STUDY OF SILICA-BASED ELECTROSPUN NANOFIBERS AS A SCAFFOLD FOR HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Abstract

Electrospun nanofibers with their nanoscale structure and ability to mimic native extracellular matrix (ECM) harbor a great potential for biotechnology, biomedicine, and tissue engineering. Inorganic nanofibers, such as silica-based nanofibers, have attracted attention for their physical and chemical stability, surface functionalities and hydrophilic nature.

In this study, we tested biodegradable SiO_2 nanofibers prepared by sol-gel method and needleless electrospinning as a scaffold for human bone marrow mesenchymal stem cells (hMSC), cells which are promising for regenerative medicine. The nanofibers were tested for cytotoxicity and capability to support cell adhesion.

For cytotoxicity testing, the nanofibers were incubated for 24 or 72 hours in phosphate-buffered saline (PBS) or cell culture media supplemented with serum. The eluate was then applied to hMSC for either 24 hours or 4 days and cytotoxicity was evaluated using MTT assay. While eluates from PBS-treated nanofibers had no effect on the cells, eluates from nanofibers treated with serum-containing media had negative impact on cell metabolic activity.

The adhesion of hMSC to nanofibers was studied using confocal microscopy. The nanofibers supported cell adhesion, however cell survival was limited.

In conclusion, silica-based nanofibers supported the adhesion of hMSC, however, they were not sufficient for extended cultivation and nanofibre eluates decreased the viability of the cells. Additional tests will be necessary to clarify the interactions of hMSC with silica nanofibers and to optimize conditions for long-term cell survival.

Keywords: Silica nanofibers, electrospinning, sol-gel method, cytotoxicity, hMSC

1. INTRODUCTION

Many biocompatible polymers, mostly organic macromolecules, were successfully electrospun as either biodegradable or non-biodegradable mats for biomedical application. Due to their properties, such as specific surface, porosity, and fiber diameter, they can mimic the extracellular matrix and could therefore be used as scaffolds in wound dressing, as drug delivery systems, or carriers for cell cultivation [1]. Silica-based nanofiber mats, an example of inorganic nanofibers, can also be electrospun, mostly via the sol-gel process and needle electrospinning [2,3,4], and can be successfully used as scaffolds for cells [5]. Recently, needleless electrospinning has emerged as a new procedure for large scale nanofiber production for broad application. Instead of a needle, nanofibers in needleless electrospinning devices, such as “Nanospider”, are produced using a roller or a string [6]. Such nanofibers could potentially offer a cheap alternative to presently used organic fibers with many additional advantageous properties, including physical and chemical stability, surface functionalities, and hydrophilic nature.

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types. They exhibit wide distribution in the body and from the perspective of function, they have a major role

in normal growth and tissue repair. MSCs can be isolated from various tissues, such as bone marrow, adipose tissue, dental pulp, etc. quite easily and can be kept and expanded in cell culture. Therefore, MSCs attract attention and interest for their great potential in tissue repair, regenerative medicine and tissue engineering [7].

Nanofibers for biomedical application as carriers for cells have to support cell attachment and function and need to match biocompatibility criteria. In this study we tested these criteria for biodegradable non-woven silica matrices prepared via the sol-gel process and used as a scaffold for human MSC (hMSC).

2. MAIN TEXT

2.1. Material and methods

SiO_2 nanofibers

Silica nanofibers were prepared by sol-gel method, needleless electrospinning, and subsequent thermal stabilization below 200°C [8,9].

Cell culture

hMSC were obtained from the Laboratory of Experimental Cell Therapy, First Faculty of Medicine, Charles University according to the Declaration of Helsinki and under the local ethical committee approval. Cell cultures were cultivated in tissue culture flasks with alpha-MEM (Gibco), supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin-streptomycin (Gibco) at 37°C in 5% CO_2/air. When cells became confluent, they were resuspended using trypsin-EDTA (Gibco).

Immunocytochemistry

Detection of hMSC adhering to silica nanofibers was performed using mouse anti-β-actin antibody. The samples were fixed for 20 min in 4% paraformaldehyde, followed by three 5-min washing steps in PBS and a 10-min incubation with 5% normal goat serum. Samples were then incubated with 5 µg/ml anti- β-actin antibody for 1 hour followed by three 5-min washing steps in PBS-Tx (0.25% Triton-X 100) and staining with anti-mouse Alexa 488 for 45 min at room temperature in darkness. After three 5-min washing steps in PBS, cell nuclei were stained with DAPI for 10 min. Leica SP5 confocal microscope was used to visualize adherent cells.

Adhesion assay

Viability and metabolic activity of adherent cells was tested with MTT assay. Cells were seeded at $2x10^4$ cells/well and cultured in cell culture medium at 37°C with 5% CO_2/air 24 h or 72h before performing the MTT assay. Samples were washed in PBS and MTT solution (1 mg/ml in medium) was added for 3 h. Then the solution was aspirated and isopropanol was added. Synergy plate reader (BioTek) was used to assess cell viability by spectrophotometry at 570 nm (reference wavelength 650 nm).

Eluates

Samples of silica nanofibers ($1cm^2$) were weighted and sterilized with UV light. Eluates were obtained by incubating 4 mg/ml of samples in PBS or cell culture medium supplemented with 5% or 10% serum at 37°C in 5% CO_2/air for 24h or 72h. PBS and culture medium supplemented with serum without silica nanofibers incubated under the same conditions served as a control.

MTT assay

Cytotoxicity was tested using the MTT assay. hMSC seeded at a density of 3×10^3/well were cultured at 37°C in 5% CO_2/air for 24 h prior to adding eluates / control. MTT assay was performed 24 or 96 hours later. Eluates were aspirated and MTT solution (1 mg/ml) was added to each well. Cells were incubated at 37°C in 5% CO_2 / air for 3 hours. Thereafter, the MTT solution was aspirated and isopropanol was added. Synergy plate reader (Bio Tek) was used to assess cell viability by spectrophotometry at λ=570 nm (reference wavelength 650 nm).

Results of the MTT assay were expressed relative to controls (cells in control medium/PBS without silica nanofibers; considered 100%).

Cell morphology analysis

Confocal microscopy images were analyzed in ImageJ and CellProfiler [10].

Statistical analysis

Statistical analysis was performed using Mann - Whitney test (differences were tested at a significance level of 0.05).

2.2. Results

Silica nanofibers prepared by needleless electrospinning were tested as a scaffold for hMSC. **Fig. 1** presents confocal microscopy images 1, 2, and 3 days after seeding the cells on the scaffold. One day after seeding, hMSC were adhering to the nanofibers and had normally distributed cytoplasm and cytoskeleton (similar to normal adherent cell culture conditions). In contrast, on the 3rd day, many cells were rounded and their area (in z-projection) decreased by almost 60% (**Fig. 2A**). The MTT assay revealed a corresponding decline of cell metabolic activity and viability of approximately 30% (**Fig. 2B**).

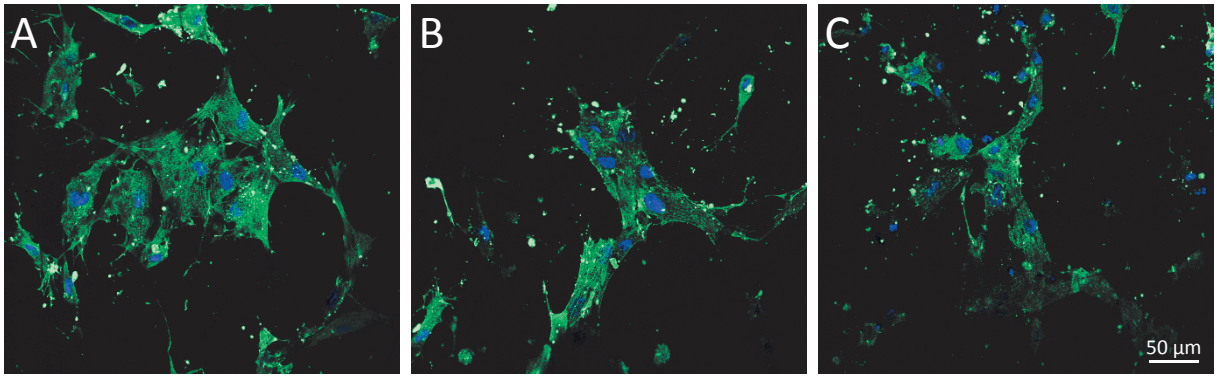

Fig. 1 Confocal microscopy images of hMSC cells seeded on silica nanofiber scaffolds: 1st day (A), 2nd day (B), and 3rd day (C). Cells were stained with anti-beta-actin antibody/Alexa 488 (green color) and DAPI (blue color)

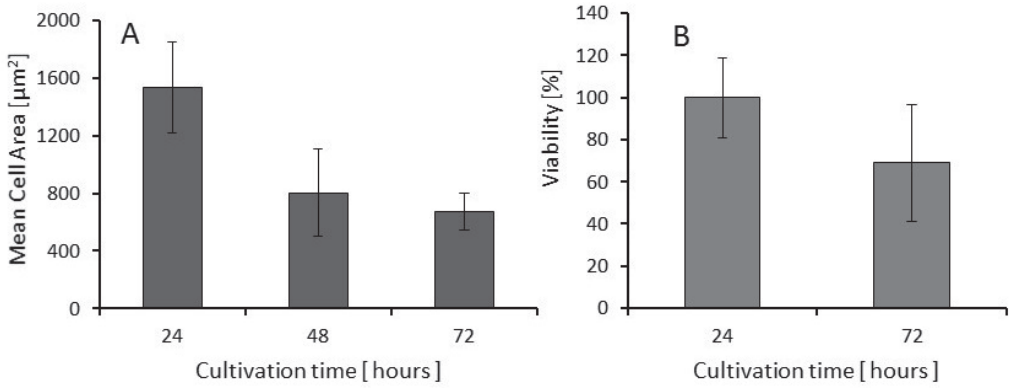

Fig. 2 Cell size (A) and viability (B) of hMSC seeded for 24-72 hours on silica-based scaffold. Cell viability was assessed using MTT assay

Cytotoxicity tests (**Fig. 3**) were performed with three different eluates of silica nanofibers incubated i) for 72 hours in PBS or culture media supplemented with 10% serum, or ii) 24 hours in culture media supplemented with 5% serum. In the first case, hMSC were exposed to eluates for 24 hours. PBS eluates did not significantly compromise cell metabolic activity and viability compared to controls (**Fig. 3A**). However, 24-hour exposure of

the cells to eluates from nanofibers incubated in culture media with 10% serum had a significant negative influence (**Fig. 3B**). Their viability was reduced to 84% (**Fig. 3A**). In the second case, the extended incubation time of 96 hours resulted in a mild but clearly pronounced decrease in cell metabolic activity and viability compared to 24-hour exposure (**Fig. 3C**).

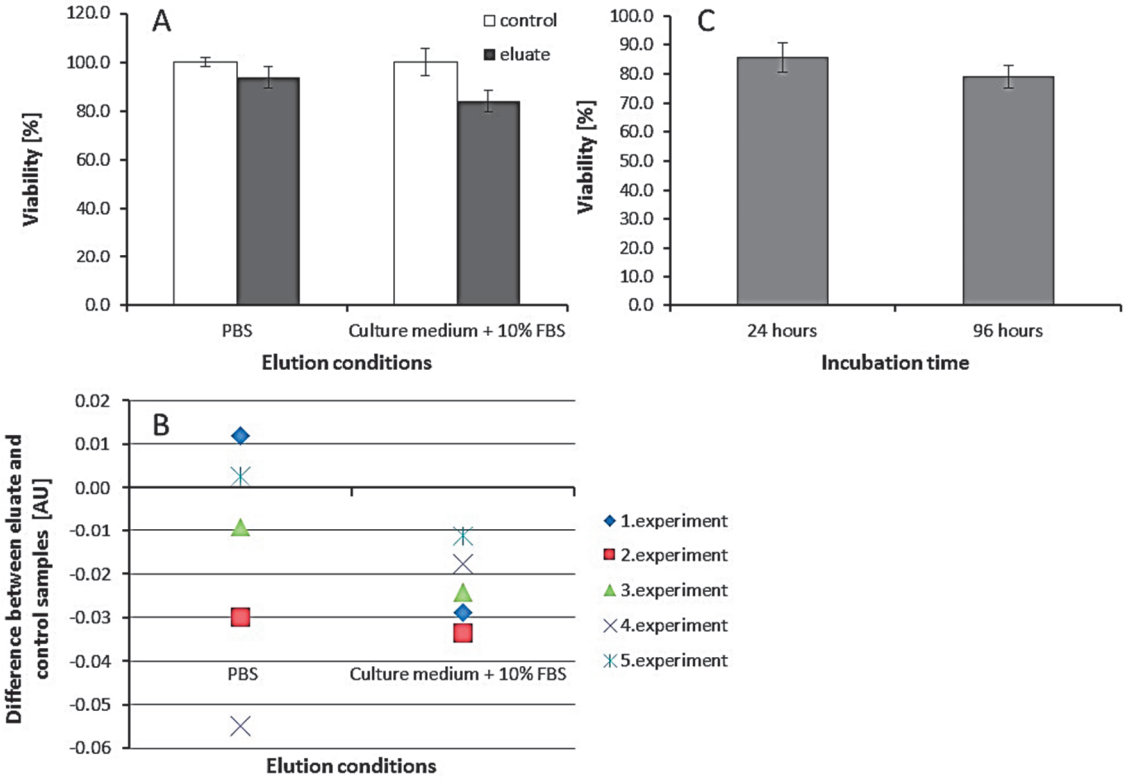

Fig. 3 Viability of hMSC exposed to eluates of silica nanofibers (MTT assay). A) After 24 hours of exposure to 72-hour eluates using two different solvents. B) Change in cell viability compared to control in individual experiments. C) Viability of cells after 24 and 96 hours of exposure to eluates of silica nanofibers incubated for 24 hours in culture medium supplemented with 5% serum

2.3. Discussion

Biocompatibility and biodegradability are two of the most important requirements imposed on materials used in biomedicine. Needle-electrospun silica nanofibers have already been successfully used as scaffold for several cell types, including Chinese hamster ovarian cells (CHO-K1), human hepatocellular carcinoma (HepG2) cells [4], or human osteoblastic MG63 cells [5]. Brázda et al. [11] have shown that needleless silica nanofibers (produced using technology identical to the one used in our study) may be biodegradable, at least when using simulated pulmonary fluids, and the nanofibers have been estimated to be safe. In our study we tested needleless silica nanofibers as a scaffold for hMSC, cells that are already used in clinical medicine.

Confocal microscopy and cell adhesion assays showed that this material can support hMSC adhesion and that hMSC cells seeded on these nanofibers are metabolically active. However, after three days in culture, the cells exhibited changes in cell morphology and a decline in viability. This finding is in contrast to the findings of Yamaguchi et al. [5] who found that silica nanofibers can support proliferation of HepG2 cells. However, they did not study cytotoxicity and they used an established cancer-derived cell line, which tend to be more resistant and have higher tendency to proliferate. They also observed a difference between heat-treated and non-heat-treated nanofibers. Whereas heat-treated fibers were better at supporting cell adhesion, non-heat treated nanofibers were better at supporting proliferation. The fact that our nanofibers were heat-treated could contribute to limited proliferation of the hMSC cells.

Regarding biocompatibility, in our research we focused on cytotoxicity tests under *in vitro* culture conditions. hMSC cells were exposed for 24 hours to eluates from nanofibers incubated for 24-72 hours in PBS or cell culture media supplemented with serum. We observed that the metabolic activity of cells was greatly influenced by the type of solvent used. Whereas PBS eluates did not significantly change cell metabolic activity and viability compared to controls, eluates from nanofibers incubated in culture medium supplemented with serum caused a significant reduction in cell viability and metabolic activity. This could be explained by the fact that in PBS the surface of the nanofibers was almost intact, whereas in cell culture medium supplemented with serum the fibers undergo partial degradation [12].

3. CONCLUSION

In conclusion, our study offers the first testing of needleless-electrospun silica nanofibers as scaffold for human bone marrow mesenchymal stem cells, cells that have already shown promising potential for regenerative medicine. In our experiments, needleless electrospun silica nanofibers revealed as a suitable scaffold to support adhesion of hMSC. However, these nanofibers in *in vitro* culture conditions tend to release eluates that are toxic to the cells and negatively impact their viability. More detailed studies aimed at optimizing the conditions for cell survival and function will be necessary.

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