SAFETY EVALUATION OF NANOSILVER USING RECONSTRUCTED HUMAN GIT TISSUES

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OVERVIEW

In our pilot 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 penetration of silver into the tissue culture medium. 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.

INTRODUCTION

Silver nanoparticles (AgNPs) have been used widely in food contact materials and cosmetics for their well known antimicrobial effects. Information on their toxicity has not been sufficiently evaluated yet, although there is a risk of accidental ingestion or misuse. Distinct studies suggest that silver nanoparticles may penetrate into the circulation system through the mucosa of the respiratory tract, gastrointestinal tract and/or through the skin. Oral ingestion of colloidal silver can increase the concentration of silver in the plasma and lead to accumulation of silver in the skin, leading to irreversible hyperpigmentation (argyria). Little is known about the metabolism of nanoparticles and their toxicity, although previous studies *in vitro* have shown that AgNPs may be cytotoxic for hepatocytes and cause oxidative stress, DNA damage and apoptosis. This pilot study was focused on mimicking the effects of AgNPs on human gastrointestinal tract *in vitro* using three novel reconstructed human GIT tissues recently made commercially available.

METHODS

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.

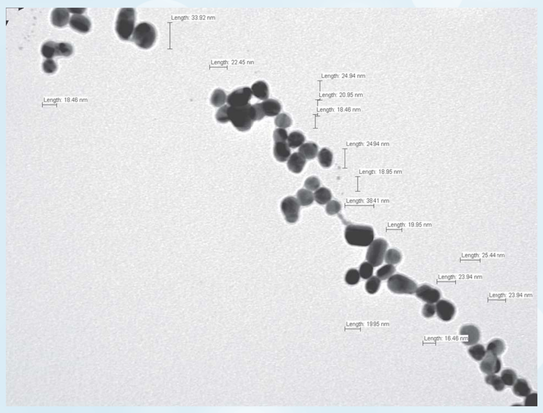

Fig. 1a TEM microphotogram of AgNPs, sample S9

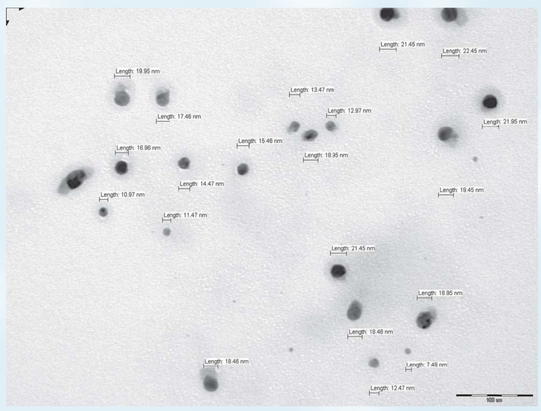

Fig. 2a TEM microphotogram of AgNPs, sample S29

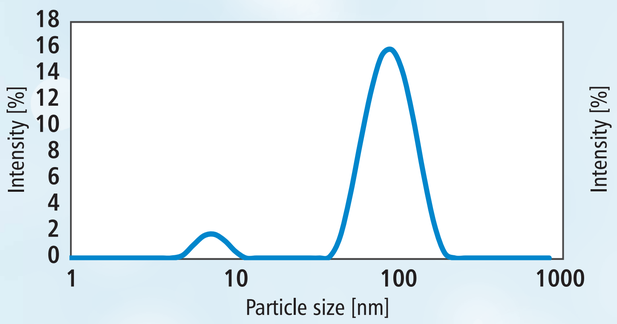

Fig. 1b Particle size and distribution, sample S9

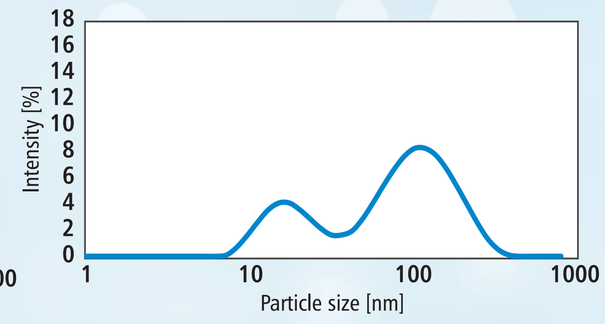

Fig. 2b Particle size and distribution, sample S29

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. 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 *in vivo* tissue. (Fig. 3a,b).

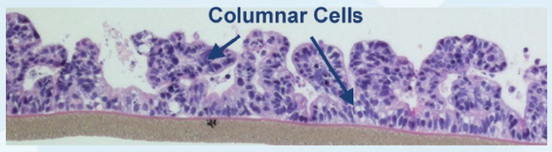

Fig. 3a EpiIntestinal™

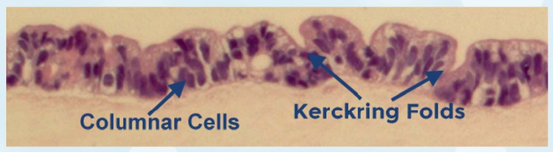

Fig. 3b EpiIntestinalFT™

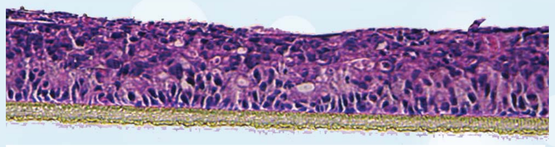

Fig. 4 Colon epithelium™

Colon epithelium™ (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).

Test procedure

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. The culture medium beneath the tissues was analysed for the presence of AgNPs by transmission electron microscope Philips Morgagni 286 (magnification 52 000X). The content of Ag in the medium was quantified using inductively coupled plasma mass spectrometry (ICP-MS). 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.

RESULTS

The results including tissue viability and cytokine release are summarized in

- Fig. 5 (EpiIntestinal);
- Fig. 6 (EpiIntestinalFT);
- Fig. 7 (Colon epithelium).

The penetration of Ag into the tissue culture media after 24 h exposure is recorded in Table 1. 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.

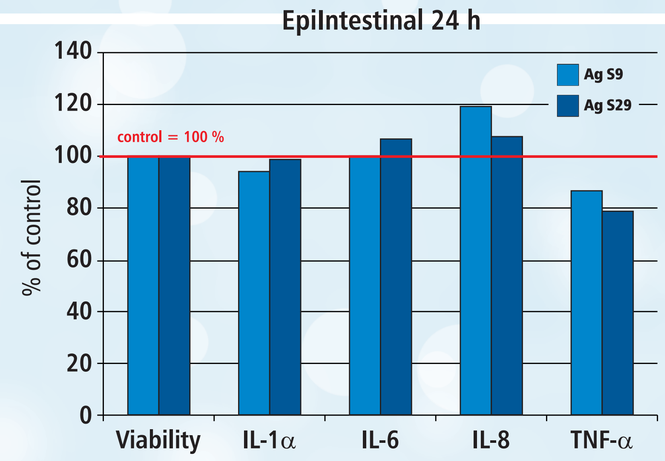

Fig. 5 ■ EpiIntestinal viability and cytokine release

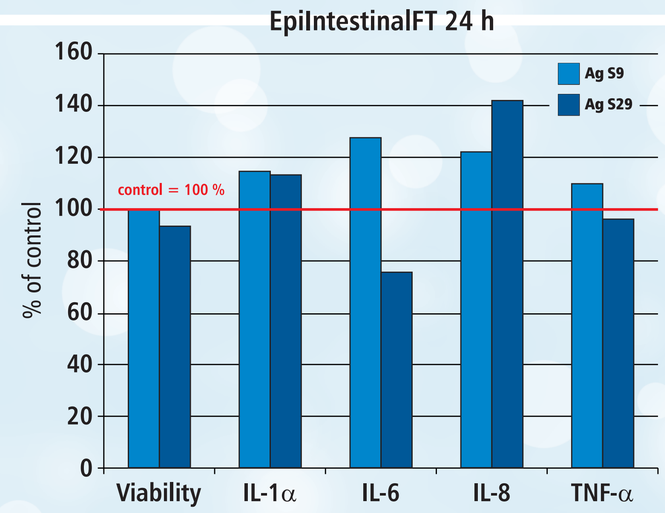

Fig. 6 ■ EpiIntestinalFT viability and cytokine release

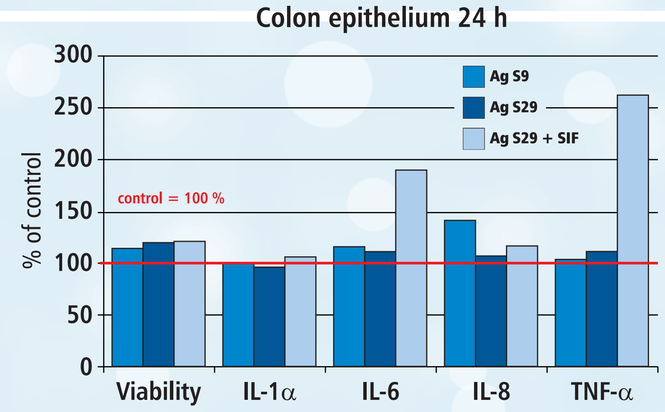

Fig. 7 ■ Colon epithelium viability and cytokine release

CONCLUSIONS

- The results of the pilot 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 S29 in simulated intestinal fluid on Colon epithelium which may suggest first signs of possible cell damage and apoptosis.
- 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 vehicle used. The simulated intestinal fluid possibly enhanced the solution of AgNPs into Ag ions, thus increasing penetration through the Colon epithelium.
- 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 Corp., 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.

Tissue	Ag S9	Ag S9 + SIF	Ag S29	Ag S29+SIF
	% of applied dose			
EpiIntestinal	0.09	not tested	3.14	not tested
EpiIntestinalFT	0.19	not tested	0.52	not tested
Colon epithelium	7.72	9.62	11.81	17.17

Table 1 ■ Ag penetration (% of applied dose) quantified in tissue medium by ICP-MS

ACKNOWLEDGEMENTS

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