что в таких концентрациях КРШ покрывают почти всю поверхность культуры и потому задерживает рост клеток.

В контроле клетки в течение всего срока наблюдения сохраняли способность равно-мерно пролиферировать и мигрировать, что играет важную роль в процессах регенерации. При культивировании с КРШ в концентрации 0,01 мг/мл заметных изменений не наблюдается (рисунок 2).

Таким образом, результаты модельных экспериментов с использованием фибробластов в культуре клеток Т3В3 показали, что КРШ не оказывают заметного негативного действия на эти клетки. Данные, полученные в модельных экспериментах, дают наглядное представление о том, каким образом процессы могут осуществляться в присутствии КРШ в опытах *in vivo*.

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Biocompatibility of carbonized rice husk with a rat heart cells line H9c2

The objective of this *in vitro* study is to explore the cytocompatibility properties of purified carbonized rice husk (CRH) with cardiomyocytes. Microscopic observation evidenced that CRH cause slight modification in cell shape and in cell count only after five days of culture. The effect of CRH bound to cells was tested by reseeding treated H9c2 cells. Cells from a CRH-treated sample showed an ability to proliferate at the small concentration of CRH. At the concentration of 0,1 mg/ml CRH-treated cell showed a limited proliferation, however overtime cells continued to grow and recovered in shape and number.

Keywords: sorbent, cell viability, cardiomyocyte, migration, proliferation.

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Биосовместимость карбонизованной рисовой шелухи (CRH) и культуры клеток кардиомицитов (H9c2)

Цель данной работы – изучение биосовместимости сорбента на основе карбонизованной рисовой шелухи (CRH) и культуры клеток кардиомицитов (H9c2). Микроскопическое исследование показало, что CRH в малых концентрациях заметного влияния на количество жизнеспособных клеток не оказывают, за срок наблюдения клетки равномерно пролиферировали и мигрировали. Таким образом, результаты модельных экспериментов с использованием кардиомиоцитов в культуре клеток H9c2 выявили, что CRH не оказывают заметного негативного действия на эти клетки.

***Ключевые слова*:** сорбент, жизнеспособность клеток, кардиомицит, миграция, пролиферация.

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Карбонизделген күріш қауызы (CRH) негізіндегі сорбент пен кардиомицит клетка дақылының (H9c2) биосыйымдылығы

Жұмыстың басты мақсаты – карбонизделген күріш қауызы (CRH) негізіндегі сорбент пен кардиомицит клетка дақылының (H9c2) биосыйымдылығын зерттеу. Микроскопиялық зерттеулер бойынша, төменгі концентрацияда CRH тіршілікке қабілетті клеткалар санына айтарлықтай өзгерістер көрсетпейді, бақылау барысында клеткалар біркелкі пролиферацияланды және миграцияланды. Олай болса, модельді зерттеудің

нәтижелері CRH сорбентінің кардиомицит H9c2 клеткасының дақылдарында кері әсер туғызбайтындығын көрсетті.

***Түйін сөздер*:** сорбент**,** клеткалардың тіршілікке қабілеттілігі, кардиомицит, миграция, пролиферация.

With the increasing interest to carbon materials obtained from vegetative raw materials by carbonization and their application in medicine and biotechnology it has become a focus to investigate the effect of these carbon materials on cardiomyocytes. Within the perspective of the in vivo use of carbon materials, biocompatibility represents an important problem as so far has not been investigated in depth [1].

Exposure to carbon materials has been associated with increased incidence of skin and lung diseases [2]. But purified carbon materials are eligible for use with biological systems. In particular, local deposition and prolonged release of appropriate agents is a promising avenue to provide effective treatments in humans [3-4].

Materials and methods

Cells. The cardiac muscle cells are a rat heart cell line H9c2 at passages from 69 to 72 were used for the experiments. H9c2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS at 37°C in 5% CO_2; the medium was changed every 2 days. Sub confluent cells were detached with trypsin and reseeded 24 h before treatment at a density of 40 000-60 000 cells cm^{-2} in a tiny Petri dishes.

Preparation of CRH. CRH were obtained in the Combustion Problems Institute at the high temperature carbonization and were washed with distilled water, rinsed with PBS, and sterilized by autoclavation at 120 °C for 30 min.

Experimental approach. Cells were seeded and let to grow for 24h before treatments.

Treatments. Untreated control cells were administered the complete medium without CRH (1); treated cells were administered the complete medium with CRH 0,001 mg/ml (2); 0,01 mg/ml (3) and 0,1 mg/ml (4). The experiments were performed in triplicate.

Light microscopy evaluation. Cardiomyo-cytes treated or not with CRH were evaluated at relevant time points by light microscopy to assess cell proliferation and viability: digital photomicrographs were recorded.

Number of viable cells. Myocytes were incubated for 24h at standard culture condi-tions to determine the viability following treatments. The number of viable cells was determined with trypan blue exclusion. In brief, cell monolayers were rinsed twice with PBS and resuspended with trypsin. The cells were immediately stained with 0,4% trypan blue and the number of viable cells was determined using a hemocytometer under a light microscope.

Results and discussion

Binding of CRH to cells. The attached cells were treated with CRH; the medium was changed after 24h. This treatment showed that CRH did not bind the cell membrane and the repeated washing is able to detach and remove them. When the cells were detached from the dish by trypsinization the absence of CRH was clearly.

Cell proliferation and shape changes. As seen in figure 1, no difference in cell growth is observed in CRH-treated (0,001 and 0,01 mg/ml) cells in comparison to untreated cells after one day of treatment. CRH-treated cells were not different from untreated cells as regards cell shape. But in the concentration of 0,1 mg/ml CRH-treated cells displayed in shape and numbers in comparison to untreated cells.

Cell viability. Cell viability of culture there was not different between CRH-treated cells in the concentration of 0,001 mg/ml and untreated cells; However, cell death was slightly higher in the concentrations of 0,01 and 0,1 mg/ml. Trypan blue exclusion confirmed these observations (figure 2).

Reseeding of CRH treated cells. To determine whether CRH bound to cell membrane affect the repeated seeding of H9c2, CRH-treated cell detached and reseeded. Three PBS washing performed before cell trypsinization to eliminate the unbound CRH. As seen in figure, 24h after reseeding, untreated cells and CRH-treated cells (2); (3) showed the average rate of exponential growth, while CRH-treated cells in the concentration of 0,1 mg/ml showed a definite difference in shape, with a high degree of cell death.

This work demonstrates that CRH interact with cardiac muscle cells do not bind to cell membrane and has limited effects on cell proliferation and viability.

Indeed this material shows no evident short-term toxicity toward H9c2 cells, as demonstrated by trypan blue positive cells.

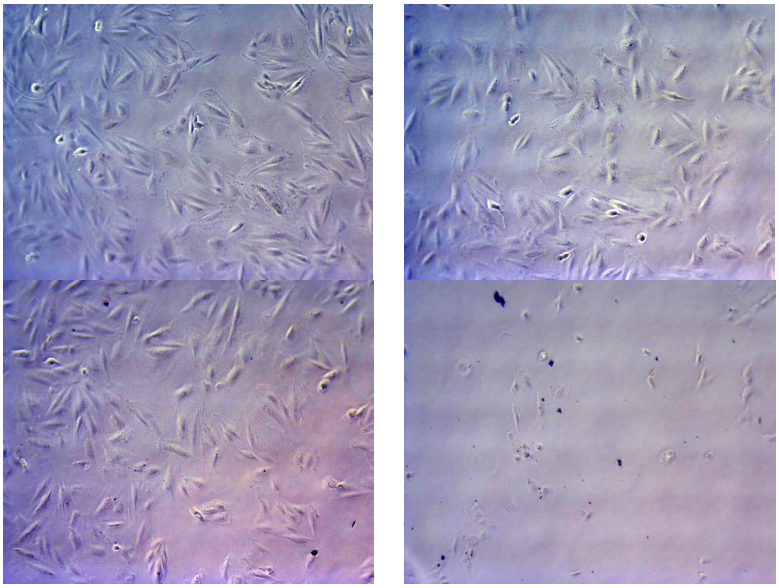

Figure 1 – Photomicrographs of H9c2 cells untreated (on the left upper) and after treatment with CRH in the concentration of 0,001 mg/ml (on the right upper); 0,01 mg/ml (on the left lower) and 0,1 mg/ml (on the right lower); magnification 40×

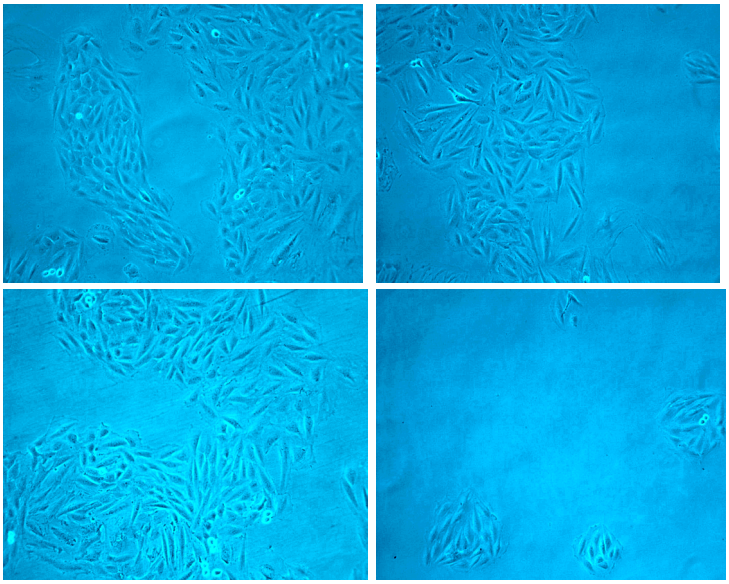

Figure 3 – Photomicrographs of H9c2 reseeded cells untreated (on the left upper) and CRH-treated cells in the concentration of 0,001 mg/ml (on the right upper); 0,01 mg/ml (on the left lower) and 0,1 mg/ml (on the right lower); magnification 40×

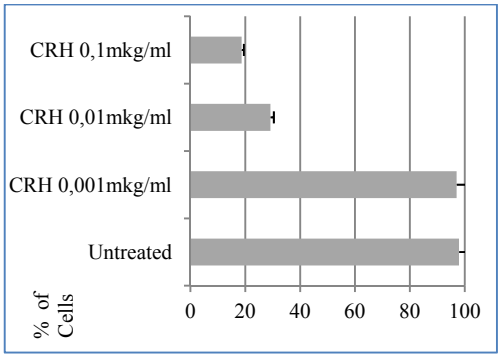

Figure 2 – CRH-treated cell viability

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