**Indian Journal of Science and Technology,** *Vol 9(18), DOI: 10.17485/ijst/2016/v9i18/93753, May 2016*
*ISSN (Print) : 0974-6846*
*ISSN (Online) : 0974-5645*

# Ion-Beam Treatment of Glass Substrates for Creation of Biomatrices

**Ramil Nailevich Kashapov*, Elmira Linovna Peres Khusaenova, Ildar Abdulkabirovich Faizrakhmanov and Lenar Nailevich Kashapov**

Kazan Federal University, Kazan, 420008, Russia; Ramil.Nailevich.k@gmail.com, Lenar.NK123@gmail.com, Elmira.Linovna12@gmail.com, Ildar.Faizrakhmanov1@gmail.com

## Abstract

**Background:** We investigated the possibility of using ion-beam processing to create biomatrix which is used to produce monolayer for cell cultures on a photo glass. **Methods:** Experimental setup with the Kaufman type ion source which produces beams of inert gases and nitrogen in diameter of 100 mm, with an energy in the range of 0.1-1.7 keV, ion current density to 500 mA/cm$^2$ has been used. **Results**: Hepatocytes of mice, which deposited on the glass surface after ion-beam treatment better adsorbed (2.8 ± 0.2 times higher) than the control (untreated) and exhibited the formation of cell aggregates. The number of human fibroblasts, which adsorbed on the matrix, in (1.5 ± 0.5) times exceeds number of cells than on a smooth glass. It has been established that fibroblasts have a normal configuration for this type of cell onto modified glass. It has also been shown that cells "Candida albicans" are 5 times more adsorbed onto the modified glass than on the control glass. **Novelty:** Extract of the hepatocytes of mice, which is incubated on a modified glass, contains protein fractions with molecular weights of 30, 43 and 120 kDa which are not present on the control sample.

**Keywords:** Adhesion, Cell Culture, Fibroblasts, Glass, Hepatocytes, Ion-Beam Processing, Surface Modification

## 1. Introduction

Matrix materials are necessary and perspective in the field of biomedical cell technologies. Correctly designed and organized matrix can't be an inert environment and will be an active medium, which regulates basic processes of living cells: Survival, proliferation, metabolism and differentiation[1].

Growing tissue or individual cells of animal and vegetable origin has not only theoretical but also great practical significance. This is precisely what led to the intensive development of this trend in biotechnology. Cell culture is a relatively simple and convenient model for studying the mechanisms of genome expression, biogenesis of cellular organelles and other theoretical and practical problems of cell biology. Cell culture of higher plants is an alternative way to obtain a plant raw material for medicine, veterinary medicine, cosmetics and food industry. Culture of animal cells, especially of human, may be used in the diagnosis of disease, determining the toxicity of various substances, transplantation medicine and other fields.

Matrices which used to obtain monolayer cultures have several advantages. They require a smaller number of cells, allow the observation of morphological changes and do not require complex culture systems[2].

Therefore, the aim of this work was to create biomatrices by using ion-beam processing techniques with an inert material for culturing cells from different sources.

Based on the objectives the following tasks were set:

- To determine the effectiveness of the matrix for culturing cells.
- To determine the factors enhancing the adhesive properties.
- Exclude toxicity and oncogenicity of the substrate.

**Author for correspondence*

## 2. Materials and Methods

Experimental samples were obtained by setting up of ion-assisted deposition (working gas - Xe). Experimental setup with the Kaufman type ion source produces beams of inert gases and nitrogen in diameter of 100 mm, with an energy in the range of 0.1-1.7 keV, ion current density to 500 mA/cm$^2$ to create a matrices that was used as photo glass (All Union State standard 683-85), the size of 1 × 1-1, 5 × 1.5 cm[3,4,5].

Process of surface treatment is performed under vacuum, at a residual pressure in the chamber of $6{,}5 \cdot 10^{-3}$ Pa and subsequent operation of the magnetron at a gas pressure of 0.1-0.4 Pa. Ion bombardment was carried perpendicularly to the surface within 5, 10, 20 and 30 minutes.

The culture of hepatocytes was obtained from liver of male and female SPF CD-1 mice and the line Balb/c. was performed for perfusion with collagenase during 15 minutes at 37° C. Thereafter, liver extrapolated and hepatocytes were obtained[6] for the deposition after centrifugation for 10 minutes at 1500 rev/min. Cell viability was assessed by staining with trypan blue. Cell viability was on average 95%[7]. Hepatocytes were cultured for 5 days in a $CO_2$ incubator at 37° C. Fibroblasts were cultured to 8th cell cycle in a $CO_2$ incubator at 37° C. Culture "Candida albicans" was grown on thioglycolic medium in which microorganisms exhibit the greatest growth rate. Incubation took place in an incubator at 37° C for two days.

Allocation of proteins from hepatocytes was carried by removing cells from the surface of glasses and their deposition in a centrifuge at 1500 rev/min for 10 minutes. The cells were treated with lytic buffer and boiled for 5 minutes. The tubes were placed on ice for 5 minutes and disrupted by sonication in the cold, adding a protease inhibitor. DNase was then added to destroy DNA and placed in a water bath at 37° C. The composition of lysing buffer: 0.25 M Tris, pH = 6.8; 6% SDS; 40% glycerol; 0.04% BFS; $dH_2O$. The protein concentration was measured by NanoDrop 2000. Electrophoresis of proteins of hepatocytes was performed by polyacrylamide gel with SDS. During the electrophoresis, 4% concentrating and 13% separating gels were used. The resulting solution of separating gel was poured into electrophoresis chamber. To this was added a thin layer of $dH_2O$ gel. Polymerization took place in 20 minutes.

Duration of electrophoresis was in general 1 hour at a current of 10 mA until output samples from the wells, 100 V and 25 watts. Then, the current amounted to 23 mA until the arrival area of the dye to the lower edge of the gel, 150 V and 24 watts. Proteins were detected in the gel by staining with silver, which is more sensitive dye in comparison with coomassie.

The surface of the samples before and after ion beam treatment was studied by scanning electron microscope MERLIN Carl Zeiss (Germany). To do this, in the process of sample preparation Pl-Au coating was applied by magnetron sputtering on the matrix.

Analysis of the matrix surface with adsorbed hepatocytes of mice was performed by using atomic force microscope Bruker (Germany). In the process of sample preparation the samples were fixed in glutaraldehyde in phosphate buffer, then the samples were washed with phosphate buffer two times for 10 minutes, dehydration was conducted in alcohol at a concentration of 30 to 100% by 2 times from 10-20 minutes.

To determine the cytotoxicity matrices and the cells were plated in 96-well plates at a concentration of 0.5 * 105 cells/ml (2000 fibroblast cells/ml). After 24 hours MTT solution was added to wells and after 4 hours of incubation was removed. Then was added DMSO to dissolve the product and to restoration of MTT by viable cells - formazan. Control group consisted of cells in the usual plastic and on the control glass. Analysis was performed on a microplate photometer (STAT FAX 2100), wavelength - 545 and 630 nm.

## 3. Results and Discussion

The results obtained after microscopic 2D-matrix in the SEM (Figures 1, 2) have shown that the matrix has a certain relief (ordered honeycomb structure) and it is possibly provide its adhesive properties. We found that on glasses which treated for 30 minutes, the cells grow

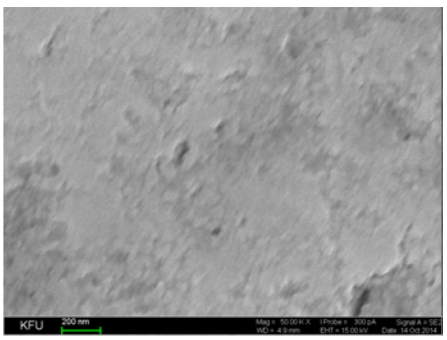


**Figure 1.** The surface of the glass before the treatment (50 000 magnification).

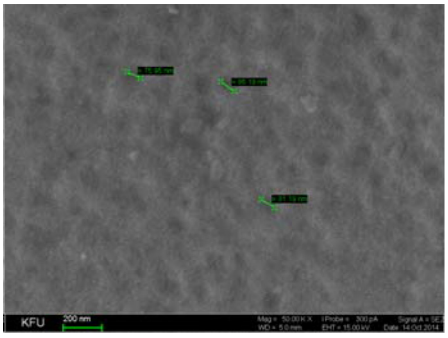


**Figure 2.** The surface of the glass until a treatment after the ion beam treatment (50 000 magnification).

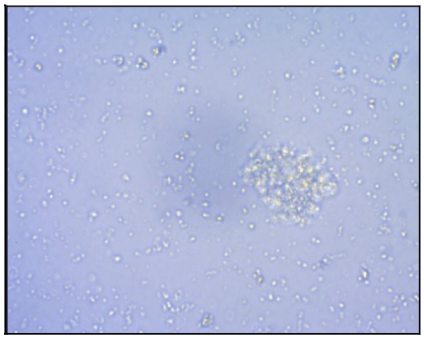

**Figure 4.** The cultivation of hepatocytes to the matrix after ion beam treatment (Zoom 40).

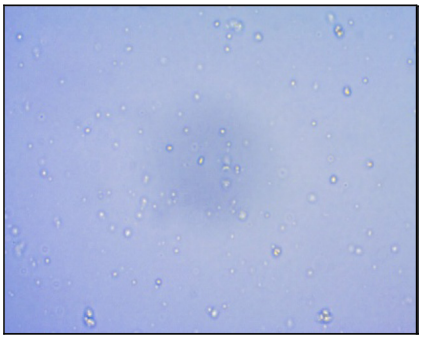

**Figure 3.** Cultivation of hepatocytes on the control glass (Zoom 40).

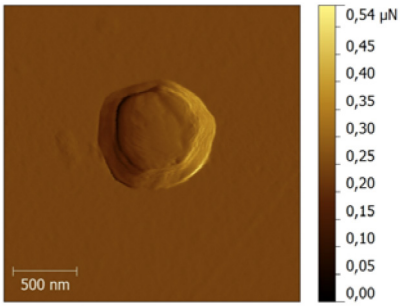


**Figure 5.** The attachment of hepatocytes to the glass surface.

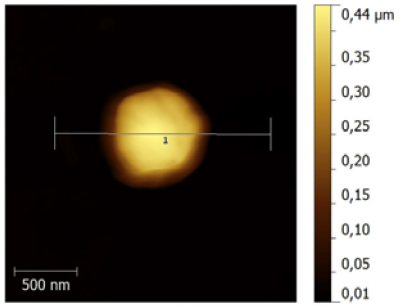


**Figure 6.** Attachment of hepatocytes to the glass surface.

much better than on the slides treated with 5, 10 and 20.5 minutes.

Work of analyzing the effectiveness of cultivation of cell lines on artificial storage media has been done. After 4 days of culture revealed that the number of hepatocytes grown on the matrix after ion beam treatment (2.8 ± 0.2) times higher than that of the reference glass (Figures 3, 4). Furthermore, it was proved that on third day the cells begin to form aggregates (Figure 4).

Hepatocytes have a diameter less than 1 micron and question arises about the proof of attachment cells to the matrix surface[8]. Due to the low resolution of light microscopes we can't observe a significant change in cell shape of hepatocytes during attachment to the surface. To solve this problem, we conducted a study on the AFM and found that the cells attached to the surface are really due to the flattening and changing the shape of the cells (Figures 5, 6).

The amount of fibroblasts on the matrix after ion beam treatment (1.5 ± 0.5) times higher than the glass without treatment (Figures 7, 8). It has been found that fibroblasts adsorbed onto modified glass had normal configuration for this type of cell[9].

To see the difference between the protein composition of cells after isolation and cell taken from 2D-matrix, it was necessary to apply the electrophoresis. Electrophoresis of proteins in polyacrylamide gel with SDS showed differences in the protein composition of cells (Figure 9):

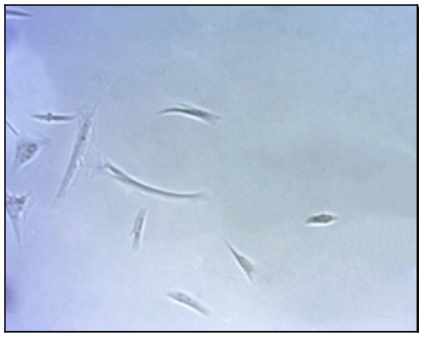

**Figure 7.** The cultivation of fibroblasts on the smooth glass (Zoom 20).

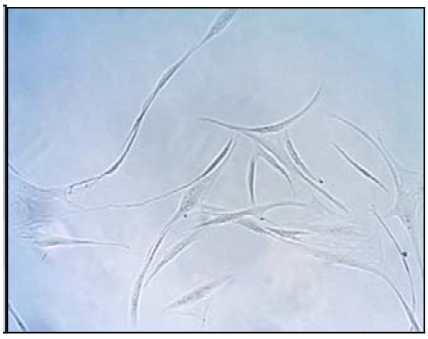

**Figure 8.** The cultivation of fibroblasts on 2D matrix (Zoom 20).

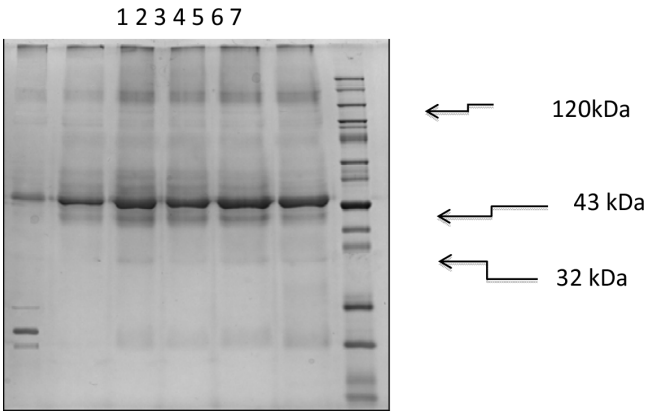


**Figure 9.** Electrophoregram of proteins in polyacrylamide gel with SDS: 1. Of hepatocytes proteins after separation; 2. Proteins of hepatocytes taken from a control glass; 3, 4, 5, 6. Proteins of hepatocytes taken from 2D-matrix with different processing time; 7. Marker.

It can be assumed that the cell extracts of hepatocytes, which intubated on 2D-matrix, contained proteins of adhesion and proteins of intercellular contacts with molecular weights of 30, 40 or 120 kDa.

## 4. Findings

Surface treatment of photoglass by means of ion-beam method is suitable for preparing biomatrix, which is used to accelerate the growth of various cell cultures, in experimental setup with the Kaufman type ion source which produces beams of inert gases and nitrogen in diameter of 100 mm, with an energy in the range of 0.1 - 1.7 keV, ion current density to 500 mA/cm$^2$.

## 5. Conclusion

Based on the results of scanning electron microscopy it is observed that the occurrence of an orderly cellular structure on the surface of the matrices after ion-beam treatment which increases cell adhesion. Toxicological tests showed the absence of cytotoxicity and carcinogenicity of glasses after the ion-beam treatment.

Hepatocytes of mice, which deposited on the glass surface after ion-beam treatment, adsorbed in an amount of (2.8 ± 0.2) times higher than before the treatment and observe the formation of cell aggregates. The number of human fibroblasts, which adsorbed on the matrix, in (1.5 ± 0.5) times exceeds number of cells than on a smooth glass. It has been established that fibroblasts have a normal configuration for this type of cell onto modified glass. It has been shown that cells "Candida albicans" a 5 times more active adsorbed onto modified glass than on the control glass. Extract of the hepatocytes of mice, which is intubated on a modified glass, contains protein fractions with molecular weights of 30, 43 and 120 kDa which are not present on the control sample.

## 6. Acknowledgements

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University. This work was financed by grants of Kazan (Volga region) Federal University of the state of tasks in the sphere of scientific activity.

## 7. References


1. Fattakhova AN, Khramova A. Belinite MA, Kashapov RN, Kashapov NF. Human and animal cells adhesion on the modified glass. Tsitologiya. 2014; 2:12–20.
2. Freshney R, Freshney M. Isolation and culture of animal and human hepatocytes. Culture of Epithelial Cells; 2nd ed. 2002.
3. Gavrilova VA, Kashapov NF, Kashapov RN. Plasma application of protective polymer-powder coatings to ultrasonic sensors. Biomedical Engineering. 2011 Dec; 45(5): 198–200.
4. Kashapov LN, Kashapov NF, Kashapov RN. Influence of plasma-electrolyte discharge to the glass surface. Journal of Physics: Conference Series. 2014; 23: 234–41.
5. Kashapov LN, Kashapov NF Kashapov RN. Research of the impact acidity of electrolytic cathode on the course of the plasma-electrolytic process surface. Journal of Physics: Conference Series. 2013; 23:201–11.
6. Guguen C. Isolation and culture of animal and human hepatocytes. Culture of Specialized Cells; 2nd ed. 2002.
7. Yin L. Wei Y, Wang Y, Xu Y. Long term and standard incubations of WST-1 reagent reflect the same inhibitory trend of cell viability in rat airway smooth muscle cells. International Journal of Medical Sciences. 2013; 10(1):68–72.
8. Biserova NM. Imaging methods of biological ultrastructures. A Practical Guide for biologists; 2013.
9. Pooryusef K, Kangarlou H. Effects of temperature on structural properties of indium oxide thin layers produced by CBD method. Indian Journal of Science and Technology. 2015 Dec; 8(35):35–42.