Supplementary Information

Self-sterilizing antibacterial silver-loaded microneedles

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Experimental

Preparation of Silver nanoparticles

Silver nanoparticles capped with 2-mercaptosuccinic acid (MSA) were synthesized following established procedures [1, 2]. Briefly 144 mL of 2 mM silver nitrate was mixed with 60 mL of 2 mM 2-mercaptosuccinic acid in an ice cold bath with vigorous stirring followed by a slow addition of 6 mL sodium borohydride at 0.05M. A distinctive solution color change from transparent to dark brown was observed upon the addition of the sodium borohydride, indicating the formation of the silver nanoparticles.

Characterization of Silver nanoparticles

The hydrodynamic diameter of the nanoparticles was detected with a Nicomp 380 particle size analyzer, Nicomp particle Sizing Systems, USA at room temperature. The samples were diluted 40 times with milli-Q water prior to each analysis. Z-potential of the as-synthesized particles was measured with a ZetaSizer Nano, Malvern instruments, UK. Disposable cuvettes were used for these measurements.

A scanning electron microscope (Carl Zeiss Microscopy Merlin with GEMINI II column) equipped with a field-emission gun operated at 2kV was used to scan the samples. The secondary electron images were recorded with an Evenhart Thornley secondary electron detector. The silver nanoparticles samples were immobilized for 10 min over Si-wafer coated with a plasma-polymerized nano-thin layer of methyloxazoline deposited at a pressure of 0,13 mbar with a plasma radio frequency power of 50W and a deposition time of 3 min,[3].

A Transmission electron microscope (TEM, JEOL-2100F Japan) was used to image the samples to determine the size of the silver nanoparticles. The samples were prepared by placing 20 µL of AgNP solution as-synthesized on a carbon coated copper grid. The grid was left to dry overnight at room temperature prior to TEM imaging.

Fabrication of Silver loaded dissolving microneedles

CMC is a water soluble, biocompatible, mechanically strong, dissolvable polysaccharide that can be transdermally applied without inflammatory effects. [4, 5] The CMC concentration was chosen to have an optimum viscosity and a convenient drying time. The silver nanoparticle load was optimized to have a measurable antibacterial effect. Suspensions containing increasing amount of AgNp (0%v (MNCMC), 50%v or 0.77 µgAg/mgCMC (MNAg50) and 100%v or 1.55 µgAg/mgCMC (MNAg100)) were prepared by mixing CMC to a concentration of 80 mg/mL and AgNP solution in Milli-Q water at room temperature for 1h.

The dissolving microneedle samples were casted on silanized poly-dimethylsiloxane (PDMS) moulds. Silanization was performed to prevent the moulds from sticking to the patches. Briefly, the moulds were activated by an oxygen plasma treatment at 0.1 mbar, 50W for 2min and loaded into a silanization chamber with a small beaker containing 200 µL of hexamethyldisilanizane. The chamber was bring to vacuum and left overnight to react. The varied mixture suspensions were poured on the MN moulds, filled by centrifugation at 1000 x g for 5 min and dried in an oven at 40 °C overnight. The microneedle patches where then gently peeled off the mold and stored under vacuum until further characterization.

Characterization of AgNP loaded CMC microneedles

1 x 1 cm^2 microneedle patches consist of conical microneedles, 750 µm in height and 400 µm in diameter. The patches were examined using an optical upright microscope (Nikon Eclipse Ci-L) and scanning electron microscope (Carl Zeiss Microscopy Merlin with GEMINI II column) operated at 2kV. In order to investigate the physicochemical properties of the silver loaded microneedle patches, thermal gravimetric analysis were carried out in a Discovery TGA (TA instruments, Waters, LLC, USA). Samples were heated at 10°C/min from room temperature to 400°C in an open platinum HT pan and Nitrogen purge rate of 30 mL/min. The temperature was held in an isothermal at 400 °C for 3 min and the purge gas was changed to Oxygen with a rate of 30 mL/min, samples were then heated at 10°C/min to 900 °C. Differential scanning calorimetry of CMC MN was carried out in a Discovery DSC (TA instruments, Waters, LLC, USA). Samples were heated at 10 °C/min from room temperature to 350°C in an Aluminum pan with pierced lid and a Nitrogen purge rate of 50mL/min.

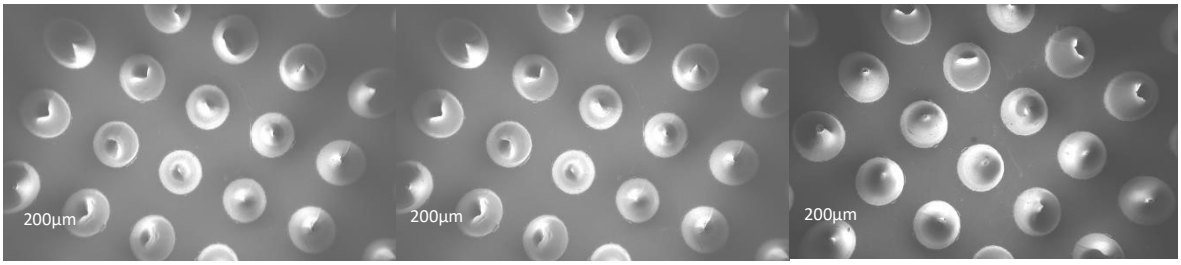

Figure S1. Scanning electron microscope pictures of the cellulose patches from left to right MNCMC, MNAg50 and MNAg100

Antibacterial assay

The anti-bacterial properties of AgNP loaded microneedles were tested against four bacterial strains including *Staphylococcus aureus ATCC 29213*, *Staphylococcus epidermidis ATCC 35984*, *Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa NCTC 6749*. All bacterial strains were chosen based on their ability to cause numerous nosocomial infections. The test samples included microneedles cut in the form of 5 mm discs loaded with AgNPs at 0.77 µgAg/mg CMC and 1.55 µgAg/mg CMC. The controls were sterile paper discs (5mm) loaded with 10µL PBS and 10µL AgNP.

The selected bacterial strains were revived from frozen stocks maintained at -80°C and plated on nutrient agar plates. After 24 hours incubation, isolated bacterial colonies were inoculated in fresh Tryptone Soy Broth (TSB) and allowed to grow to mid log phase (OD600nm ~ 0.3-0.5). The bacterial suspension was then diluted to $1X10^6$ colony forming unit (CFU) / ml using sterile TSB. 100 µl of the diluted bacterial suspension was added to separate nutrient agar plate and spread uniformly with a sterile spreader. The test samples and control paper discs were placed on top of agar plates and were incubated overnight at 37°C. The antibacterial activity was determined as the mean diameter of inhibition zones (mm) or zone of inhibition (ZOI) developed around the samples. Photographs were taken to further support these results.

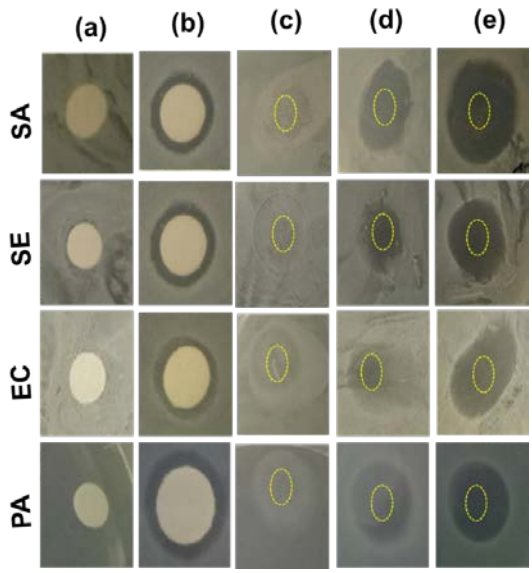

Figure S2. Zone of inhibition study (106 CFU/mL) for Straphyloccocus aureus (SA), Escherichia coli (EC), Straphyloccocus epidermis (SE) and Pseudomona aeruginosa (PA). (a) PBS diffusion disc (b) AgNP diffusion disc (c) MNCMC (d) MNAg50 (e) MNAg100

Dissolution of CMC microneedles

Microneedles should be hard enough to penetrate the *stratum corneum* without breaking or curving. This mechanical strength is influenced by the material and the needle sharpness and geometry.[6] In order to study the microneedle penetration in a human skin model, gelatin hydrogels were tailored to mimic the elastic modulus of human skin.[7] 5% w/v gelatin hydrogels were prepared by dissolving 0.5g in 10mL Milli-Q water at 40°C. 2mL of gelatin solution was then poured on Costar 6 well plate and allowed to settle for 12h at 4°C. The microneedles patches dyed with Brilliant Blue R were pressed on the gelatin and pictured before insertion and after different time points pressed in gelatin.

Cell culture

Cells of the monocyte cell line THP-1 and human dermal fibroblast (HDF) were used in this study. The THP-1 cells were maintained in RPMI (Sigma Aldrich) and HDF in DMEM (Life Technologies) containing 5% heat-inactivated fetal bovine serum (FBS, Thermo Scientific) and 1% (v/v) penicillin/streptomycin (Life Technologies) in a humidified atmosphere containing 5% CO2 at 37 °C. The cells were passaged using trypsin to dislodge them from the surface of the flask.

Cytotoxicity assay

The cells viability were determined by resazurin assay (sigma Aldrich). HDF cells were seeded on 24 well plate at a density of 5 x 104 cells/well. After overnight growth, the medium was removed and cells were washed with PBS. Fresh medium was added to the wells along with control and silver loaded microneedles. The cells were allowed to grow for 6 and 24 hours, after which the culture medium was replaced with media containing 10% resazurin and incubated for an hour. 200 µL of the media were pipetted to a 96 well plate and the fluorescent intensity was measured in a microplate spectrophotometer (λex = 544 nm and λem = 590 nm). The percentage of the cell viability was calculated

as:

Cell viability % = ((fluorescent intensity of treated)/ (fluorescent intensity of control)) X 100

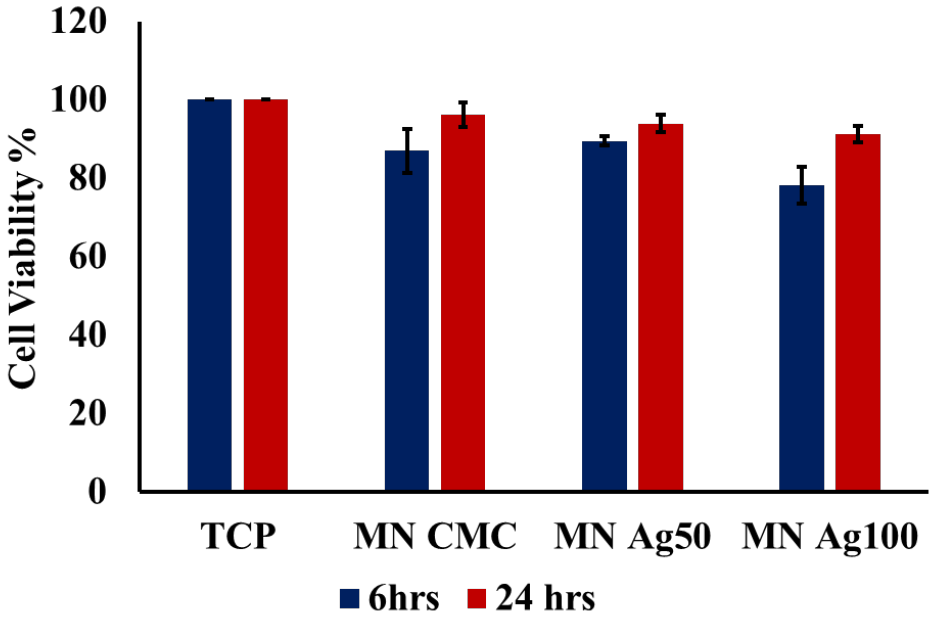

Figure S3. Viability of human dermal fibroblast assessed by resazurin metabolic activity assay. After 6 and 24 h of HDFs exposure to AgNPs containing dissolving microneedles, the cytotoxic effect was evaluated. As negative control untreated cells were used.

HDF's cells were used as a mammalian model cell to explore the cytotoxicity effect of silver loaded microneedles. After 6 hours the metabolic activity of the samples did not show significant difference compared with the control group (culture medium without microneedles). MNAg100 the group with the highest AgNPs concentration demonstrated a mild effect on decreasing the metabolic activity of the HDF cells after 6 hours study. However after 24 hour all the test samples show more than 90% viability, this indicate that silver loaded microneedles are not toxic to the mammalian cells.

Inflammatory response of silver loaded microneedles

THP-1 cells were differentiated into macrophages d THP-1 using PMA (phorbol-12-myristate 13-acetate) according to the protocol previously reported 8, and seeded on 24 well plate at a density of 5 x 104 cells/well. After overnight growth, the medium was removed and cells were washed with PBS. Fresh

medium were added to the wells along with control and silver loaded microneedles. Experiments were repeated with LPS (1µg/ml) to activate inflammatory macrophages and to give an infectious or inflammatory environment. After 6 hours of incubation the conditioned media were collected and centrifuged to remove the cell debris and silver nanoparticles. Supernatant were collected and analyzed for pro inflammatory cytokines of TNFα, IL-1β, and IL-8 using ELISA kits (BioLegend, San Diego, CA, USA) following the manufacturer's instructions.

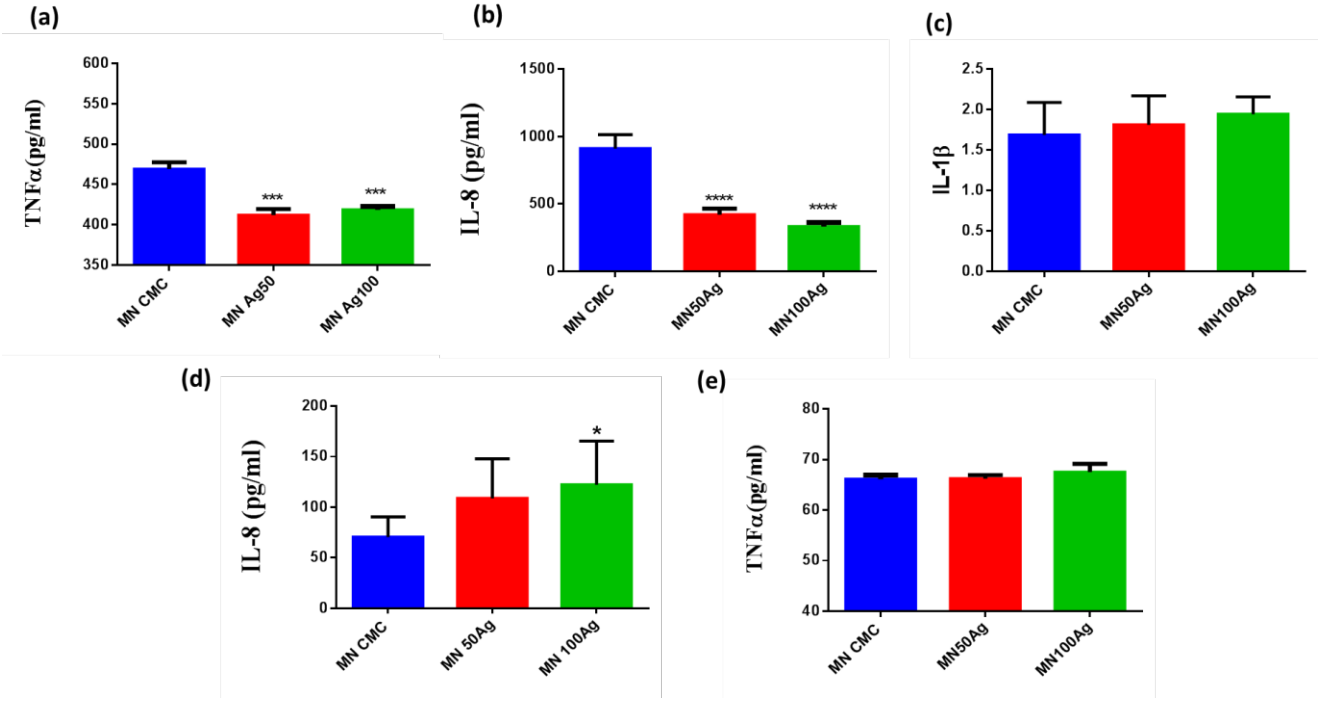

Figure S4. Activation response of macrophages (d THP-1) on AgNP loaded microneedles and controls, macrophages were incubated on control and AgNP modified microneedles, then stimulated with LPS for the detection of (a) TNF-α, (b) IL-8, and (c) IL-1β secretion. Pro inflammatory cytokine secreted by non-activated macrophages for (d) TNF-α, and (e) IL-8. Results represent two independent experiments, with a one way ANOVA statistical analysis being performed using a Dunnett's post-test (CMC control comparison group) $p > 0.05$ (mean ± SEM).

AgNPs loaded microneedles show decreased levels of pro-inflammatory cytokine production from d THP-1 macrophages activated with LPS (infectious or inflammatory environment). This anti-inflammatory properties are essential for microneedles since inflammation could slow down the healing rate and lead to the formation of chronic wounds. Pro-inflammatory cytokine production of TNF-α and IL-8 significantly decreased on both concentrations of silver loaded microneedles, while the production of IL-1β was not affected significantly. These findings of antiinflammatory properties of silver nanoparticles are consistent with our previous studies reported on BMDM.9 For the non-activated macrophages pro inflammatory cytokine production of TNF-α did not show significant changes for silver loaded microneedles compared to the control microneedles, however IL-8 cytokine production slightly increased for silver loaded microneedles. IL-1β were not detected on non-activated macrophages.

Inflammation is a part of complex biological response of the human body to protect against invading pathogens and foreign particles. On non-activated macrophages AgNPs-containing microneedles

encourage a small amount of cytokine production. However on an infectious or inflammatory environment the activated macrophages produced significantly less pro-inflammatory cytokines, this would be beneficial for wound healing and prevent the formation of chronic wounds. Thus silver loaded microneedles have been proved to be efficient in reducing inflammation and also protecting against bacterial infections.

References

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