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Use of single particle ICP-MS to estimate silver nanoparticle penetration through baby porcine mucosa

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ABSTRACT

Children are potentially exposed to products that contain nanoparticles (NPs). In particular, silver NPs are commonly present both in products used by and around children, primarily due to their antibacterial properties. However, very few data are available regarding the ability of silver NPs to penetrate through the oral mucosa in children. In the present work, we used baby porcine buccal mucosa mounted on vertical Franz diffusion cells, as an *in vitro* model to investigate penetration of silver NPs (19 ± 5 nm). Permeability experiments were performed using pristine physiologically-relevant saline solution in the receiver chamber and known concentrations of NPs or ions in the donor chamber; conditions mimicked the *in vivo* physiological pH conditions. After physicochemical characterization of silver nanoparticles dispersed in physiological solution, we evaluated the passage of ions and NPs through the mucosa, using single particle inductively coupled plasma mass spectrometry. A flux of 4.1 ± 1.7 ng $cm^{-2} min^{-1}$ and a lag time of 159 ± 17 min were observed through mucosa exposed to silver nanoparticles. The latter suggests nanoparticle penetration through the baby porcine mucosa and release Ag^{+} ions in the receptor fluid, as confirmed by computational model. Due to physiological similarity between human and pig membranes it is reasonable to assume that a trans-oral mucosa penetration could occur in children upon contact with silver nanoparticles.

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KEYWORDS
Silver nanoparticles; SP-ICP-MS; baby porcine; oral exposure; Franz cell model

1. Introduction

Silver nanoparticles (Ag NPs) are widely sold and used in different applications that babies and young children can come into contact with. These include food packaging, containers, toothpaste and tooth brushes, alcohol free mouthwash, nasal sprays, textiles, medical devices, water purification devices and others. The wide use of Ag NPs in many applications that involve human occupational and consumer exposure is justified by their known antibacterial, antiviral and antifungal properties to prevent infections. Despite its previous use (for instance colloidal silver 'Collargol' has been used for medical applications and has been manufactured commercially since 1897), Ag NPs gained renewed interest when its use as antibacterial and antiviral nanotechnology became more widespread and better supported by scientific evidence (Dung et al. 2020; Schneider 2017). In addition to their benefits, there are concerns about potential human and environmental hazard (Bouwmeester et al. 2009; Epstein et al. 2014; Lead et al. 2018). Regarding the exposure limits already available, the US Environmental Protection Agency (EPA) (U.S. Environmental Protection Agency Chemical Assessment 1991) suggests a value of bulk silver of <5 μg kg^{-1} daily as reference dose (Rfd) for oral

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Supplemental data for this article can be accessed here.

exposure, corresponding to a total daily amount of 350 μg of silver for a 70 kg adult. While the European Chemicals Agency (ECHA) recommended as silver Derived No Effect Level (DNEL) for human long oral exposure a value of 1.2 μg kg^{-1} daily, corresponding to 84 μg of silver for a 70 kg adult, slightly below the previous value (Silver - Registration Dossier - ECHA n.d.). While there are some reference values are bulk or total Ag, these are lacking for NPs. In this regard. The Scientific Committee on Consumer Safety (SCCS) recommended a maximum concentration limit of 1% w/w in products, considering reasonably foreseeable exposure conditions used in cosmetics including toothpastes and skin care products. However, the SCCS is not in the position to draw a conclusion on the safety of Ag NPs when used in oral and dermal cosmetic products due to a number of major data gaps (European Commission 2018). Moreover, silver intoxication (*argyria*) is reported in literature and has been described through oral (Chang, Khosravi, and Egbert 2006; Johnston et al. 2010) or dermal (Trop et al. 2006) exposure. The US Agency for Toxic Substances and Disease Registry (ATSDR) describes argyria as a 'cosmetic issue', since it consists mainly in a blue-gray discoloration of the skin (Agency for Toxic Substances and Disease Registry (ATSDR) 1999). Nevertheless, these are irreversible. There are also isolated reports of more serious neurologic, renal and hepatic complications caused by the ingestion of colloidal silver (Singh et al. 2011; Stepien et al. 2009), and there are concerns about microbiome changes from Ag NPs ingestion, affecting organism health (Li, Tang, and Xue 2019).

Ag NPs are therefore able to come in contact with human targets and specifically with the oral mucosa (Lansdown 2006; León-Silva, Fernández-Luqueño, and López-Valdez 2016)' especially in applications from the food and personal care sectors. The oral mucosa traditionally acts as a first barrier to xenobiotics in the digestive tract (Harris and Robinson 1992; Wertz and Squier 1991). However, due to its histological structure, the oral mucosa shows a permeability 20 times higher to water (Lesch et al. 1989) and up to 4000 times higher to certain pharmaceuticals when compared to skin (Fox and Modak 1974). In addition, investigation of penetration through oral mucosa could support toxicokinetic studies related to Ag NPs exposure after oral administration, if linked with toxicity studies by the gastrointestinal tract (GIT) route (Bergin and Witzmann 2013). Despite much research, trans-oral sorption and penetration rates and mechanisms of NPs through mucosa are not completely understood (Roblegg et al. 2012). Experimental data are in most cases derived from animal or human adult donor skin and are likely to be unsuitable to predict transdermal penetration in children. The porcine lining mucosa is the most similar to the human one in terms of structure and enzyme activity and is a thoroughly investigated *in-vitro* model to estimate human buccal absorption and drug penetration (Lesch et al. 1989). Currently, NP penetration through oral mucosa is not fully quantified for adults and even fewer data are available for children (Mauro et al. 2015; Mauro 2018). Children can come into contact with Ag NPs embedded in commercial products such as pajamas, baby blankets, soft toys and also in items used around children, such as disinfectant sprays, surface wipes and kitchen cleaners (Quadros et al. 2013), exposing skin and the buccal cavity of children to silver ionic and NPs form. In some applications and products addressed to children and adults, the exposure range is a few mg day^{-1} (values calculate considering different European disinfecting sprays products for oral application – see Table S1 ESI). In addition, children's skin and oral mucosa significantly differ from adults and so available nanotoxicological knowledge cannot predict infant's dermal and oral sorption. Finally, infant's transdermal exposure can lead to systemic effects, due to the differences in permeability and in the activity of metabolic pathways, potentially transforming substances which are of low toxicity in adults into more toxic substances for children (Fröhlich and Roblegg 2016; Tang et al. 2015; Tulve et al. 2015).

For the above reasons, in this work, we performed experiments to investigate the possible penetration of Ag NPs into/across the children buccal mucosa, using a standard dermatological *in-vitro ex-vivo model* (Franz cell), applied to oral baby porcine mucosa (Adams 1974; Bergman, Siegel, and Ciancio 1968). In particular, we coupled for the first time in this application, the Franz cell model with the use of single particle – inductively coupled plasma – mass spectrometry (SP-ICP-MS) technique and with the computational model Visual MINTEQ

3.1 (Visual MINTEQ – Visual MINTEQ – a Free Equilibrium Speciation Model 2021). The use of SP-ICP-MS as a powerful analytical method is growing and reached different application fields dealing with complex biological and environmental matrices (Dan et al. 2015; Dimitrova et al. 2017; Laborda et al. 2013; Lee et al. 2014; Lee and Chan 2015; Merrifield, Stephan, and Lead 2017a; Merrifield, Stephan, and Lead 2017b; Mitrano et al. 2014; Mitrano et al. 2012). SP-ICP-MS analysis allowed us to perform the quantitative and simultaneous estimation of Ag NPs and Ag^+ ions in the receptor fluid, avoiding the limitations of ultrafiltration process. While, the computational model Visual MINTEQ 3.1 was applied in support to the SP-ICP-MS measurements, confirming the silver speciation in the receptor fluid.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade. Sodium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, glutaraldehyde (50% v/v), nitric acid (69% v/v), hydrochloric acid (36.5–38.0% v/v) and hydrogen peroxide (30%) were purchased from Sigma Aldrich (Milan, Italy), while ammonium hydroxide (25%) from J.T. Baker (Milan, Italy). Water reagent grade was produced with a Millipore purification pack system (MilliQ water). The physiological solution used as receptor fluid was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 9 g of NaCl into 1 L of MilliQ water (final pH = 7.35). Silver NPs suspensions for this study were supplied by NanoAmor Materials Inc. (Houston, TX, USA) and were stabilized with PVP - polyvinylpirrolidone (content of silver: 25% w/w, polymer 75%). Ionic Au and Ag standard were used for calibration of ICP-MS, respectively ion mass of 197 and 107 a.m.u. Au 60 nm citrate coated NPs were used as standard for single-particle calibration and transport efficiency calculations (Table S2). All standards used were purchased from Sigma Aldrich (Milan, Italy).

2.2. Characterization of Ag NPs

Dynamic light scattering (d_{DLS}) and Zeta potential (ZP - ζ-pot_{ELS}) measurements on Ag NPs were performed using a ZetasizerNano ZS (Malvern Instruments Ltd.). Ag NPs suspension was diluted 1:5 in physiological solution and loaded into low size disposable cuvette. Zeta potential was calculated using Henry's equation. Summary statistics were obtained using quadruplicate 3 min analysis (total analysis time = 12 min). Transmission electron microscopy (TEM) was carried out on an EM208, operating at 200 kV (Philips, Eindhoven, The Netherlands), with a high definition acquisition system based on a side-mounted TEM camera OSIS Morada and an iTEM software platform (Olympus Soft Imaging Solutions GmbH, Münster, Germany). Ag NPs, dispersed in physiological solution, were placed onto a carbon-coated grid and dried at room temperature under vacuum.

2.3. Static Franz diffusion cell system

In vitro permeation studies were performed using a Static Franz Diffusion Cell System (see Figure S1), an *in vitro* model for testing static diffusion through biological membranes (Franz 1975). In mucosal studies, the receptor compartment has a mean volume of 4.5 mL and was maintained at 37 °C by means of circulation of thermostated water in the jacket surrounding the cells throughout the experiment. This temperature value was chosen in order to reproduce oral physiological conditions. The concentration of the salt in the receiver fluids was approximately the same that can be found in the blood. The solution in each cell was continuously stirred using a Teflon-coated magnetic stirrer. Each excised sheet of mucosa was clamped between the donor and the receptor compartment, in this way the epithelium faced the donor and the connective tissue region faced the receiver compartment. The mean exposed area of the mucous membranes was 0.95 cm^2. Four cells were used as controls, using physiological solution as donor fluid.

2.4. Preparation of stock suspensions

In order to discriminate between Ag NPs and silver ions, released from the NPs, two different donor phases were prepared just before the experiments. The stock suspensions of donor fluid 1 and 2 were prepared, accordingly to the following procedure.

For the stock 1, 200 mg of PVP-coated Ag NPs were dispersed in 100 mL of physiological solution and sonicated, at the aim to obtain a concentration of 500 mg L^{-1} (as silver content). Stock 2 was prepared by ultrafiltering the stock 1 (5000 rpm for 30 min by means of Amicon Ultra-4 centrifugal filters, 10 KDa MWCO), in order to separate and collect the water-soluble silver species of the stock 1. The ultrafiltered solution (stock 2) was analyzed by ICP-OES, and the concentration of ions was quantified (25 mg L^{-1} − 5% of the stock 1 silver content) and checked, also after the end of the experiments (240 min), to confirm that it remained stable. Stock 2 aims to reproduce the behavior of Ag ions fraction in the permeation process, comparing to stock 1 results. Five ultrafiltered aliquots (of 4 mL each) were mixed and used during the experiments as stock 2. The two donor fluids were prepared by adding 0.5 mL of the two stocks to 1 mL of physiological solution, before feeding the Franz Cell, reaching daily exposure concentrations comparable to the ones reported in some applications and products.

2.5. Preparation of oral mucosa

Due to its morphological and enzymatic similarities with the children mucosa, baby porcine oral mucosa was used for the *in-vitro* experiments (Adams 1974; Bergman, Siegel, and Ciancio 1968; Lesch et al. 1989). The membranes were provided by a local butcher's shop and obtained immediately after the pigs were sacrificed (porcine age between 4 and 8 months). During the transport to laboratory, the tissue was stored at 4 °C and then in freezer at −80 °C for a period of time up to, but not exceeding, 1 week. On the day of the experiment, the tissue was removed from the freezer and thawed in a physiologically-relevant solution (NaCl 9 wt.%), at room temperature, for approximately 30 min before the experiment. It has been shown that this method of storage does not affect the mucous barrier properties, since no change in the permeability has been previously mentioned (Nicolazzo, Reed, and Finnin 2003). Mucous membranes integrity was tested as suggested by Lestari (Lestari, Nicolazzo, and Finnin 2009). Mucosa integrity was checked even at the end of experiment, finding no alterations or damages in the oral mucosa.

2.6. Experimental set-up used for in vitro permeation studies

The experimental set-up used for testing Ag concentration in different compartments of Franz Cell System is shown in Figure 1.

The first experiment was performed with the donor fluid 1 (1 mL of physiological solution and 0.5 mL of Ag NPs stock 1 suspension). At time 0, the exposure chambers of 4 Franz diffusion cells were filled with donor fluid 1 (260 μg cm^{-2}). At selected intervals (20, 40, 60, 90, 120, 150, 180, 210, 240 min), 1 mL of the receptor fluid was removed and collected for the analysis, and immediately replaced with an equal volume of fresh physiological solution. The experiment was performed for 240 min, as suggested in other studies (Mauro et al. 2015; Roblegg et al. 2012). At the end of the experiment, the mucosal pieces were removed, washed with MilliQ water, and subsequently stored in the freezer together with donor and receptor solutions for performing ICP-OES, ICP-MS/SP-ICP-MS analysis. The experiment was repeated twice for a total of 8 Franz cells. The second experiment was performed with the donor fluid 2 (1 mL of physiological solution and 0.5 mL of the Ag ultra-filtered stock 2 solution). The exposure chambers of 4 Franz diffusion cells were filled donor fluid 2(13 μg cm^{-2}). The other test conditions were the same of the experiment 1. The experiment was repeated twice for a total of 8 Franz cells.

2.7. Mucosal digestion

All the mucosal exposed samples were collected and stored individually in freezer at −25 °C for the following digestion and analysis. At the time of the analysis, the skin membranes were dried for 2 hours at room temperature, weight, and then acid-digested in a closed microwave system (Multiwave PRO, Anton Paar) using 2.5 mL of HNO_3 (69%) and 0.5 mL of H_2O_2 (30%) for digestion. The obtained solutions diluted to a final volume of 10 mL with MilliQ water for the ICP-OES analysis.

2.8. Quantification of total metal content

Total Ag concentration in the solutions resulting from the mineralization of the mucosa samples were performed by ICP-OES using an Optima 8000

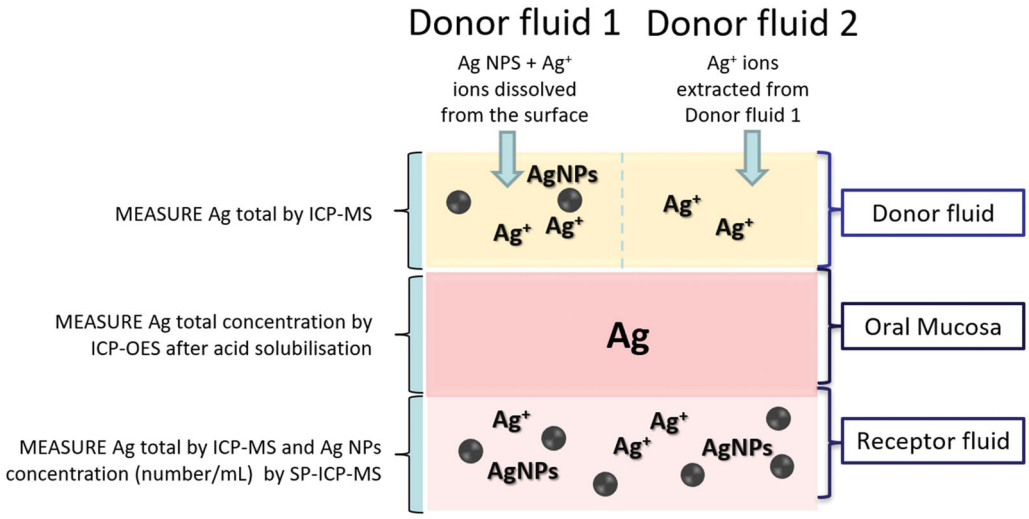

Figure 1. Scheme of experimental set-up used for testing Ag concentration in different compartments of Franz Cell System.

Spectrometer (PerkinElmer, U.S.A.), equipped with an S10 Autosampler. Analyses were conducted using a calibration curve obtained by dilution (range: $0-10$ mg L^{-1}) of Ag standard solution for ICP-OES analyses. The limit of detection (LOD) for Ag at the operative wavelength of 328.068 nm was 0.01 mg L^{-1}. The precision of the measurements expressed as RSD % for the analysis was always less than 5%. An Inductive Coupled Plasma Mass Spectrometer, ICP-MS Nexion 350X with an ESI autosampler (Perkin Elmer, USA instrument), was used to determinate the total silver concentration in the donator and receiver phases, after solubilization with 1 mL of HNO_3 (69%) for 10 mL of sample. The analysis was carried out in KED mode using ultra-high purity helium (flow rate of 4.8 mL min^{-1}) to minimize polyatomic interferences. The Ag calibration curve for dissolved metal element content was performed using Ag ionic standard (0.5, 1.0, 5.0 and 10.0 μg L^{-1}, ion mass 107 a.m.u.).

2.9. Sp-ICP-MS instrumentation and working conditions

The SP-ICP-MS analysis were run using the Nano Application Module in Syngisitx™ software (version 2.1). A pump speed 20 rpm for flush, wash and delay, and nebulizer gas flow of 1.12 mL min^{-1} were used for measurements. The sample uptake was 0.34 mL min^{-1} and a dwell time of 50 μs was used with 60 s sampling times. To calculate the transport efficiency (1.5 and 10 μg L^{-1}) ionic Au standard was used for SP-ICP-MS ionic calibration (ion mass 197 a.m.u.). While Au 60 nm NPs citrate coated suspension were used as standard for NPs size dimension in Single-Particle software calibration material. The 60 nm Au particles were diluted in MilliQ water (18.2 MΩ cm), for SP-ICP-MS size calibration, to approximately 50,000 ± 10,000 particle mL^{-1}. We derived this value, considering the NPs number reported in the Sigma Aldrich datasheet (1.76–2.16 10^{10}). NPs concentration and other parameters provided by the supplier are reported in Table S2. We calculated a transport efficiency of 4.0 ± 0.2%. NPs dimension detectable by SP-ICP-MS is NPs dimension >15 nm, while Ag ions are not measured by SP-ICP-MS directly or by difference accurately for several reasons (Merrifield, Stephan, and Lead 2017b). Calibration curve obtained for Ag dissolution concentration is reported in Figure S2. Ag concentration data (μg cm^{-3}) in the receptor solution were converted to the Ag amount that penetrated per skin surface unit (μg cm^{-2}). Data analysis was performed with Excel for Windows, release 2007 and Stata Software, version 11.0 (StataCorp LP, College Station, TX, USA). Skin absorption data were reported as mean Ag total concentration ± SD. The difference among independent data was assessed by means of the Mann-Whitney test. A p value <0.05 was considered significant. Silver speciation was estimated with the use of computational model Visual MINTEQ 3.1 (Visual Basic 2012). Using as input data: pH, temperature, ions concentration and media

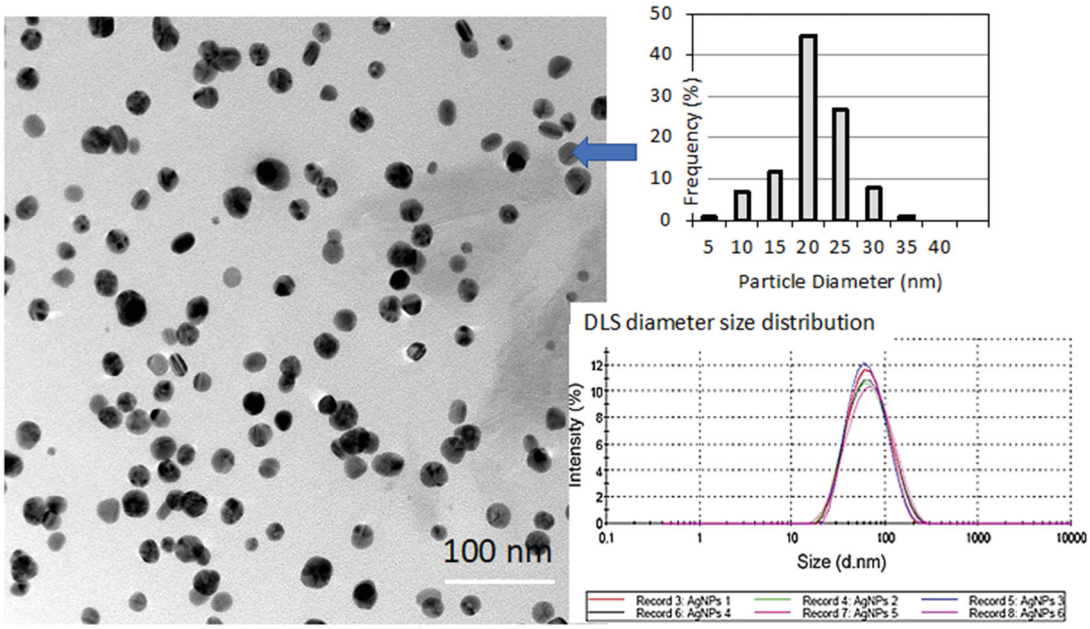

Figure 2. TEM images and DLS size (diameter) distribution of the Ag NPs dispersed in physiological solution.

composition, the computational model can provide as output information about possible soluble and insoluble species (K_{ps} and ΔH_f thermodynamic constants are reported in Table S4). This calculation allowed a more accurate estimation of the ions fraction present in the receptor fluid, once subtracted the amount of Ag NPs measured by SP-ICP-MS.

3. Results and discussion

3.1. Characterization of Ag NPs

The particle size distribution of Ag NPs in water and in exposure medium (donor fluid 1) was evaluated. TEM images show spherical NPs with a quite narrow distribution of size, quantified as 19 ± 5 nm ($n = 150$). TEM measurements were compared to DLS diameter size distribution (Figure 2).

The expected difference between the TEM and DLS sizes was the result of various factors, as elsewhere reported (Blosi et al. 2011). In principle, the DLS technique measures the hydrodynamic size and is influenced by polydispersity and aggregation.

3.2. Ag penetration in oral mucosa

The concentration of Ag detected in the mucosa after 240 min of exposure of donor fluid 1 and 2 are reported in Figure 3 and Figure S3. The higher Ag concentration found after exposure to donor fluid 1 (0.32 μg cm^{-2}), in comparison with donor fluid 2 (0.08 μg cm^{-2}), is explained by the translocation of both Ag ions and NPs. However, in both cases, the concentration of silver able to penetrate into the mucosa is very low (less than 1%). The higher percentage of penetration is found for the donor fluid 2, indicating the higher mobility of Ag ions compared to Ag NPs. In both cases (donor fluid 1 and 2), the dose extrapolated values and reported in Table S3 were below the limits recommended by EPA and ECHA for adult population exposed to silver (see extrapolation reported in ESI). In addition, comparing our results with the penetration of Ag (NPs and ions) reported in literature for adult porcine mucosa at the similar exposure condition (Mauro et al. 2015), we found an higher content of silver in baby tissues. The result is consistent with differences between young and adult mucosal membranes. Oral mucosa thickness, in fact, is age-dependent (Burns et al. 2004) and as a consequence the skin/mucosa permeability of baby tissues is higher than in adult individuals, especially during the first year of life, justifying the observed higher accumulation of silver in the mucosa.

3.3. Ag permeation in receptor fluid

The total silver concentration detected in the receptor fluid is reported in Figure 4. The permeation of

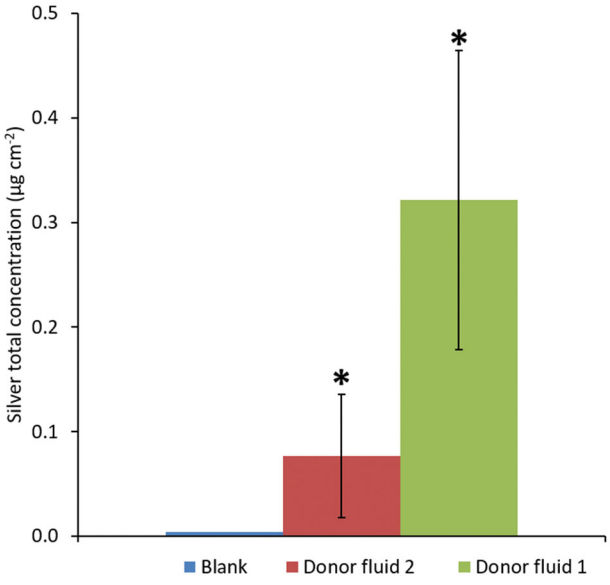

Figure 3. Total Ag detected in oral mucosa from the two donor fluids after 240 min exposure. The donor fluid 2 contains the Ag^+ ions extracted by donor fluid 1, so the differences are due to the penetration of Ag NPs of Donor fluid 1. *: values significantly different from the blank, $p < 0.05$.

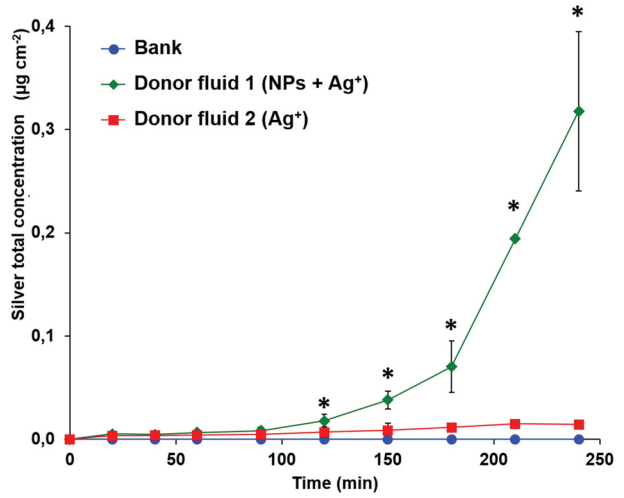

Figure 4. Silver permeation in the receptor fluid. The value of silver total concentration after 240 min of exposure correspond to a silver content of 65.7 ± 16.4 µg L^{-1} in receptor fluid (*: values are significant different from the blank, $p < 0.05$).

silver from donor fluid 2 is negligible and not significantly different to the blank (p values > 0.05). This suggests that Ag^+ ions are most likely precipitated and/or stopped and absorbed into mucosa, probably by the binding with -SH group present in the oral membrane(Iurina, Pil'tiaĭ, and Protsenko 1986; Toto 1965) and are not able to reach the receptor fluid. While for donor fluid 1 (Ag NPs + Ag^+), we observed a permeation of silver in the receptor fluid with a flux of 4.1 ± 1.7 ng $cm^{-2} min^{-1}$ and a lag time of 159 ± 17 min, most likely attributed to the NPs fraction. These results confirm the capability of PVP coating to mask the Ag NPs surface and allow a better dispersibility and stability, promoting NP permeation through tissue (Merrifield and Lead 2016; Tejamaya et al. 2012), even if in a very low concentration compare to the initial concentration (ca. 0.04% wt). Thus, this behavior could potentially promote toxic effects, through a Trojan horse mechanism, that would justify the higher reported cytotoxicity of Ag NPs if compared with the same concentration of Ag ions (Park et al. 2010).

The results of SP-ICP-MS analysis of the receptor fluid, exposed to donor fluid 1, are reported in Table 1 and in Figure 5. As shown in Figure 4, the permeation of Ag has a time lag of ca. 150 min. The size of particles detected by SP-ICP-MS, after this time (23 ± 8 nm), is comparable with TEM size, suggesting a penetration through the oral mucosa of untransformed NPs. The size distribution histograms of Figure 5 and Table 1 show how NPs detected are distributed in terms of size and relative frequency. The observed variation of particle number has not statistically significant ($p = 0.28$), so we can conclude that the particles number does not seem to be affected by time of exposure (Table 1). In addition, the nonsignificant change in most frequent size and mean diameter vs time of exposure shown in Figure 6, stays within the standard deviation, suggesting the absence of aggregation phenomena or of Ostwald ripening growth during the time of experiment. Computational analysis, using the speciation code MINTEQ, was also performed to estimate dissolved silver speciation in the receptor fluid (Table S4). The computational outputs suggest that the dissolved fraction in the receptor fluid, exposed to donor fluid 1, is mainly characterized by the presence of soluble species as $AgCl_2^-$ and $AgCl_3^{-2}$ (more than 93% of total ionic silver). The small amount of insoluble AgCl hypnotized by the computational model, ca. 6%, only slightly affect the estimation of Ag NPs size on Ag ions content (difference between Ag total and Ag NPs concentration), made by SP-ICP-MS technique.

Therefore, we found that Ag NPs are able to penetrate the baby porcine oral mucosa, reaching the receptor fluid and being potentially available for translocation processes after *in-vivo* exposure.

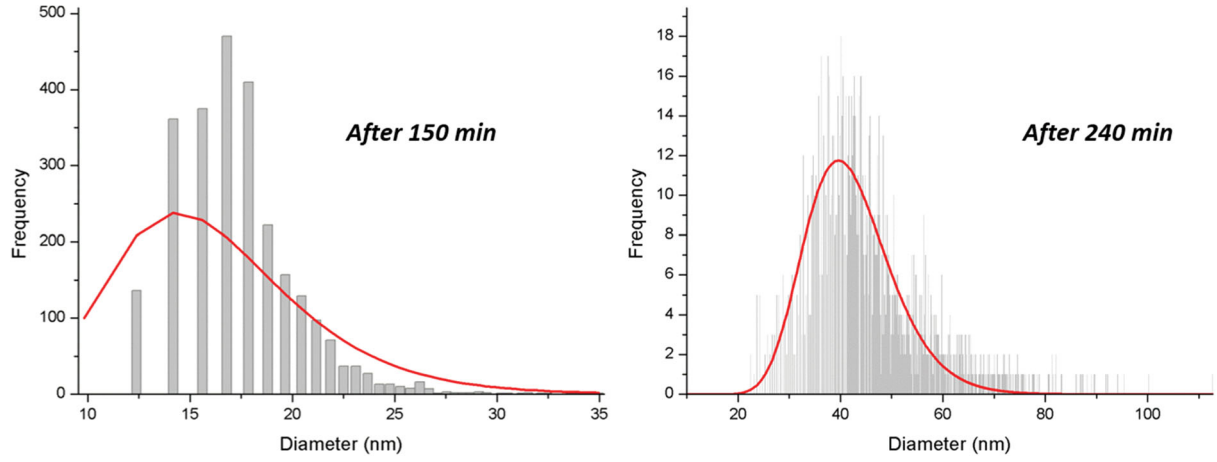

Figure 5. Ag NPs size distribution by frequency detected by SP-ICP-MS, at different time of exposure.

Table 1. SP-ICP-MS measurement of receptor fluid exposed to donor fluid 1.

Time (min)	Mode Size (nm)	Mean Size (nm)	Ag NPs (number mL^{-1})	Ag dissolved ($\mu g\ L^{-1}$)	Fraction Ag dissolved (%)	Fraction of Ag NPs (%)
150	19 ± 7	23 ± 8	215576 ± 120408	0.35 ± 0.29	25 ± 9	75 ± 9
180	21 ± 7	26 ± 9	155136 ± 120250	0.39 ± 0.22	23 ± 6	77 ± 6
210	24 ± 0	29 ± 1	322290 ± 78447	0.51 ± 0.07	16 ± 1	84 ± 1
240	31 ± 8	38 ± 9	177445 ± 87374	1.45 ± 1.25	35 ± 10	65 ± 10

The increase in ions amount, from 5% in donor fluid, to ca.25% in the receptor fluid can be most likely attributed to Ag NPs surface transformation (more likely adsorption of bio-surfactants when crossing mucosa), that could justify the higher amount of ions released that stay in equilibrium with solid surface (Gardini et al. 2018). Due to similarity between human and pig membranes, as reported in literature (Adams 1974), it is reasonable to assume that a trans-oral mucosa passage could occur also in children mucosa once in contact, voluntary or accidentally, with silver NPs.

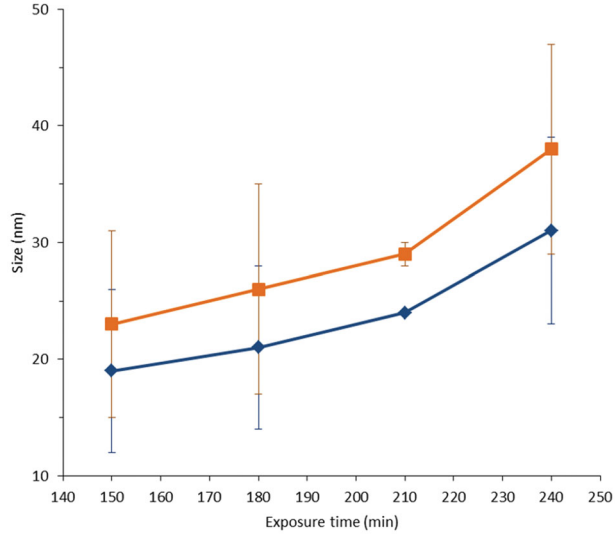

Figure 6. Size distribution of Ag NPs permeated in the receptor fluid as a function of time: ■ most frequent size (size at the maximum of the size distribution histogram) and ◆ mean size (Z-average). There are no statistically relevant differences between the two size populations (blue and orange) ($p < 0.05$ only for sample at 210 min) and comparing different times (ca. $p = 0.17$).

4. Conclusions

To our knowledge, this work presents one of the first attempts to evaluate Ag NP permeation and penetration through baby porcine oral mucosa to predict the effect of potential oral absorption of Ag NPs in children. Franz *ex-vivo in-vitro* diffusion cell was used to investigate the total amount of silver penetrating the oral mucosa and achieving the receptor fluid, during the first 240 min of exposure. The comparison between the two donor fluids, the first containing Ag NPs dispersion and the second only the ionic fraction, collected from the first donor fluid, suggested that PVP coated Ag NPs are untransformed and cross the mucosa, reaching the donor fluid, whilst Ag^+ ion speciate with Cl^- and cannot easily translocate. The SP-ICP-MS analysis of the receptor fluid and the use of computational

model allowed us to estimate the content of Ag NPs and Ag^+ ions and the NPs degree of aggregation/agglomeration, revealing that the Ag NPs reaching the donor fluid are quite well dispersed and able to release a higher concentration of ions if compared to the pristine donor fluid. Due to the similarity between human and pig oral mucosa, these findings would support in baby individuals the potential permeation of Ag NPs through oral mucosa, even at level below the reference doses recommended by existing authorities (EPA and ECHA).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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