Safety evaluation of nanosilver using reconstructed human GIT tissues

Dagmar Jirova[1], Marketa Dvorakova[1], Kristina Kejlova[1], Lucie Kasparova[1], Daniel Krsek[1], Hana Kolarova[2]

Abstract**—**Silver nanoparticles (AgNPs) have been used widely in food storage materials and cosmetics for their well known antimicrobial effects. Information on the toxicity have not been sufficiently evaluated, although there is a risk of accidental ingestion or misuse. Recent studies revealed that silver nanoparticles may penetrate into the circulation system through the mucosa of the respiratory tract, gastrointestinal tract and by penetration through the skin. Penetration of nano-silver may lead to interactions with plasma proteins, coagulation factors, erythrocytes, white blood cells and thrombocytes. Oral ingestion of colloidal silver can increase the concentration of silver in the plasma and save the silver particles in the skin, which is later reflected by irreversible hyperpigmentation (argyria). According to previous studies *in vitro*, silver nanoparticles may be cytotoxic for hepatocytes and may cause oxidative stress, DNA damage and apoptosis. For safety evaluation of nanosilver, reconstructed human GIT tissues Epi Oral, Epi Intestinal, Epi Intestinal FT and Colon epithelium were used as a model mimicking the human gastrointestinal tract. Biological methods *in vitro* were applied for evaluating the viability, cytotoxicity and penetration in tissue cultures. MTT viability assay was employed for evaluating the cytotoxicity and viability of the tissues. ICP-MS and TEM was used for detecting the penetration of AgNPs. ELISA method was used for investigation of inflammatory reactions. The results confirmed negative effect of nano-silver on viability of the tissues and neither cytotoxicity effect was revealed. Evaluating the penetration of nano-silver through the tissue, ICP-MS method confirmed the presence of nano-silver in the medium after exposure time of 24h of the Epi Intestinal FT tissue and Colon epithelium tissue. The results confirmed no adverse effect of nanosilver on the viability of the tissues even after exaggerated exposure. Penetration of nanosilver through the tissues, probably in the form of Ag ions, was confirmed by ICP-MS in a rate depending on the tissue type and application vehicle. No significant (at least two-fold) increase of the inflammatory cytokines was recorded by ELISA method. The tested AgNP samples did not elicit any adverse effects in the available reconstructed human GIT tissues.

Keywords**—**GIT tissues, inflammation, nanosilver, penetration.

Dagmar Jirova[1], Marketa Dvorakova[1], Kristina Kejlova[1], Lucie Kasparova[1], Daniel Krsek[1] National Institute of Public Health, Srobarova 48, Prague 10, Czech Republic, e-mail: jirova@szu.cz

Hana Kolarova[2], Department of Medical Biophysics, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic, e-mail: hana.kolarova@upol.cz

I. Introduction

SILVER nanoparticles (AgNPs) represent most frequently applied nanomaterials and are used in everyday consumer products due to their antifungal, antibacterial, antiviral, and antimicrobial effects caused by their enormous surface area and reactivity [1]. Due to their general use in cosmetics, liquids, textiles, food storage products, paints, plastics, toys etc., silver nanoparticles have become a target of deeper investigation focused on better understanding of their risk potential in case of human exposure [2]-[4]. In our study focused on safety evaluation of nanosilver, three novel commercially available reconstructed human tissues were used for mimicking the human gastrointestinal tract (GIT). The evaluated endpoints included tissue viability, cytokine release and permeation of silver through human tissues.

II. Materials and Methods

A. AgNP dispersions

Aqueous dispersions of silver nanoparticles were obtained from KC Rulc company (http://www.kcrulc.cz/en) with the declared Ag concentration of 20 ppm. Two samples designated S9 and S29 were characterized by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS), see Fig. 1a,b and Fig. 2a,b.

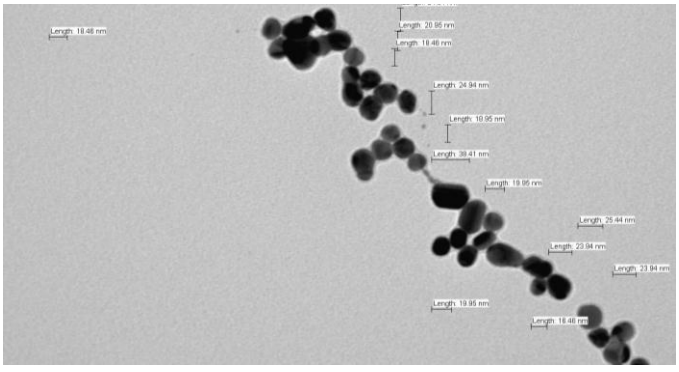

Fig. 1a TEM microphotogram of AgNPs, sample S9

http://dx.doi.org/10.15242/IICBE.C0615099

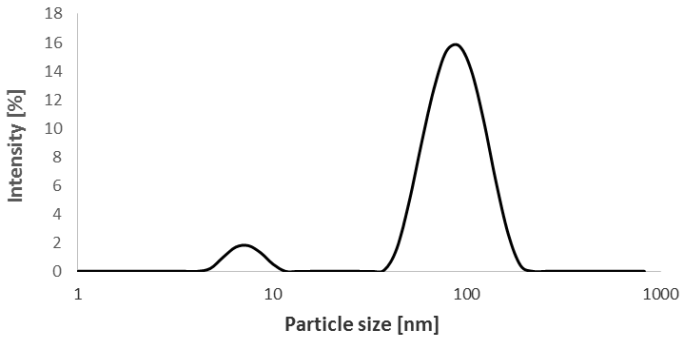

Fig. 1b Particle size and distribution, sample S9

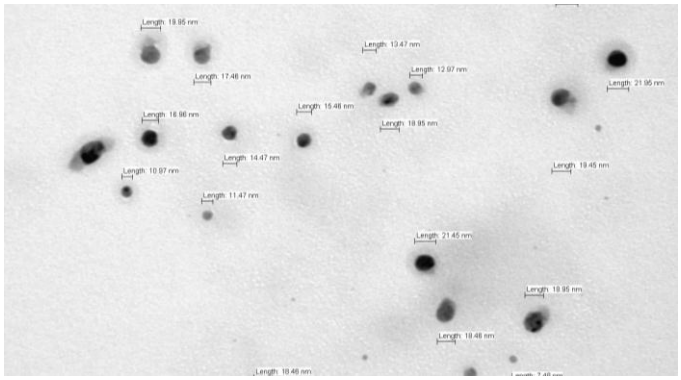

Fig. 2a TEM microphotogram of AgNPs, sample S29

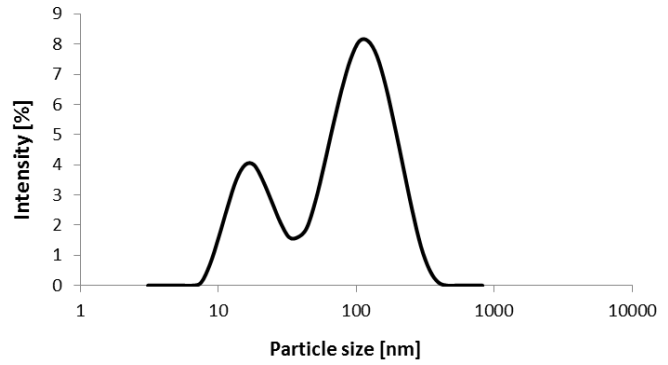

Fig. 2b Particle size and distribution, sample S29

B. Tissue models

EpiIntestinal™ tissues consist of either partial thickness (SMI-100™) or full-thickness EpiIntestinal tissues (SMI-100-FT™) (MatTek Corp., USA). While the partial thickness small intestinal tissue model consists of only epithelial cells, the full-thickness tissue model is composed of epithelial cells, fibroblasts and endothelial cells. Both tissue models are 3-dimensional, highly differentiated, and stratified reconstructed tissues derived from normal, human small intestine cells [5], [6]. The 3D intestinal tissues have declared structure of columnar shaped basal cells, Kerckring folds, brush borders, functional tight junctions, drug transporters, metabolizing enzymes and mucous secreting granules similar to tissue (Fig. 3a,b).

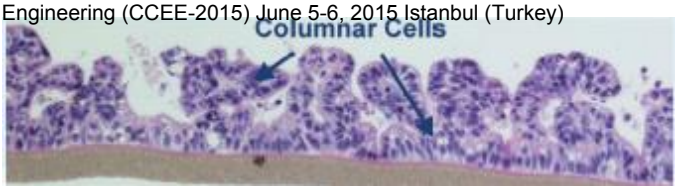

Fig. 3a EpiIntestinal tissue model

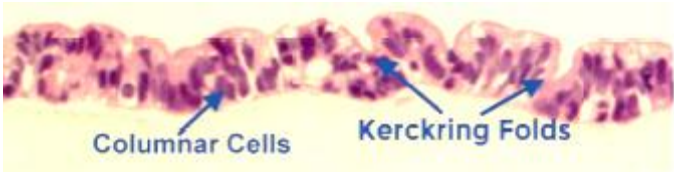

Fig. 3b EpiIntestinal tissue model

Colon epithelium™ tissue (Sterlab, France) is a multi-layered columnar simple epithelium with striated plate cells that absorb nutrients and goblet cells (mucous cells) secreting mucus (Fig. 4).

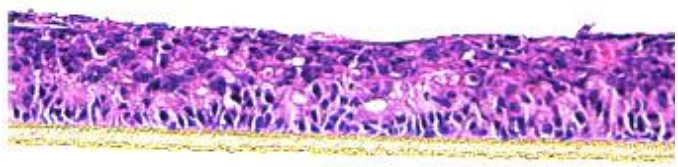

Fig. 4 Colon epithelium model

C. MTT assay

The models of reconstructed human tissues were exposed for 24 h to the tested samples as such or diluted (1:0.5) in simulated intestinal fluid (SIF, Sigma-Aldrich). After exposure the viability of tissues was determined using MTT assay, based on the enzymatic reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan salt by living cells.

D. Transmission electron microscopy

The culture medium beneath the tissues was analysed for the presence of AgNPs by transmission electron microscope Philips Morgagni 286 (magnification 52 000X).

E. ICP-MS analysis

The content of Ag in the medium was quantified using inductively coupled plasma mass spectrometry (ICP-MS).

F. Cytokine release analysis

The Quantikine™ ELISA method (RnDSystems.com) was used for quantitative determination of human IL-1α, TNF-α, IL-6 and IL-8 concentrations in tissue culture media for investigation of inflammatory reactions.

III. Results

The ELISA method did not confirm any significant increase of the inflammatory cytokines with the exception of TNF-α after application of S29 in simulated intestinal fluid on Colon epithelium which may suggest first signs of possible cell damage and apoptosis.

The results including tissue viability and cytokine release (IL-1α, IL-6, IL-8 and TNF-α) are summarized in Fig. 5 (EpiIntestinal); Fig. 6 (EpiIntestinalFT); Fig. 7 (Colon epithelium).

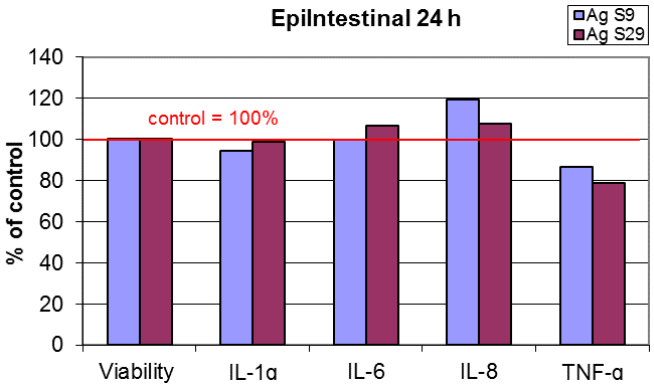

Fig.5 EpiIntestinal viability and cytokine release

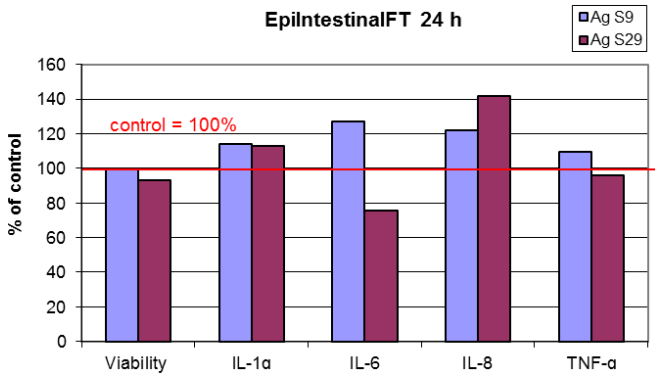

Fig. 6 EpiIntestinal FT viability and cytokine release

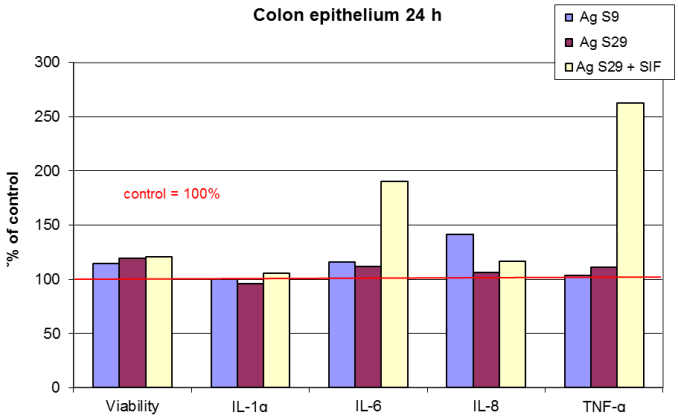

Fig. 7 Colon epithelium viability and cytokine release

Permeation of nanosilver through the tissues, probably in the form of Ag ions, was confirmed by ICP-MS in a rate depending on the tissue type and vehicle used. The simulated intestinal fluid possibly enhanced the solution of AgNPs into Ag ions, thus increasing permeation through the Colon epithelium. TEM investigation did not confirm any presence of nanoparticles in the medium, the content of silver detected by ICP-MS is probably in the form of Ag ions. The permeation of Ag into the tissue culture media after 24 h exposure is recorded in Table 1.

TABLE 1
AG PENETRATION (% OF APPLIED DOSE)
QUANTIFIED IN TISSUE MEDIUM BY ICP-MS

Tissue	Ag S9	Ag S9 + SIF	Ag S29	Ag S29+SIF
	% of applied dose			
EpiIntestinal	0.09	not tested	III.14	not tested
EpiIntestinal FT	0.19	not tested	0.52	not tested
Colon epithelium	VII.72	IX.62	XI.81	17.17

IV. CONCLUSION

The results of the study confirmed no adverse effect of nanosilver on the viability of the reconstructed human GIT tissues even after exaggerated exposure of 24 h. The ELISA method did not confirm any significant increase of the inflammatory cytokines with the exception of TNF-α after application of the sample S29 in simulated intestinal fluid on Colon epithelium which may suggest first signs of possible cell damage and apoptosis. The study of AgNPs effect on reconstructed intestinal and colon tissues will continue with the use of simulated intestinal and gastric fluids in combination with pepsin and pancreatin in order to mimick the human digestive system more closely. Reconstructed human oral cavity tissue models (EpiOral and EpiGingival, produced by MatTek Org., USA) will be employed in a future study of AgNPs diluted in simulated saliva for investigation of another part of oral exposure of humans to silver nanoparticles.

ACKNOWLEDGMENT

The research was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic (NT 14060-3/2013).

REFERENCES

[1] G. A. Sotiriou, S. E. Pratsinis. Antibacterial activity of nanosilver ions and particles. *Environ. Sci. Technol.*, 44 (14), 2010, pp. 5649–5654. http://dx.doi.org/10.1021/es101072s

[2] N. S. Tulve, A. B. Stefaniak, M. E. Vance, K. Rogers, S. Mwilu, R. F. LeBouf, D. Schwegler-Berry, R. Willis, T. A. Thomas, L. C. Marr. Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposures. *Int J Hyg Environ Health.*, 218, 2015, pp. 345-357. http://dx.doi.org/10.1016/j.ijheh.2015.02.002

[3] B. Nowack, H. F. Krug, M. Height. 120 years of nanosilver history: implications for policy makers. *Environ. Sci. Technol.*, 45(4), 2011, pp. 1177-83. http://dx.doi.org/10.1021/es103316q

[4] F.F. Larese, F. D'Agostin, M. Crosera, G. Adami, N. Renzi, M. Bovenzi et al. Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology*, 2009;255, pp. 33–37. http://dx.doi.org/10.1016/j.tox.2008.09.025

[5] M. Klausner, S. Ayehunie, B.A. Breyfogle, P.W. Wertz, L. Bacca, J. Kubilus. Organotypic human oral tissue models for toxicological studies. *Toxicol. In Vitro.* Aug;21(5), 2007, pp. 938-49.

[6] S. Ayehunie, Z. Stevens, T. Landry, M. Taimi, A. Armento, M. Klausner, P. Hayden. Organotypic 3-D Human Small Intestinal Tissue to Assess Drug Safety and Intestinal Inflammation and Restitution. Mattek Corporation, http://www.mattek.com/news/sot-2015-posters.