Effect of Ag nanoparticles on cryopreserved mesenchymal stem cells from cartilage tissue

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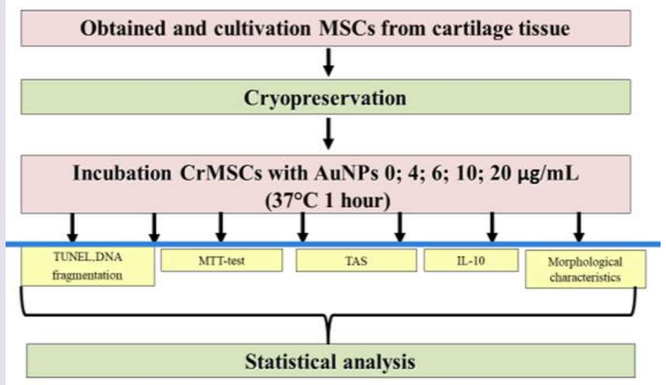
Introduction

The nanoscale materials have a dose-dependent effect on biological objects. The finding of the presence/absence of Ag nanoparticles (AgNPs) cytotoxic action is an important direction of nanobiotechnological research. The effect of AgNPs on morphological characteristics, proliferative and antioxidant potentials of cryopreserved mesenchymal stem cells (CrMSCs) from cartilage tissue was investigated.

Materials and Methods

The AgNPs (Sigma-Aldrich, USA) with an average size of 15 nm were used in the study. CrMSCs from cartilage tissue were incubated with AgNPs at final concentrations of 4, 6, 10, 20 μg/mL for 1 hour. The control was cells without the influence of AgNPs (0 μg/mL). On the 1, 3, 7 and 10 days of cultivation the number of viable cells was determined by MTT-test. Analysis of total antioxidant status (TAS) was performed using Randox test kit (UK). DNA fragmentation was evaluated by TUNEL-method ("BioVision", USA). Determination of interleukin-10 (IL-10) in the culture medium of CrMSCs on the 10th day of cultivation was carried out using the IL-10 test-kit (Sigma-Aldrich, USA). On the 10th day, the CrMSCs were fixed in a 4 % solution of paraformaldehyde followed by azure-II and eosin staining by Romanovsky-Giemsa. The results were processed with Student's t-test using Excel software.



Results

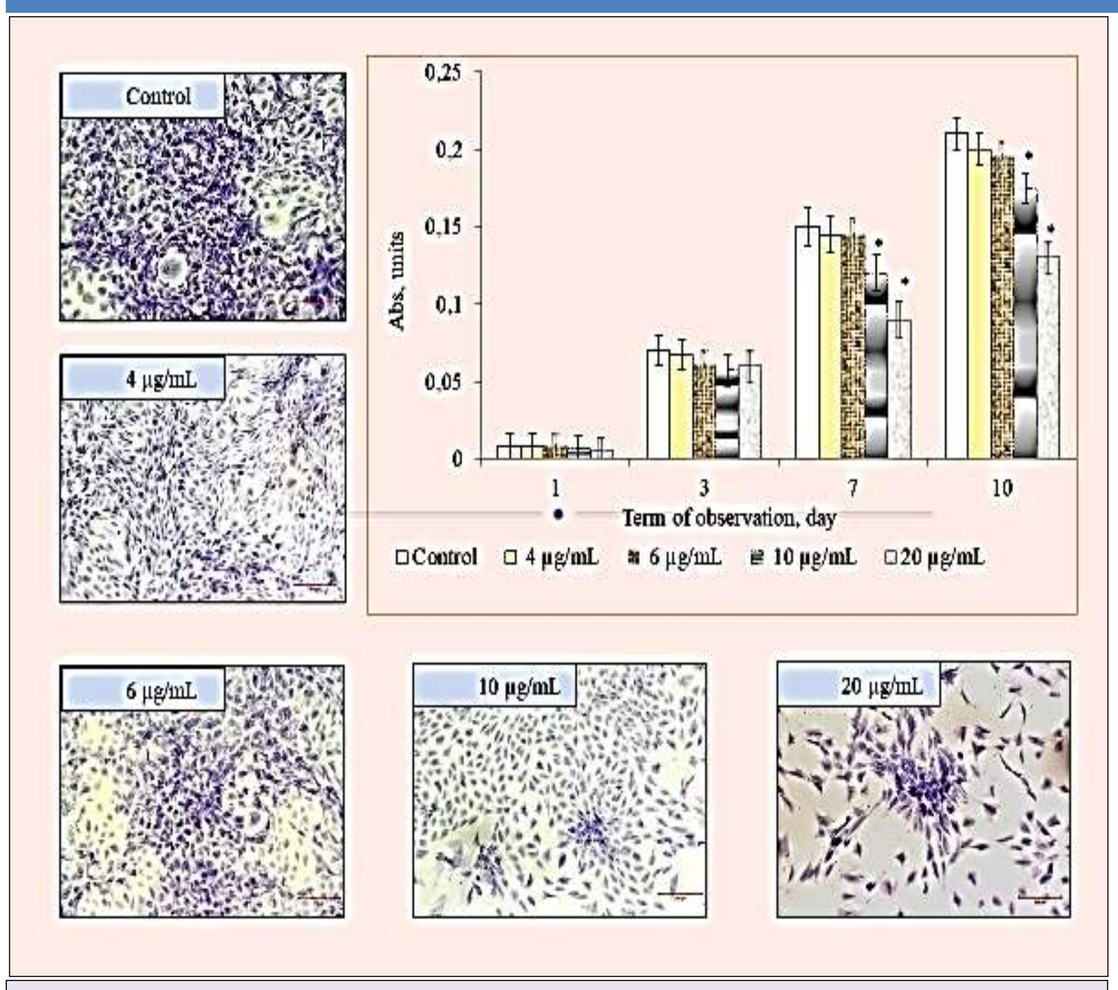
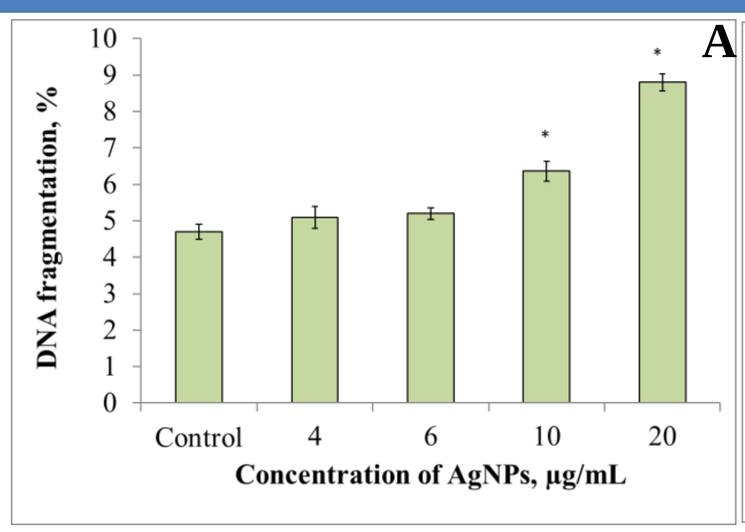
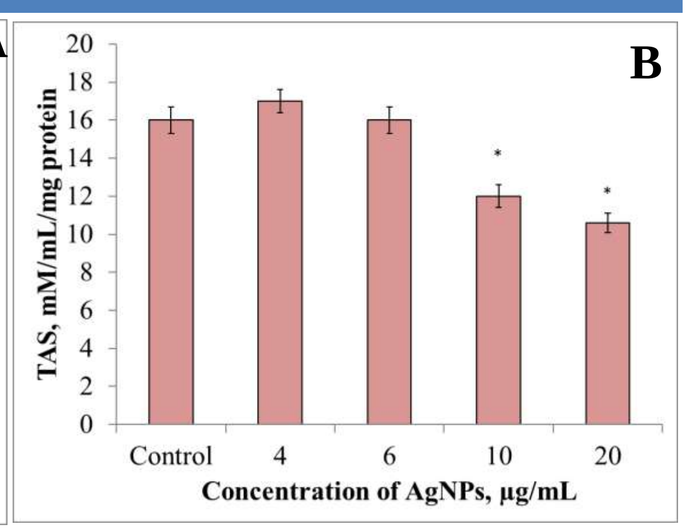


Fig.1 Influence of AgNPs on proliserative and morphological characteristics of CrMSCs, 10 days of culturing. Light microscopy. Azure&eosin staining.





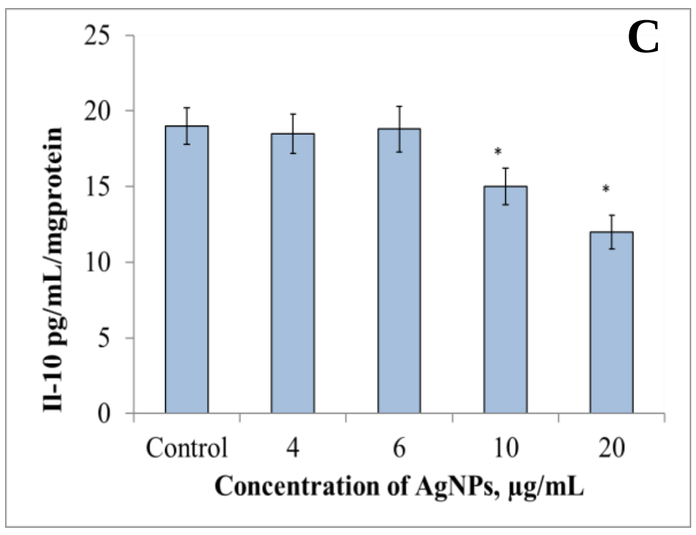


Fig.2 Influence of AgNPs on state of CrMSCs:
A – DNA-fragmentation;
B –TAS activity;
C – synthesis of IL-10.
* – the difference is statistically significant relative to the control ($p<0.05$)

Conclusions

The use of 4 and 6 μg/ml AgNPs did not influence on morphological characteristics, DNA fragmentation, synthesis of IL-10, proliferative and TAS activity in CrMSCs from cartilage tissue. AgNPs at concentrations of 10 and 20 μg/mL decreased the TAS index (1.3 and 1.5-fold respectively), proliferative potential (1.2 and 1.6-fold respectively) and synthesis of IL-10 (1.3 and 1.7-fold respectively) if compare with the control. Under the conditions of using of AgNPs at concentrations of 10 and 20 μg/mL there was an increase in the number of cells with signs of DNA fragmentation in 1.4 and 1.7 times respectively compared to the control. The use of AgNPs at concentrations of 10 and 20 μg/ mL led to changes in the morphological characteristics of CrMSCs from cartilage tissue in relation to control samples, namely signs of cytoskeletal dystrophy, cytoplasmatic granularity and vacuolization of nuclei. It was found that AgNPs in concentrations of 4 and 6 μg/mL are safe for CrMSCs from cartilage tissue, while increase up to 10 μg/mL has a toxic effect manifested by the change of proliferative, antioxidant activities and morphological characteristic. Thus, the presented results are belonged to the field of applied nanobiotechnology, that spreads to field restorative medicine, mostly in creation of cartilage bioimplants.

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