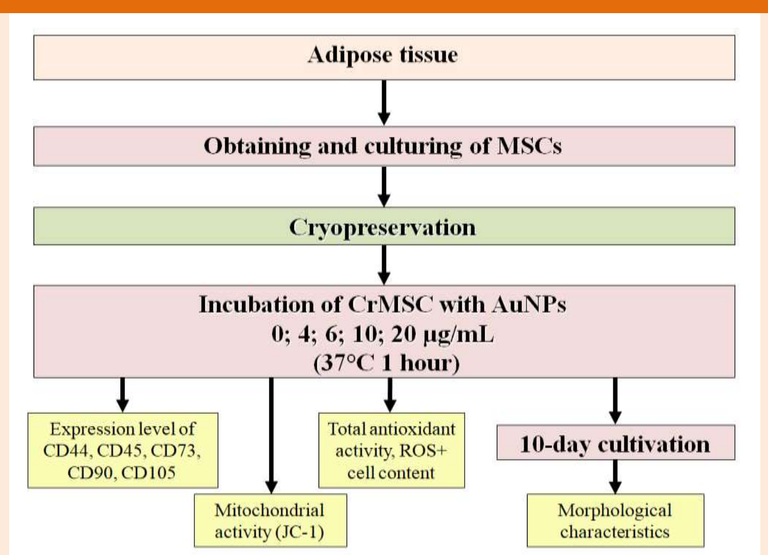
The influence of Au nanoparticles on metabolic state of cryopreserved mesenchymal stem cells from adipose tissue

N.O. Volkova, M.S. Yukhta, L.V. Sokil, L.G. Chernyshenko, L.V. Stepanyuk, A.M. Goltsev
Institute for Problems of Cryobiology and Cryomedicine, Natl. Acad. of Sci. of Ukraine, Kharkov
e-mail: volkovana781@gmail.com

Introduction

The optimum quantity of nanoparticles is defined by the balance between pronounced direct action and low side effects like unwanted cytotoxicity. Gold nanoparticles are very attractive for usage in biomedical technologies due to their unique properties and conventional methods of synthesis. This nanoscale metal can have different impact on both physical and chemical properties of cells, depending on their quantity or therapeutic dose. The influence of Au nanoparticles (AuNPs) on phenotypic characteristics, mitochondrial activity and antioxidant status of cryopreserved mesenchymal stem cells (CrMSCs) from adipose tissue was investigated.

Materials and Methods



The AuNPs (Sigma-Aldrich, USA) with an initial metal concentration of 45 µg/mL and an average size of 15 nm were used in the study. CrMSCs from adipose tissue were incubated with AuNPs at final concentrations of 4, 6, 10, 20 µg/mL for 1 hour. The control was cells without the influence of AuNPs (0 µg/mL). For phenotypic analysis CrMSCs were stained with CD45-FITC, CD44-FITC, CD73-FITC, CD90-FITC, CD105-PE monoclonal antibodies (BD Biosciences, USA). The functional state of mitochondria was investigated using JC-1 detection kit (BD). Analysis of total antioxidant status (TAS) was performed using Randox test kit (UK). The results were processed with Student's t-test using Statistica 8.

Results

Table. Influence of AuNPs on phenotype of CrMSCs

Samples	Expression level , %				
	CD 44	CD 45	CD 73	CD 90	CD 105
Control	97.71±0.22	1.12±0.11	92.04±0.82	97.78±1.21	94.64±0.45
AuNPs 4 µg/mL	97.23±0.41	1.09±0.12	92.30±0.41	97.47±0.51	94.48±0.36
AuNPs 6 µg/mL	97.15±0.36	1.06±0.13	92.88±0.31	97.62 ±0.35	94.14±0.45
AuNPs 10 µg/mL	**85.98±0.42***	1.08±0.08	92.92±0.32	97.51±0.24	93.17±0.51
AuNPs 20 µg/mL	**82.52±0.31***	1.10±0.15	92.26±0.65	97.86±0.11	**86.12±0.44***

* – the difference is statistically significant relative to the control ($p<0.05$)

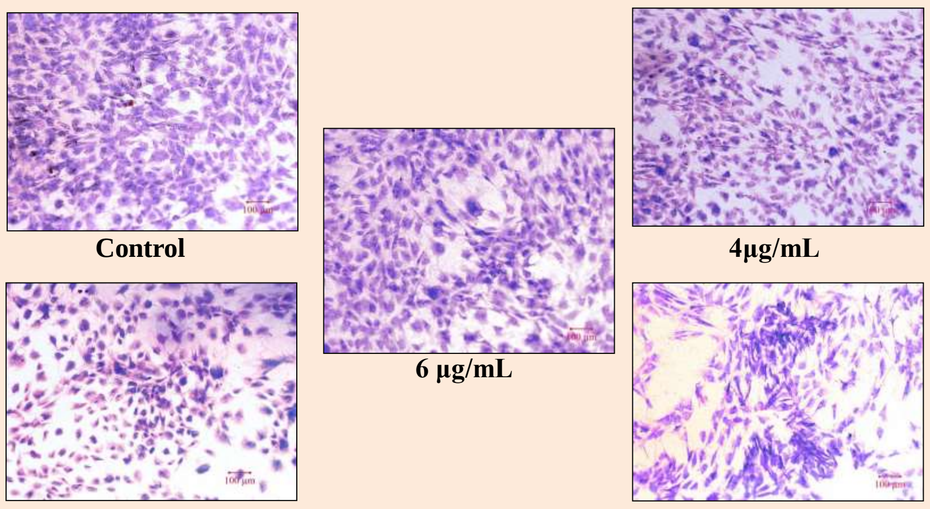
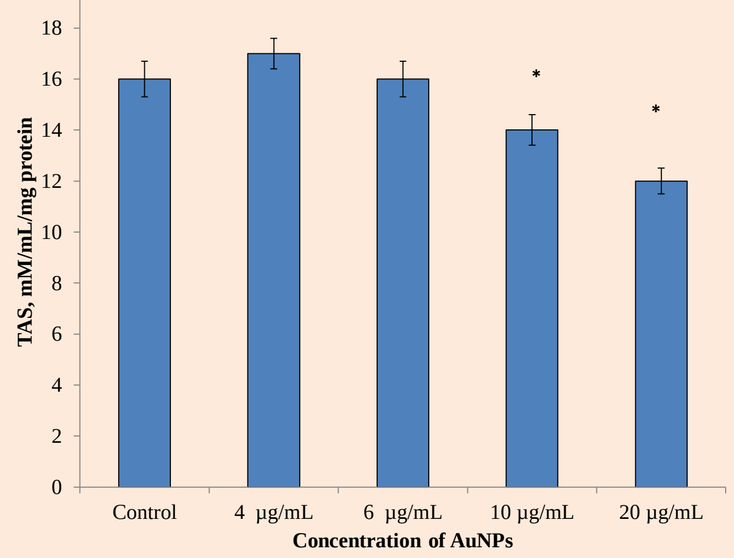
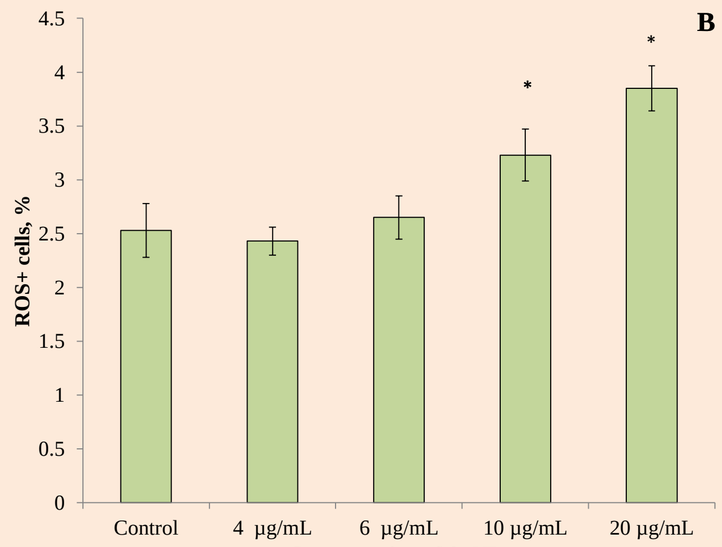


Fig.1 Influence of AuNPs on morphological characteristics of CrMSCs, 10 days of culturing. Light microscopy. Azure&eosin staining.





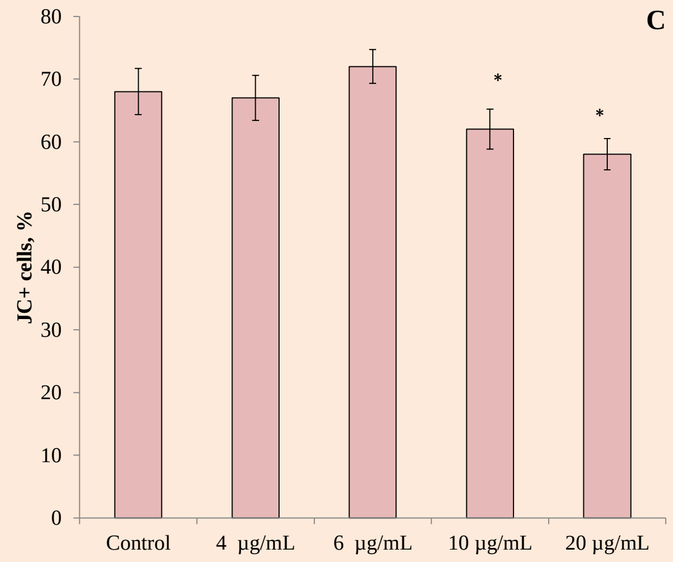


Fig.2 Influence of AuNPs on metabolic state of CrMSCs:
A – TAS activity; B –content of ROS+ cells; C – content of JC-1+ cells.
* – the difference is statistically significant relative to the control ($p<0.05$)

Conclusions

Adding of AuNPs at studied concentrations did not lead to any significant changes of the CD45, CD73, CD90 expression level of CrMSCs. Using of 10 and 20 µg/mL AuNPs resulted in reduction of $CD44^+$ cells by 12% and 18% respectively compared to the control. Content of $CD105^+$ cells were reduced by 9% in the case of 20 µg/mL AuNPs concentration. The use of 4 and 6 µg/mL AuNPs did not influence mitochondrial activity and TAS in CrMSCs. AuNPs at concentrations of 10 and 20 µg/mL decreased the TAS index (14 and 22% correspondently) and $JC\text{-}1^+$ cells content compare with the control samples. It was found that AuNPs in concentrations of 4 and 6 µg/mL are safe for CrMSCs from adipose tissue, while increase up to 10 µg/mL has a toxic effect manifested by the change of phenotype, mitochondrial and antioxidant activities. Thus, the obtained results are related to the field of applied nanotechnology and extend to clinical medicine, especially in development of addressed drug delivery to target cells or organs.