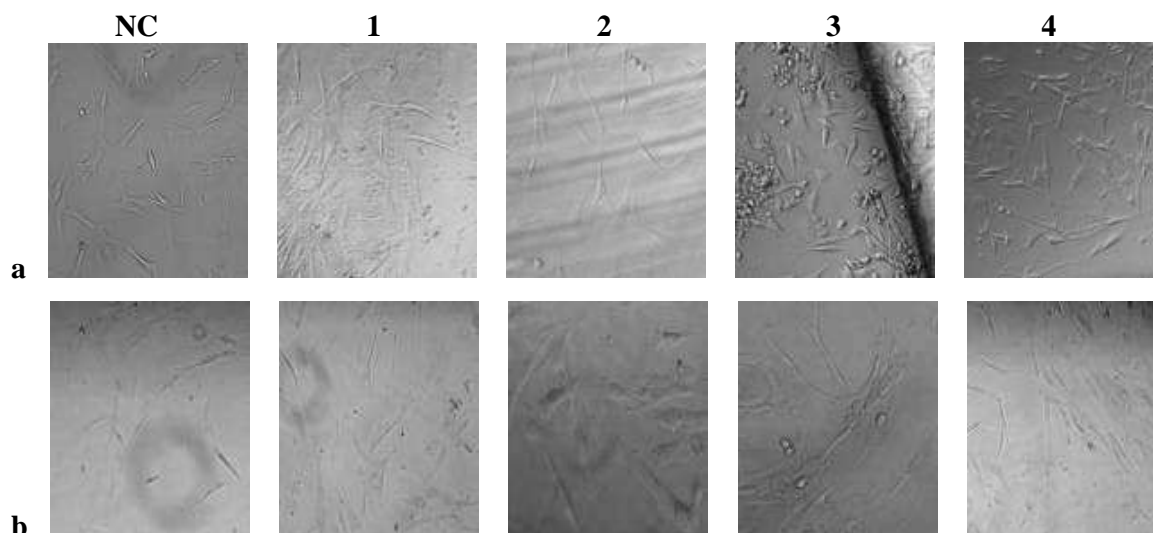

Fig. 4. The morphology of VH10 cells (a) and B-HNF-1 cells (b) growing 144 h on the source of bioglass without P_2O_5 (1) and with 5% (2), 12% (3) and 14% (4) P_2O_5 content evaluated by light microscopy. The cells growing on the surface of Petri dish were used as negative control (NC). Magnification: 25×.

The effects of bioglass-ceramic composite with different P_2O_5 content on level of LDH release from VH10 and B-HNF-1 cells after 144 h treatment are shown in Fig. 6. The percentage of LDH release was determined as the ratio of released LDH amount and total LDH amount. As can be seen in figure, slight percentage increase of LDH release was found in comparison to the negative control in both used cell lines cultivated on all types of bioglass. The amount of LDH release from cells proportionately increased with increasing content of P_2O_5 in bioglass. The percentage of LDH release was in the range from 17.9% to 27.3% (for VH10 cells) and from 22.9% to 28.6% (for B-HNF-1 cells). The lowest level of LDH release was found at the bioglass sample without P_2O_5 content. These results correlated with the results obtained in the experiments of cytotoxicity assessment by direct cell counting (Fig. 5).

From the study of relationship between P_2O_5 content in bioglass-ceramic composite samples and their cytotoxicity can be concluded, that with increasing P_2O_5 content cytotoxicity of tested samples increased, too.
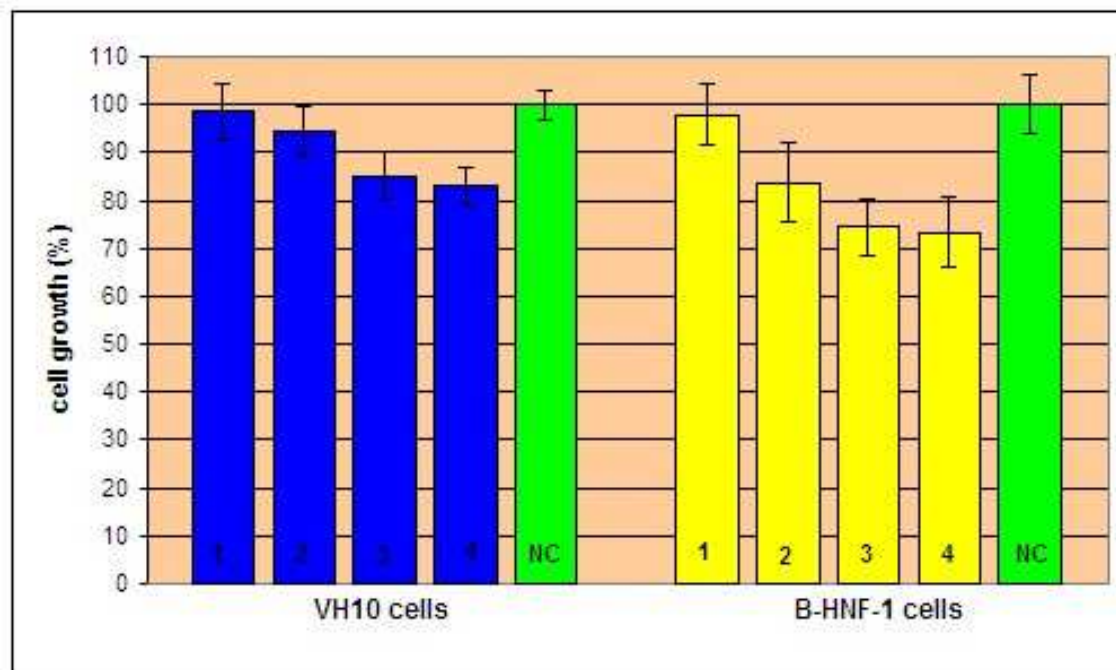
Fig. 5. The effect of bioglass without (1) and with 5% (2), 12% (3), 14% (4) P_2O_5 content on proliferation of VH10 and B-HNF-1 cells after 144 h of incubation. The cells grown on the surface of Plastic dishes were used as the negative control (NC).

It is generally known that P_2O_5 is one of the essential components of calcium phosphate bioglasses and bioglass-ceramics. Glasses and glass-ceramic materials based on the SiO_2-CaO-P_2O_5 system constitute an important group of biomaterials that have found wide application in medicine as bone implants. These biomaterials are able to bind with bone in a living organism through the formation of apatite like layer on the implant site or surface. Bone replacements by autografts and allografts have gained significant interest from the researchers during the recent years owing to the huge demand. Since the discovery of Bioglass (45S5) by Hench et al. (1971) which had proved its favourable features on the formation of new bone tissue at the implant site, several other glass and glass-ceramic compositions have been attempted aiming at better suit for the bone related surgical operations. Such type of bioactive material upon implantation is supposed to chemically interact with the body fluid in the tissue rehabilitation process. The behavior of bioactive glasses in the formation of new bone tissue depends on the chemical composition and textural properties (pore size and volume) (Balamurugan et al. 2007).

The effect of P_2O_5 on the surface reactivity of glasses and glass-ceramic has been reported by Lebecq et al. (2007). Authors demonstrated that the addition of small quantities of

phosphorus to bioglass compositions (up to 5 mol %) would improve its reactivity. By contrast, some phenomenological models show that even small quantities of phosphorus would diminish bioglass bioactivity. Moreover, when P_2O_5 is the glass former (45, 50 mol %), the formulations are not bioactive.
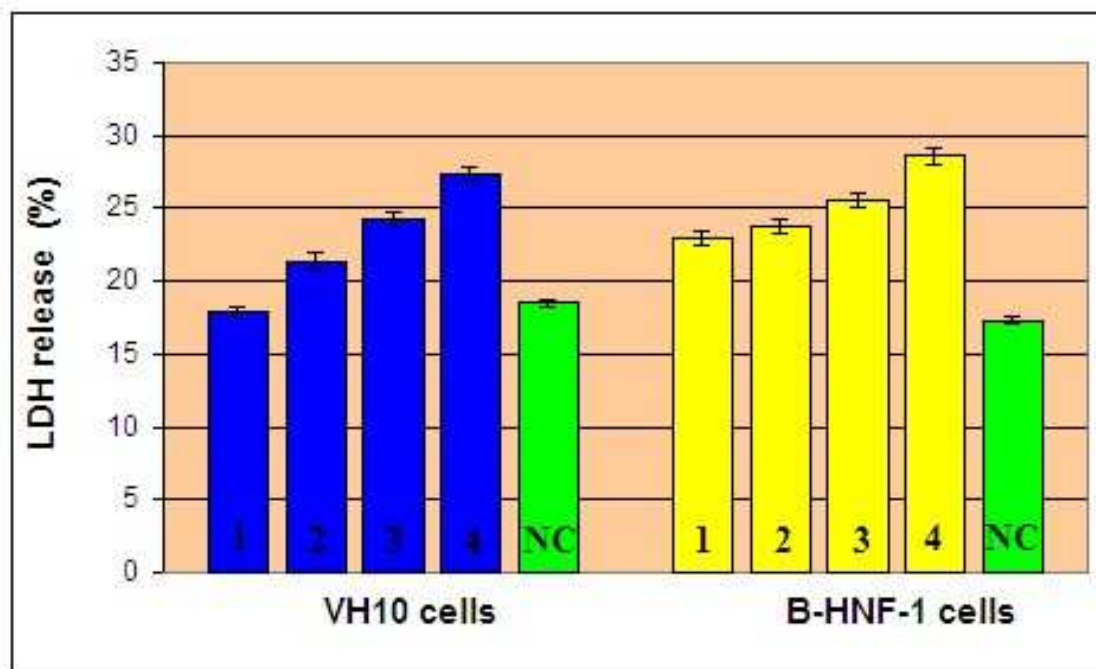

Fig. 6. The effect of bioglass without (1) and with 5% (2), 12% (3), 14% (4) P_2O_5 content on LDH release in VH10 and B-HNF cells after 144 h of cultivation. The cells grown on the surface of standard plastic dish (microplate) were used as the negative control (NC).

The biological effects of glasses and glass-ceramics with different P_2O_5 content in Si-Ca-Na-P system were presented by many authors. The original 45S5 Bioglass, as described by Hench (1971), is a silica-based melt-derived glass characterized by a high CaO : P_2O_5 ratio and very well biocompatibility. Balamurugan et al. (2007) prepared a sol-gel derived CaO-P_2O_5-SiO_2-ZnO bioglass with 5% a 10% P_2O_5 content. The osteoblast cells cultured on the bioglass discs consistently showed a high alkaline phosphatase activity and cell counts compared to cells cultured on either polystyrene plates or the base of CaO-P_2O_5-SiO_2 bioglass. Porous bioactive glass contained 6% P_2O_5, 45% SiO_2, 24.5% Na_2O, 24.5% CaO were synthesized by Livingston et al. (2002). This bioglass has been shown as a bioactive scaffold for tissue engineering. Preparation and *in vivo* evaluation of newly developed bioglass

ceramic with 12% P_2O_5 content demonstrated Lin et al. (1993). The rabbit condyle test showed that material formed a tight chemical bond with biological texture and had good biocompatibility. Vallet-Regi et al. (1999) prepared sol-gel bioglasses with 0% and 3% P_2O_5 content in the system SiO_2-CaO-P_2O_5 and influence of P_2O_5 on crystallinity of apatite formed *in vitro* on surface was studied. The obtained results showed that the apatite crystals formed on surface of the glass containing P_2O_5 in the composition were larger. Therefore, the presence of P_2O_5 in the sol-gel glass composition promotes the crystal growth of the apatite. Kitsugi et al. (1986) presented a new type of apatite-containing glass ceramic for the MgO-CaO-SiO_2-P_2O_5-CaF_2 system with 16.3% P_2O_5 content which can form a tight chemical bond with bones and has a high mechanical strength. Franks et al. (2001) have shown that glasses with a fixed P_2O_5 content of 45 mol% give a good range of glasses which melt and cast easily and show good biocompatibility as a biomaterial *in vitro*.

Based on the obtained results we can conclude that all used cell lines were sensitive to bioglasses. All used methods: cell proliferation, morphology, LDH level, protein and DNA cell content have indicated slight cytotoxicity. The inhibition of cell proliferation was concentration- and time- dependent and was in the range from 1.4% to 28.4%. The difference was observed among bioglasses with various content of P_2O_5. Bioglasses were biocompatible, all of cell types of fibroblasts adhered on their surface very well, the adhered cells did not have morphological difference to control VH10 a B-HNF-1 cells and their proliferation was very well. The microscopic observations shown that control cells grew on the surface of the plastic Petri dishes. The vast majority of them were scattered and exhibited a typically fibroblast morphology with an elongated and polygonal shape. In some areas, cells in mitosis were observed. VH10, B-HNF-1 and NIH-3T3 cells growing on the surface of bioglass-ceramic composite were homogeneously distributed on the substrate with good colonization. No significant morphologic changes were found in treated cells, their morphology was completely similar to that of the control cells. The amount of released LDH in cells cultured with bioglass-ceramic composite was increased in comparison to the control and was P_2O_5 content- and time-dependent.

Bioglass with various content of P_2O_5 in oxide system Li_2O-SiO_2-CaO-CaF_2-P_2O_5 showed a slight cytotoxicity and very good biocompatibility. Other studies are necessary for their next practical utilization.

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