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Ag modification of PLC-COOH nanofibers provides mesenchymal cells proliferation

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Motivation and Aim: Currently, wound healing materials with protective and regenerative properties are in great demand, the development of which is actively being carried out all over the world. To date, active research is underway on regenerative materials with an antibacterial effect. Silver is actively studied as an antibacterial component and is used in various creams, substances and nanomaterials for regenerative medicine [1, 2]. In addition to the antibacterial effect, silver has a regenerative effect, provided by stimulating the proliferation of cells involved in regeneration. Antimicrobial properties of silver have been known for a long time, but there is also cytotoxicity of high concentrations of silver [1]. Therefore, it is important to select the concentration and shape of silver depending on the goals. The ideal wound dressing should ensure that the wound remains moist, protected from infections, and has no toxic compounds. In present work, we obtained a series of polycaprolactone-based nanomaterials fabricated by electrospinning, modified with COOH groups and incorporated with silver ions (up to 0.6 at. %). By adjusting the magnetron current (0.3 A) and implanter voltage (5 kV), the deposition of TiO2 and Ag+ implantation into PCL/PEO nanofibers was optimized in order to achieve implantation of Ag+ without damaging the nanofibrous structure of the PCL/PEO [3]. It was shown that the maximum amount of silver is released from the PCL-Ti0.3-Ag-5 kV sample, and in the first hours there is a rapid release, then a smoother one, and for 7 days it continues to be released slower [3]. The aim of this work is to determine the effect of the obtained samples on the viability and proliferative potential of mesenchymal stromal cells.

Methods and Algorithms: PCL nanofibers were created by the method described in the article [3]. Here we used PLC nanofibers with the following characteristic: A1678 (0.3 A * 2 min + Ag 8 kV*3 mA*12 min) 180 mm, A1679 (0.3 A*2 min + Ag 5 kV*3 mA*15 min) 180 mm, A1677 (0.5 A*3 min + Ag 15 kV*3 mA*10 min) 120 mm. Membranes were cut in pieces with 0,5 diameter and used in set of experiments. MTT test was conducted to test cytotoxicity of used Ag concentrations. $5*10^3$ hMSC were seeded in 96-wells plate on plastic, Ag-PCL membranes samples were soaked in culture medium for 24 hours, then this medium was transferred to cells for 3 days incubation. Results were measured by optical density by used Multiscan FC spectrophotometer. To test proliferation rate human mesenchymal stromal cells were seeded on Ag-PCL membranes by $5*10^3$ cells per sample and incubated for 3 days in standard conditions (95 % humidity, 5 % CO_2). To further evaluate total amount, cells were dyed with Hoechst reagent to visualized cells nuclei and with EdU Click iT-reagent to count number of proliferating cells. *Results*: Ag+ ion release differed in samples depending on the initial Ag concentrations and was presented in the article [3]. Ag concentrations in all samples had no toxicity on human mesenchymal cells, even more Ag+ ions released in culture medium from Ag1677 and Ag1679 resulted in increased the percentage of living cells in contrast with the control (Fig 1). Total cell count was significantly higher on Ag1678 samples in comparison to PLC-ref membranes. However, proliferation rate was higher on all samples in comparison to PLC-ref (Fig. 2).

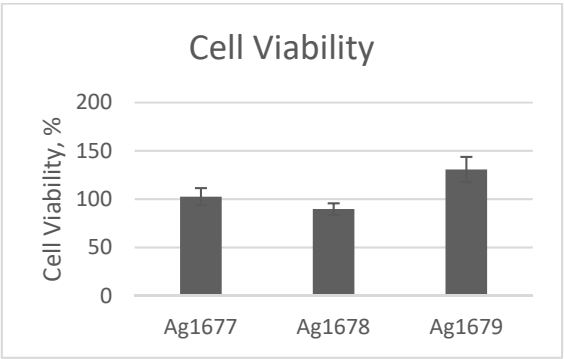

Fig. 1. Cell viability after incubation in culture medium from Ag-PLC nanofibrous samples, soaked for 24 hours in relation to 100 % viability in control wells. Results presented as mean ± std

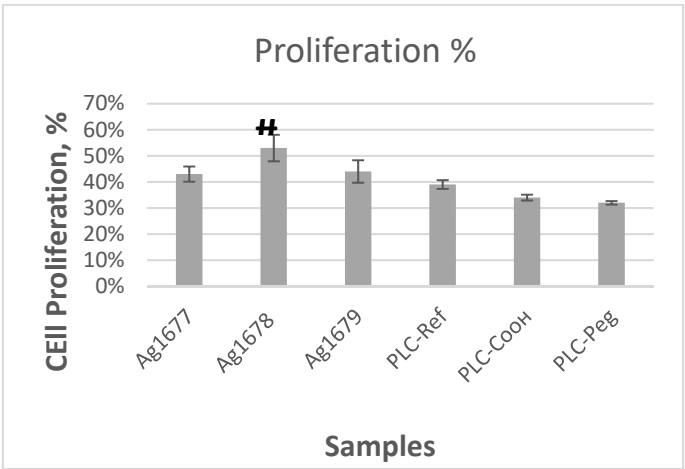

Fig. 2. Proliferation rate of hMSC on Ag incorporated PLC nanofibers in comparison with PLC-ref, PLC-COOH and PLC-Peg modifications. Results presented as means ± std. # - statistical significance ($p < 0,05$). Ag1677 – (0.5A*3 min + Ag 15kV*3mA*10 min) 120 mm, Ag1678 – (0.3A * 2 min + Ag 8 kV*3 mA*12 min) 180 mm, Ag1679 – (0.3A*2 min + Ag 5 kV*3 mA*15 min) 180 mm

Conclusion: Ag modification of PLC nanofibers membrane with the following parameters: A1678 (0.3A * 2min + Ag 8kV*3mA*12min) induces proliferation of human mesenchymal cells, though other concentration has a tendency to promote proliferation too, that we can see by the results of MTT test. Besides that, fast Ag+ ion release from PLC nanofibers (Ag1677) in the first 24 hours could've possibly reduce bacterial activity in wound dressings, but is low enough for a longer period of time and did not cause cytotoxicity. Obtained results allows us to speculate about that material application in biomedical field and in tissue engineering as it can provide effective protection not only from mechanical influence but also has antimicrobial effect because of Ag, which is important for chronic wounds and injuries with large area of damage, and can activate host cells proliferation.

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