Contact Lens & Anterior Eye 37 (2014) 149–152

Contents lists available at ScienceDirect

Contact Lens & Anterior Eye

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

Preparation, characterization and antimicrobial study of a hydrogel (soft contact lens) material impregnated with silver nanoparticles

Bibi Sedigheh Fazly Bazzaz[a], Bahman Khameneh[b], Mohammad-mehdi Jalili-Behabadi[c], Bizhan Malaekeh-Nikouei[d], Seyed Ahmad Mohajeri[c,*]

[a] Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Students Research Committee, Department of Food and Drug Control, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Targeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Nanotechnology Research Centre, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 10 December 2012
Received in revised form
16 September 2013
Accepted 17 September 2013

Keywords:
Antimicrobial
Disinfection
Soft contact lens
Hydrogel
Keratitis
Microbial keratitis
Silver nanoparticles

ABSTRACT

Purpose: Contact lenses that incorporate antimicrobial properties may reduce the risk for microbial-associated adverse events for lens wearers. The aim of this study was to assess the antimicrobial effects of silver nanoparticles (NP) when impregnated in a hydrogel material.
Methods: Hydrogel disks, used as a proxy for soft contact lenses, were prepared with silver NPs to add an antimicrobial effect to the polymer. Six groups of disks were created, each with a different concentration of silver NPs. The antimicrobial effect of the hydrogels against *Pseudomonas aeruginosa* (ATCC15442) and *Staphylococcus aureus* (ATCC6538) was evaluated at 6, 24, 48 and 72 h.
Results: Silver NP concentrations ranged from 20.71 to 98.06 μg/disk. All groups demonstrated excellent antibacterial effects against *P. aeruginosa* at each time point. After 6 h all disks didn't exhibit desirable antibacterial activity against *S. aureus*; whereas except those with 20.71 μg silver NPs showed antibacterial activity at 24 h and only the disks with 57.13 and 98.06 μg silver NPs showed antimicrobial activity at 48 and 72 h.
Conclusions: The development of contact lenses made of a silver NP-impregnated hydrogel material may bring antimicrobial effects sufficient to decrease the risk of microbial-related adverse events for lens wearers.

1. Introduction

Soft (hydrogel) contact lens wear is associated with non-infectious, but microbial-related, forms of keratitis such as Contact Lens-induced Acute Red Eye (CLARE) and Contact Lens-induced Peripheral Ulcers (CLPU) and with infectious keratitis ("microbial keratitis", or "MK") [1]. Some of the many approaches that have been pursued to decrease the risk for developing such adverse events are improved. These approaches are included in lens and lens case hygiene, using lens care solutions that provide effective disinfection, and frequent replacement of lens cases. The development of antimicrobial surfaces for contact lenses or contact lens storage cases may offer an effective strategy to decrease the risk of MK. A contact lens or lens case could incorporate antimicrobial agents on the surface or infused into the bulk of the lens or lens case material to provide antimicrobial properties [2,3]. Coating contact lenses with a cationic peptide, such as melimine, has demonstrated that the antibacterial properties of the peptide could reduce the prevalence of bacteria-associated contact lens-related adverse responses and events in *in vivo* models [4]. Contact lenses coated with a cationic peptide have also shown a broad-spectrum antimicrobial activity which could impede the development of CLARE and CLPU in animal models [4,5]. Selenium that was covalently bonded to silicone hydrogel contact lenses has been shown to reduce the colonization of *P. aeruginosa in vitro* and demonstrated a degree of safety in animal model testing for up to 2 months of extended wear [6]. In other studies, the inclusion of norfloxacin, fimbrolide [3] or silver nanoparticles [7–12] on the surfaces of contact lenses and lens cases for antibacterial effect has also been investigated. Nano-sized silver particles provide a broad-spectrum antimicrobial effect through mechanisms such as inhibition of replication by interfering with DNA and RNA, disruption of the cell membrane, interference with cell respiration, and inactivation and alteration of enzyme conformation [13]. Silver nanoparticles also show a low toxicity in therapeutic doses to human tissues [14,15]. Silver coated contact

* Corresponding author. Tel.: +98 511 7112611; fax: +98 511 7112470.
E-mail addresses: mohajeria@mums.ac.ir, seyedahmad_mohajeri@yahoo.com (S.A. Mohajeri).

1367-0484/$ – see front matter © 2013 British Contact Lens Association. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.clae.2013.09.008

lenses have been shown to be effective against *P. aeruginosa* and *Staphylococcus aureus*, the microorganisms most commonly associated with the serious adverse event of MK [16,17], by impeding biofilm formation [7] and bacterial colonization [8]. Silver nanoparticles also have a pronounced activity against *Acanthamoeba* sp. [7]. The effectiveness of incorporating silver ions into the plastic of a contact lens case has been reported [9–11] and such lens cases have been commercially available for a number of years [2].

Regardless of the technology used, an effective antimicrobial lens or lens case could potentially reduce or eliminate infectious or inflammatory adverse responses and events that are associated with the presence of microorganisms. The aim of this study was to evaluate the *in vitro* antimicrobial efficacy of hydrogel lens proxies prepared with varying concentrations of nano-sized silver particles.

2. Methods

2.1. Hydrogel disks preparation

Radical polymerization technique was used in the preparation of the hydrogel disks (soft contact lens proxies). EGDMA (ethylene glycol dimethacrylate, Aldrich [Milwaukee, USA]) (140 mM or 1.70 mol%, as a cross-linker) and MAA (methacrylic acid, Aldrich [Milwaukee, USA]) (200 mM, equivalent to 2.40 mol%) as comonomer were dissolved in HEMA (2-hydroxyethyl methacrylate, Aldrich [Milwaukee, USA]) (95.90 mol%, as a backbone monomer) with the addition of different amounts (1, 2, 3, 4, 5 and 7 mg) of nano-sized silver (particle size <100 nm) (Sigma–Aldrich [St. Louis, MO, USA]) to create 6 hydrogel groups (A–F). AIBN (2,2′-azobisisobutyronitrile, Acros [Geel, Belgium]) (10 mM) was added as a free-radical initiator. Nitrogen was purged through the mixture to remove oxygen for 5 min; each monomer mixture was immediately injected into a mold (0.4 mm thick, 3.2 ml volume) made of two polypropylene plates. The mold was then placed in an incubator overnight at 60 °C. After polymerization, each polymer was immersed in boiling water to remove unreacted monomers and polymer film was then punched into disks with a diameter of 14 mm (similar to commercial contact lenses). The control hydrogel was prepared, in the absence of nano-sized silver, under the same condition described above. The disks were washed in borate-buffered saline and then soaked for 10 min in sodium hypochlorite (5.25%) as an oxidation reagent; then rinsed 5 times in 0.85% physiological saline solution.

2.2. Atomic absorption spectrometry (AAS)

2.2.1. Determination of silver in hydrogel disks

Samples were taken into solution by acid digestion in a Kjeldahl flask. The standard solutions (0.5, 1, 1.5, 2 and 2.5 mg/L of silver NPs) were prepared in 2% H_2SO_4. The calibration curve was plotted based on atomic absorption. Acidic digestion method was applied to digest hydrogel matrix [18]. Each disk was suspended in nitric acid and perchloric acid at a ratio of 1:1 and boiled to obtain clear solution. Resulting solutions were analyzed by atomic absorption spectrometry (AAS) [19].

2.2.2. Determination of silver in culture media

For determination of silver in culture media, each disk was soaked in the culture media for 72 h and amount of silver in each sample was analyzed by AAS.

2.3. In vitro antibacterial activity assessment

Antibacterial properties of the disks were assessed by biological broth method, whereby the disks were washed with Dulbecco's phosphate buffered saline without calcium chloride and magnesium chloride, then soaked into 1000 μl Mueller Hinton Broth containing approximately 10^8 CFU/ml *P. aeruginosa* (ATCC 15442) or *S. aureus* (ATTC 6538); and finally incubated at 37 ± 0.2 °C for 24, 48 and 72 h. The soaking solutions were cultured using Pour Plate Method to enumerate the bacteria.

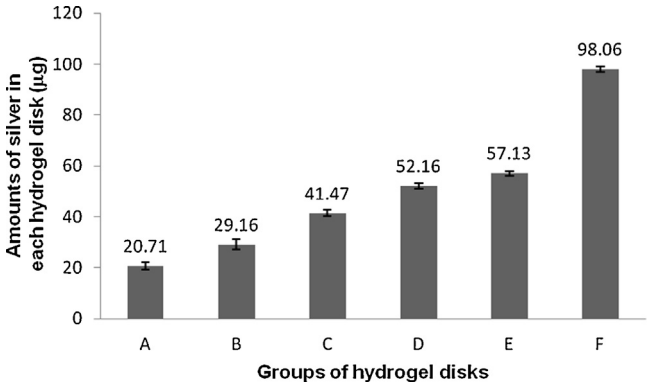

Fig. 1. Micrograms of silver nanoparticles (mean ± SD) for triplicate testing of each group of hydrogel disks. Groups A, B, C, D, E and F representing the hydrogel proxies that were prepared with 1, 2, 3, 4, 5 and 7 mg of silver nanoparticles, respectively.

2.4. Statistical analysis

The data were log transformed prior to data analysis. Differences between the groups were analyzed using ANOVA test. Statistical significance was set at 5%.

3. Results

3.1. Determination of silver in hydrogel disks and culture media

The amount of silver NPs used for preparation of hydrogel series of A, B, C, D, E and F was 1, 2, 3, 4, 5 and 7 mg, respectively. According to AAS data the amount of silver in each hydrogel disk of series A–F varied from 20.71 to 98.06 μg/disk (Fig. 1). After 72 h of incubation, the amount of silver in the culture media of each series of disks was 16.5, 18.4, 27.9, 29.1, 30 and 32.5 μg, respectively. No silver was detected in the culture of control disks. The percent of silver released, for the above mentioned disks, was 79.7%, 63.1%, 67.3%, 55.8%, 52.5% and 33.1%, respectively.

3.2. In vitro antibacterial activity assessment

The control group, with no silver NPs, showed no antibacterial activity against *P. aeruginosa* or *S. aureus* after 6, 24, 48 and 72 h incubation. Against *S. aureus*, disks with 20.71 μg of silver NPs had no significant effect compared to control group (Fig. 2). Although, the reduction of bacteria was between one and two logs after 6 h for all other disk groups, the logarithmic reduction was more than two logs after 24 h (except the disks with 20.71 μg silver NPs). However, there was no significant difference between disks containing 29.16 and 41.47 μg/disk silver NP ($p > 0.05$). Also no difference was observed between disks with 52.16 and 57.13 μg silver NPs/disk ($p > 0.05$). After 48 and 72 h of incubation time, only disks with 57.13 and 98.06 μg silver NPs/disk showed significant reduction in bacterial growth, with no significant difference between the two groups ($p > 0.05$). Against *P. aeruginosa*, all of hydrogel disk groups showed a 3 log reduction or greater at 6 h and remained effective at 24, 48 and 72 h (Fig. 3).