Supporting Information for "*Safety and Efficacy of Composite Collagen-Silver Nanoparticle Hydrogels as Tissue Engineering Scaffolds*"

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Absorption spectra of AgNP@collagen: In order to insure particle stability before and after the lyophilization process, the absorption spectra for AgNP@collagen were recorded before and after lyophilization (see experimental details). Figure S1 shows that the absorption spectrum for both solutions have a similar shape and maximum position. The inset in Figure S1 indicates a decrease of around 10% in the area under the curve (AUC) for the reconstituted AgNP@collagen solution.

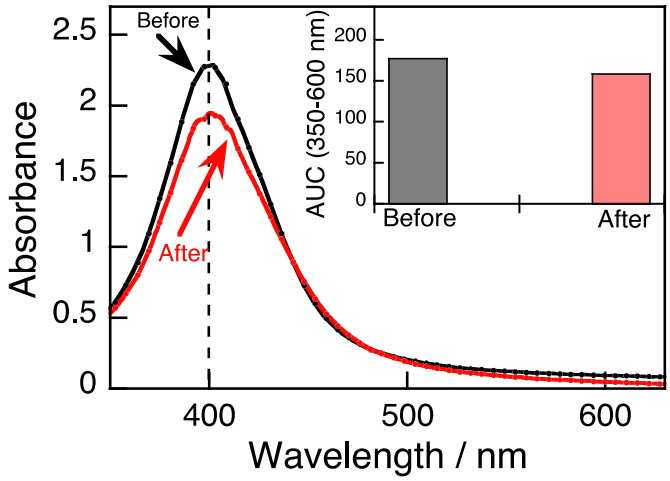

Figure S1. Representative absorption spectra for AgNP@collagen nanoparticles before (black) and after (red) lyophilization. The inset shows the calculated area under the curve between 350 to 600 nm for the two solutions (see experimental in main text). All measurements were carried out at room temperature.

Hydrogels washing procedure: Once the hydrogels were crosslinked they were washed daily with 15 ml PBS. Figure S2 is a representative example of the absorption spectra obtained with the washes of a 1.0 µM AgNP hydrogel over the course of five days. Note the spectra suggest that most of the side products were removed after the first two washes.

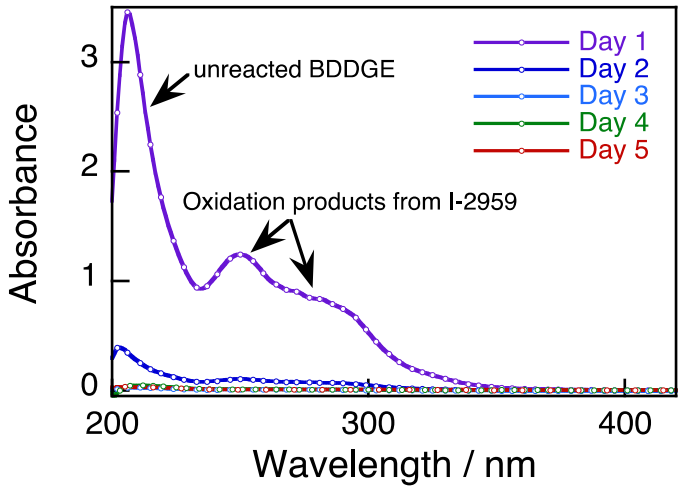

Figure S2. Absorption spectra of the different washes obtained from a 1.0 µM AgNP hydrogel over the course of five days. The arrows in the figure denote the most likely assignments for the absorption maxima peaks observed at day 1. All measurements were carried out at room temperature in 1x1 cm quartz cuvettes.

Variation of the area under the curve for AgNP-surface plasmon band as a function of the AgNP@collagen concentration: Once the hydrogels were washed and chemical debris removed; absorption spectra for the samples prepared at different concentrations of AgNP@collagen were measured.

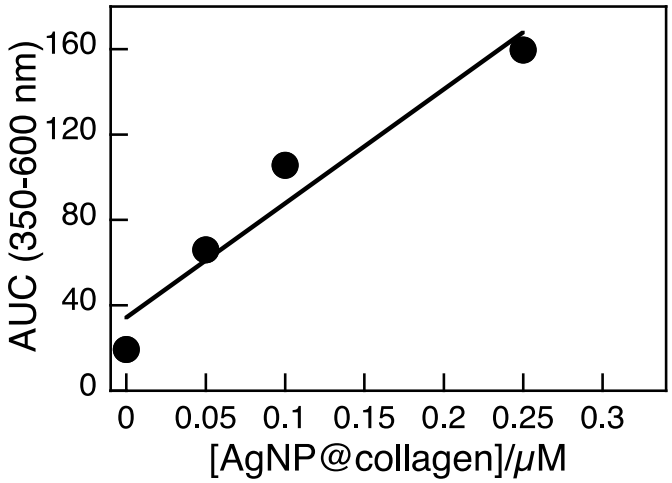

Figure S3. Area under the curve (AUC) as calculated from the absorption spectra of 500 μm thickness collagen hydrogels prepared using different concentrations of AgNP@collagen. Absorption spectra were measured in a microplate reader, see experimental, using 6 mm circular pieces immersed in 100 μl PBS buffer pH 7.4. All measurements were carried out at room temperature. Line shown in the plot corresponds to the best linear fit (R=0.97) calculated in KaleidaGraph® 4.5.

Changes in hydrogel topography upon AgNP incorporation: In order to better elucidate if the decrease in the mechanical properties showed in Table 1 resulted from changes in the crosslinking patterns due to AgNP incorporation; Cryo SEM imaging of 1.0 µM AgNP and control hydrogels was performed (Figure S4).

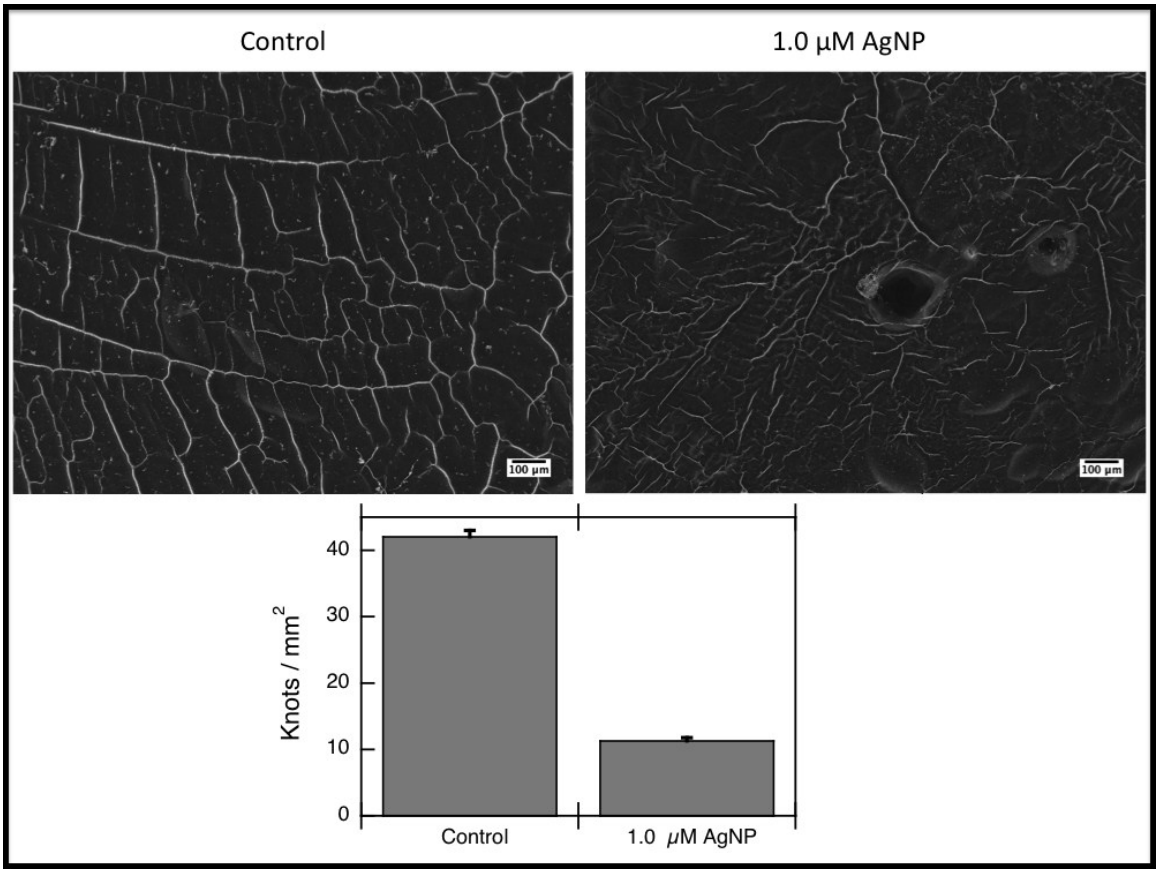

Figure S4. Top panel. Selected Cryo-SEM images for BDDGE type I collagen-based hydrogels in the absence (top left) or presence (top right) of 1.0 µM AgNP. Bottom panel. Histogram showing the number of points were two or more collagen fibrils intersect in a single point or knots within the two hydrogels.

Direct incorporation of $AgNO_3$ within collagen-based hydrogels: 494 μM Silver nitrate was also incorporated within the hydrogel to explore the biocompatibility and antimicrobial activity of ionic silver. However, as shown in Figure S5, the limited stability of $AgNO_3$ leads to the formation of black solid aggregates, most likely silver oxide, inside the material. Interestingly, human skin fibroblasts were unable to proliferate in the near vicinity of the black aggregates.

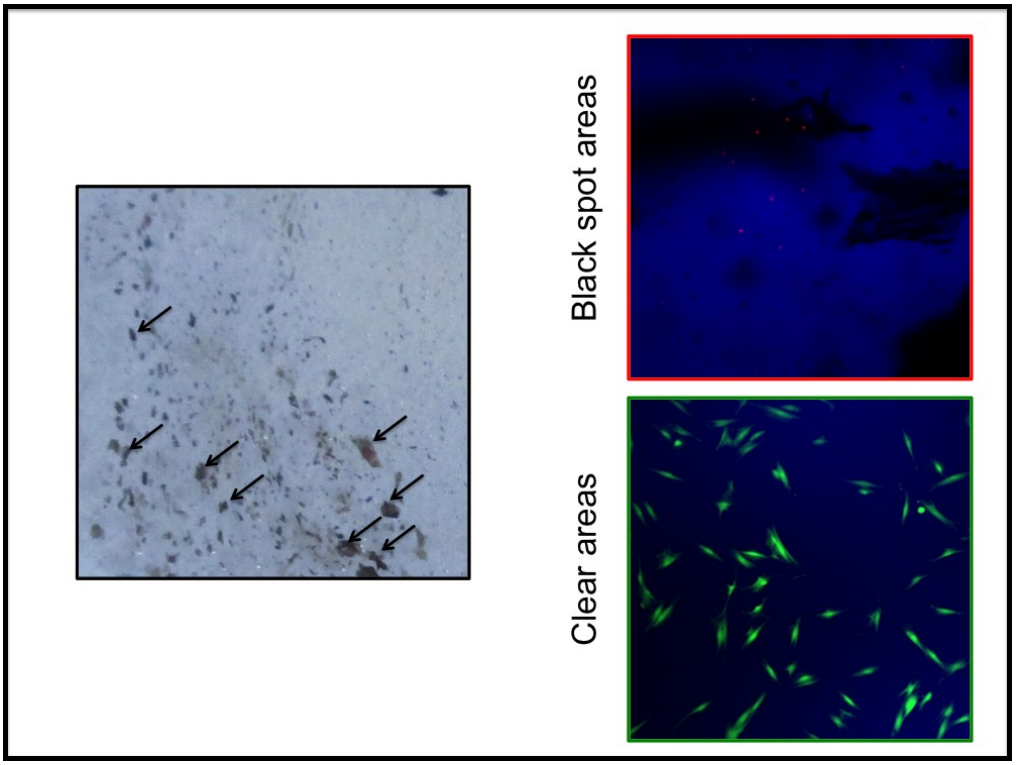

Figure S5. (left) Actual image of a selected area of the collagen-based hydrogel prepared using $AgNO_3$ instead of AgNP@collagen nanoparticles. The black arrows in the left panel denote the presence of some selected black aggregates produced after injection of $AgNO_3$. (right) Live/dead staining for human skin fibroblasts performed 24 hours after seeding the cells on the $AgNO_3$ hydrogel (initial cell density $1x10^4$ cells/ml). Images were taken in the areas corresponding to regions with (top right) or without (bottom right) aggregates.

Growth inhibition profiles for bacterial strains: In order to elucidate the ability of the materials prepare here to delay bacteria growing was assessed by following the increment in light dispersion measured as the increase of the absorption at 600 nm as a function of the incubation time.

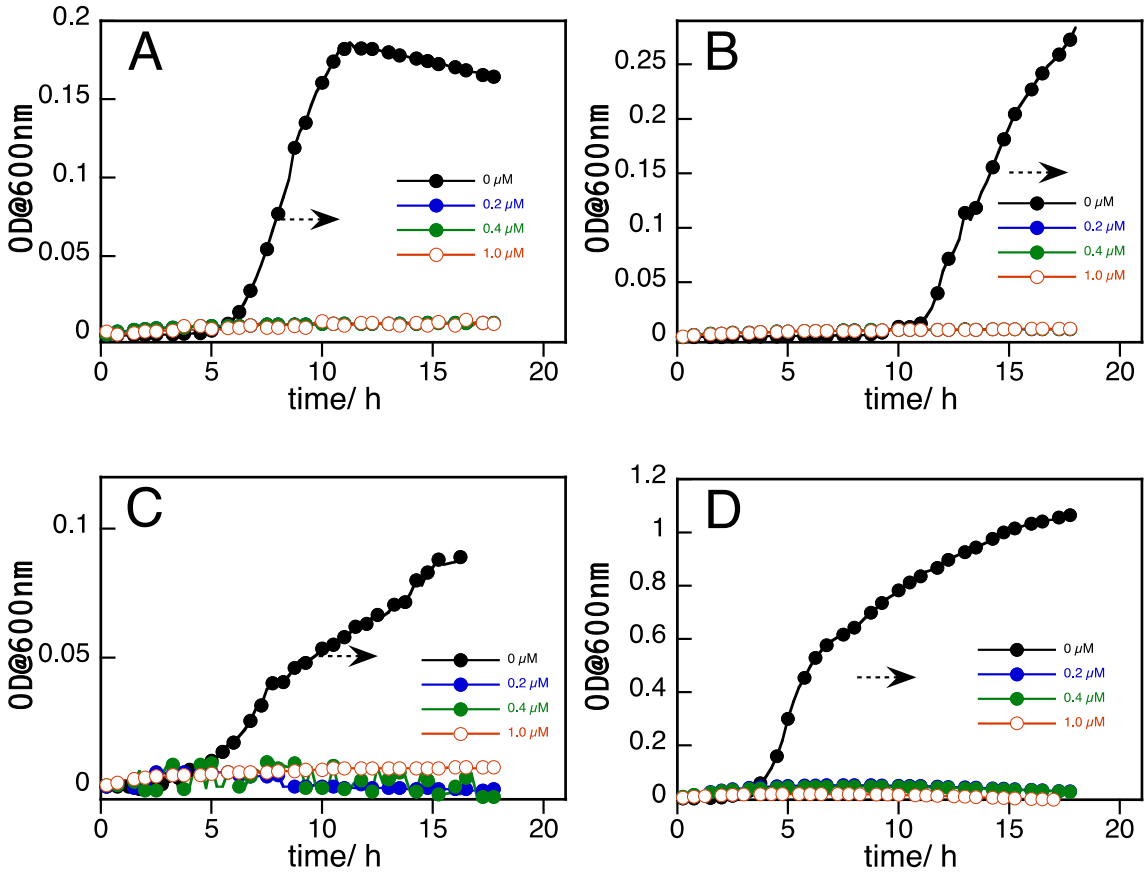

Figure S6. Growth inhibition profile for *E. coli* (**A**), *S. aureus* (**B**), *S. epidermidis* (**C**) and *P. aeruginosa* (**D**) in the presence of hydrogels containing either different concentrations of AgNPs measured every 15 min over an 18 h time interval. OD_{600} estimates obtained from continuously monitored 96-well plates with approximately $1x10^5$ cfu/mL bacteria initially seeded. Cells were incubated at 37ºC and under constant shaking for the whole experiment.