Materials Science and Engineering C 80 (2017) 624–630

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

The application of silver nano-particles on developing potential treatment for chronic rhinosinusitis: Antibacterial action and cytotoxicity effect on human nasal epithelial cell model

Shyuan-Yow Chen [a], Min-Tsan Huang [b,1], Sylvia L.F. Pender [c], Muhammad Ruslin [d], Hsin-Hua Chou [b,e,*], Keng-Liang Ou [a,b,f,g,h,*]

[a] Department of Dentistry, Cathay General Hospital, Taipei 106, Taiwan
[b] School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei 110, Taiwan
[c] Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
[d] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[e] Dental Department of Wan-Fang Hospital, Taipei Medical University, Taipei 116, Taiwan
[f] Department of Dentistry, Taipei Medical University Hospital, Taipei 110, Taiwan
[g] Department of Dentistry, Taipei Medical University-Shuang Ho Hospital, New Taipei City 235, Taiwan
[h] Biomedical Engineering Research and Development Center, China Medical University Hospital, Taichung 404, Taiwan

ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 25 December 2016
Accepted 21 March 2017
Available online 22 March 2017

Keywords:
Chronic rhinosinusitis
Silver nano-particles
Human nasal epithelial cell
Cytotoxicity
Antibacterial agent

ABSTRACT

Chronic rhinosinusitis (CRS) has raised attentions both in many countries due to its high relapsing rate and the resistance of certain treatments especially antibiotics application on both acute and chronic bacterial rhinosinusitis. The aim of this research is stressing on developing an effective alternative treatment for treating CRS and reducing the use of antibiotics to avoid further resistance forming. The antibacterial functions of silver nano-particles (AgNPs) are well known according to previous reports and studies. However, for developing a suitable treatment for further clinical application, a variety of AgNPs cell cytotoxicity experiments and AgNPs antibacterial properties experiments were examined *in vitro* in this study. For imitating the clinical condition of CRS, the human nasal epithelial cell line (RPMI2650) has chosen as experimental model. Moreover, Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) were selected for antibacterial function experiments. The analytical results demonstrated that 5 ppm of AgNPs not only maintains >80% of cell activity to RPMI2650, but also possesses >80% of antibacterial function to *S. aureus* and 100% of antibacterial function to *E. coli*. Therefore, 5 ppm of AgNPs might be considered as a promising antibacterial agent for treating CRS.

1. Introduction

CRS has been considering as one of severe prevalent chronic health problems for both Western and Asian countries, which impacts all age groups of people due to its high relapsing rate. CRS is a condition which can be classified according to various symptoms of sinus inflammation with the time period at least two months or longer [1]. There are numerous factors can potentially induce rhinosinusitis including infection, allergy, inflammation or obstruction of the sinuses [1]. Moreover, in terms of bacteria-infected rhinosinusitis which covers four main kinds of bacterial strains such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* [2,3]. Based on a meta-analysis study that collected data from 1990 to 2006, which involved various antibiotic treatment studies of acute bacterial rhinosinusitis in the United States, the result conducted a potential fact that *S. aureus* could be considered as the main pathogen in acute bacterial rhinosinusitis [2]. Additionally, a clinical outcome of research conducted by Niederfuhr et al. [4] indicated that *S. aureus* might play an important role with respect to CRS.

In terms of the pathogenesis of CRS which might due to unsolved bacterial-infected and inflammation on/inside nasal mucous membrane inside the nose [4]. A number of medical studies have applied the RPMI2650 cell line as testing model because of its high similar descriptions of human nasal mucous membrane cell including the function of yielding mucoid material on the cell surface, creating an enzymatic metabolic encumbrance, and also possessing comparable permeability with human nasal mucosa [5].

Nowadays, antibiotics have been considered as one of common treatments at the first stage for treating acute bacterial rhinosinusitis.

* Corresponding authors at: School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei 110, Taiwan.
E-mail addresses: hhchou@tmu.edu.tw (H.-H. Chou), klou@tmu.edu.tw (K.-L. Ou).
[1] Co-first author: Min-Tsan Huang.

http://dx.doi.org/10.1016/j.msec.2017.03.189
0928-4931/

It is evident that the most common treatment for bacterial infection rhinosinusitis is prescribed antibiotics [6–8]. However, resistance of antibiotics is recognized as an issue that arises when an antibiotic has lost the ability to control or eliminate bacterial growth within human bodies from time to time [2,6]. Additionally, untreated acute bacterial rhinosinusitis might possess high probability of developing into CRS due to uncompleted antibiotics therapy for treating sinusitis [6].

Ag is a well known element with potential germicidal functions which has applied as antibacterial agents by releasing as metal Ag^+ ions or nano-particles [9–12]. AgNPs are considered as possessing the ability to perforate the bacterial cell wall and afterward run through it. Accordingly, inciting structural transforms in the cell membrane such as its permeability and death of the cell [11,13]. A dedicate study conducted by Panáček et al. [6] which combined various antibiotics with low concentration AgNPs for improving antibacterial efficiency. The outcomes demonstrated that AgNPs increased the efficiency of antibiotics and decreased the usage amount of antibiotics for eliminating bacteria. Nevertheless, there was a lack of research that has stressed on investigating or applying the antibacterial capacities of AgNPs as a potential clinical antibacterial agent for especially treating CRS. Therefore, this research aims to develop a promising antibacterial agent of treating CRS by applying different concentrations of AgNPs as the intermedium to inhibit *S. aureus* and using RPMI2650 cell groups as the examinational model. The antibacterial functions and cell cytotoxicity response of AgNPs will be examined in this study.

2. Materials and methods

2.1. Preparation of AgNPs

The 100 ml of aqueous buffer solution with concentration of 20 ppm and diameter of 10 nm AgNPs was purchased from Sigma-Aldrich, Taiwan. The phase identification of AgNPs was analyzed by a high resolution transmission electron microscope (TEM, JEM-2100) equipped with an energy-dispersive X-ray spectrometer (EDS, INCA). The 20 μl suspension was pipetted onto copper grid coated with carbon film. Subsequently, the grid was dried thoroughly in an electronic dry cabinet and then observed at 200 kV. For cell cytotoxicity and antibacterial testing, the solution was diluted into different concentrations of 1, 3, 5 and 7 ppm and which were defined as group of AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7, respectively.

2.2. TEM observation of RPMI2650 cell cultured with AgNPs

To investigate the phenomenon of AgNPs entering RPMI2650 cell (ATCC CCL-30, Taiwan), the RPMI2650 cells were cultured first within formulated Minimum Essential Medium (MEM; Gibco, USA), and further, initial medium were supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS, Gibco USA) mixed with 1% Penicillin Streptomycin (P/S, Gibco USA) in a highly humidified atmosphere. Subsequently, the RPMI2650 cell suspensions with a 1×10^5 cells ml^{-1} were cultured with 5 ppm of AgNPs (ultra-sonicated for 30 min) and without AgNPs in an incubator at 37 °C with 5% CO_2 for 24 h, respectively. Sample preparations for TEM were followed by fixing, dehydrating, resin embedding, ultra-microtome sectioning. Cross-sectional appearance of the samples was observed using the Hitachi HT-7700 TEM at 80 kV.

2.3. Morphology observation of RPMI2650 cell cultured with S. aureus

To evaluate the cell morphology of RPMI2650 when suffering the invasion of *S. aureus* (ATCC6538P, Taiwan), the RPMI2650 cells were cultured first within formulated medium as mentioned before. Meanwhile, *S. aureus* strain was grew and maintained in a formulated Tryptic Soy Broth (TSB, Acumedia USA) with anaerobic atmosphere in a dry anaerobic indicator strip (GasPak™, Becton, Dickinson and Company USA) under 37 °C for incubating 24 h. For tracking the potential invasive methods of *S. aureus* on RPMI2650 cells, *S. aureus* had processed by fluorescein isothiocyanate before culturing with RPMI2650 cells. Hereafter, the 1×10^4 cells ml^{-1} RPMI2650 cell suspensions were cultured with 1×10^4 CFU ml^{-1} *S. aureus* and without *S. aureus* in an incubator at 37 °C with 5% CO_2 for 1 h, respectively. Morphology observation was conducted by Olympus IX71 optical microscope (OM) and fluorescence microscope (FM).

2.4. Cell cytotoxicity assay

The cell cytotoxicity assay was followed by ISO 10993-5 specifications. The different concentrations of AgNPs (*i.e.* AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7) were ultra-sonicated for 30 min to avoid self-aggregation and were sterilized by sterile filtration using syringe filters (pore size ≤ 0.22 μm). Next, added into culture medium then were gentle shaking at 37 °C for 24 h on an orbital shaker. Meanwhile, to culture the RPMI2650 cells with 1×10^5 cells ml^{-1} to each well in a 96-well micro-plate. Subsequently, cultured with AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7 samples for three independent experiments ($N = 3$) at 37 °C with 5% CO_2 for 24 h, respectively. Cells were then incubated in 50 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution for an additional 3 h to form the formazan solvent precipitates. Then, the MTT solution was decanted and 100 μl of isopropanol were added in each well. To sway the plate for 10 min to release the formazan and subsequently applied ELISA Reader (BioTek-Epoch, USA) with a 570 nm filter to read the optical density (OD) of cells. Zinc dibutyldithiocarbamate (ZDBC) as positive control and high density polyethylene (HDPE) as negative control, respectively. Control (blank) cells were exposed to FBS medium only.

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of blue-violet formazan formed, as monitored by the OD at 570 nm. The reduction of viability was calculated by Eq. (1) which represented the cell activity:

$$\text{Viability}\% = OD_{570e}/OD_{570b} \quad (1)$$

where OD_{570e} is the mean value of the measured OD of the test sample; OD_{570b} is the mean value of the measured OD of the blank. The sample has considered as acute cytotoxic potential when viability value of the sample is reduced to <70% of the blank.

2.5. Antibacterial evaluation

Before the examination, all experimental tools were sterilized at 120 °C for 15 min by autoclaving. The bacterial strains of *S. aureus* which cultured in Tryptic Soy Broth (TSB, Acumedia USA), and *E. coli* (ATCC8739, Taiwan) which cultured in lysogeny broth (LB, Acumedia USA) were conducted in the tests. For *S. aureus*, nutrition agar plates were streaked out with a loop from the frozen stock and grown in anaerobic environment with GasPack system overnight at 37 °C, more so, *E. coli* were cultured under aerobic environment overnight at 37 °C. A single colony was incubated into nutrition broth in a conical flask and prepared the bacterial suspension at 1×10^5 CFU ml^{-1}. Subsequently, both bacterial *S. aureus* and *E. coli* suspensions were incubated with ultra-sonicated AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7 samples ($N = 3$) at 37 °C for 24 h under anaerobic and aerobic environment, respectively. After incubation, the colony of each petri-dish was counted to calculate the number of bacteria. The antibacterial rate (AR) determined by Eq. (2) was considered as the antibacterial effect [11].

$$AR\ (\%) = 100 \times \left[N_{(control)} - N_{(sample)}\right]/N_{(control)} \quad (2)$$

where $N_{(control)}$ is number of bacteria adhering on control petri-dish after 24 h incubation, and $N_{(sample)}$ is number of bacteria adhering on AgNPs medium contented petri-dish after 24 h incubation.