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# Surface modification of silver nanoparticles for biomedical applications

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## Introduction

A major challenge in surgical procedures involving scaffold implantation, particularly corneal replacements, is to prevent nosocomial infection. Although prophylactic use of antibiotics, impregnated within the implant, would appear as the first alternative, they are not recommended due the risk of developing antibiotic resistance. Thus, one alternative strategy to provide anti-microbial protection to the implant is the use of silver nanoparticles (AgNPs) embedded within the actual material. However, spherical AgNPs present an intense yellow color that has hindered their use in corneal implants, due to cosmetic reasons. Thus, in this project a new post-synthesis stabilization approach has been developed for the preparation of collagen-based hydrogels containing different shapes of AgNPs that are able to confer colors like blue and green to the material. The materials have been characterized for their use as corneal replacements (physical and biological compatibility) and work is ongoing to determine antimicrobial characteristics.

## Methods

AgNO<sub>3</sub> in solution with sodium citrate was reduced in a photochemical reaction using I-2959 as source of radical electrons with exposure to 40 minutes of UVA radiation.

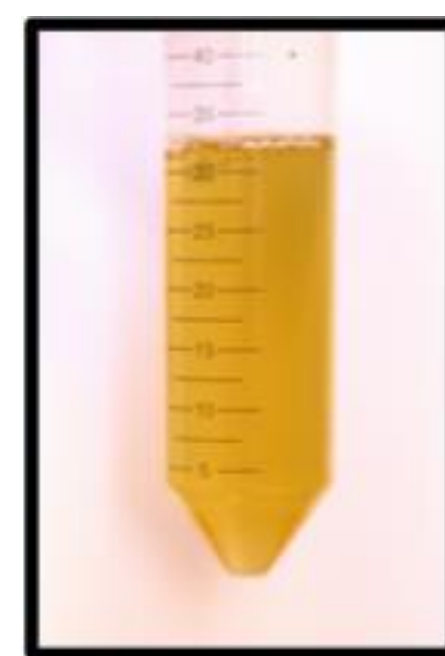


Figure 1. AgNP solution before reshaping.

The resulting AgNPs were then irradiated using an LED. Changing the wavelength of light produced led to different shapes and thus colours of AgNPs.



Figure 2. Irradiation of AgNP using an LED.

After approximately 72 hours, the reshaped nanoparticles were stabilized using the peptide LL37-SH as a capping agent. The stabilized AgNP solution was concentrated via freeze drying.



Figure 3. Concentrated AgNP precipitate after freeze drying.

The freeze dried solid AgNP were resuspended in a small volume of water to concentrate the nanoparticles. The resulting solution was injected into a syringe system containing collagen, BGGDE cross-linker, NaOH and AgNP solution.



Figure 4. Resuspended AgNP (left) and syringe system (right) used for hydrogel preparation.

The properties of the resulting AgNP hydrogel were tested.



Figure 5. Completed blue hydrogel.

## Results

Hydrogels incorporating both blue and green AgNPs were successfully produced. The blue AgNP hydrogels produced much more intense colours whereas the green hydrogels were very faint. Absorption spectra of both AgNP hydrogel types were taken.

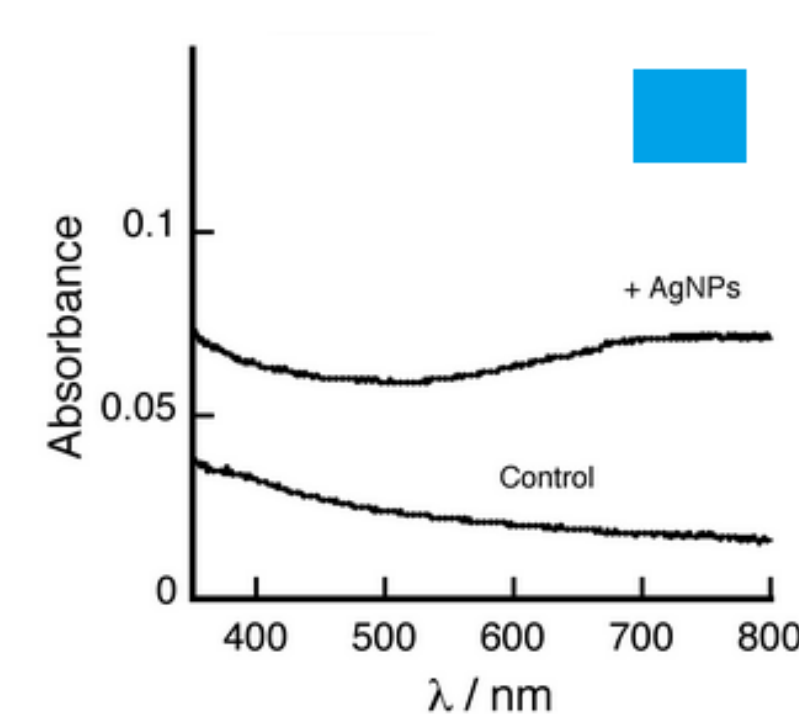


Figure 6. Absorption spectrum for blue AgNP hydrogel.

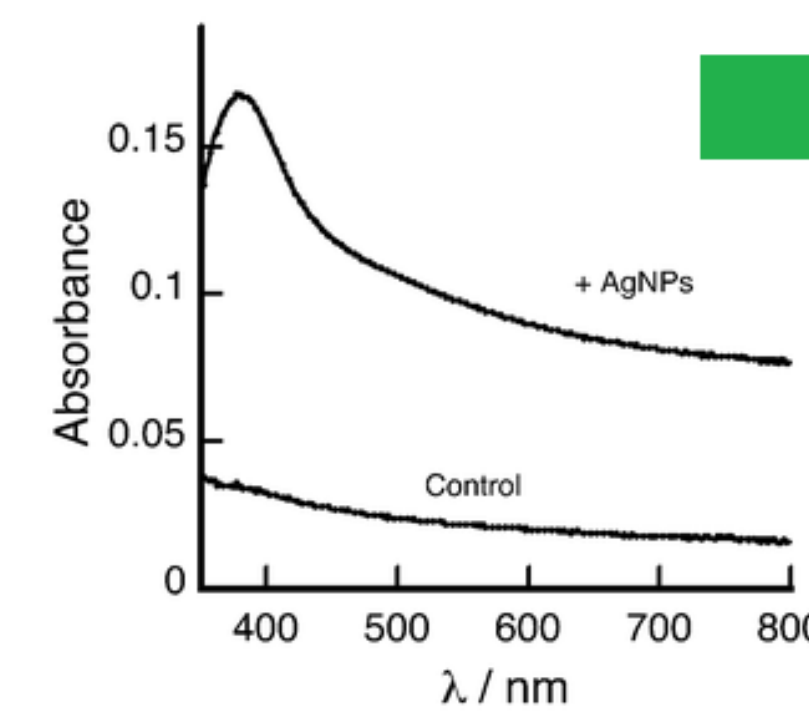


Figure 7. Absorption spectrum for green AgNP hydrogel.

The mechanical properties and denaturation temperature were also characterized for the AgNP hydrogels. Washes were performed with phosphate buffered saline solution and showed no significant change in absorption spectra after each wash.

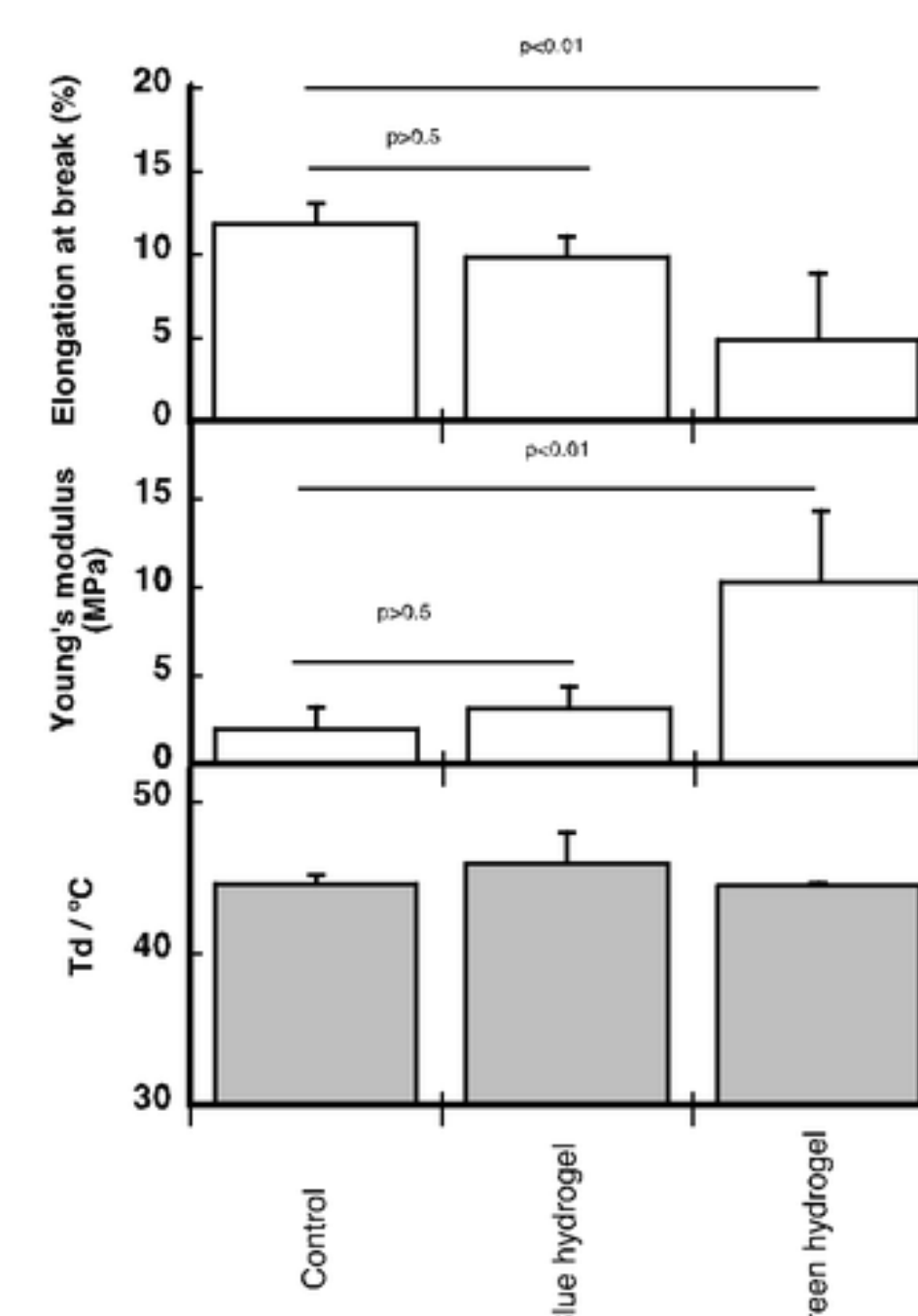


Figure 8. Mechanical properties (strain breaking point, Young's modulus) and denaturation temperature for a control (water) hydrogel and hydrogels injected with green/blue AgNPs.

A key aspect of the project is the stabilization of the AgNPs by replacing the citrate coating used during synthesis with peptide capping agents. The effect of various peptides on the nanoparticles was studied by taking the absorbance spectra of AgNP + peptide solutions.

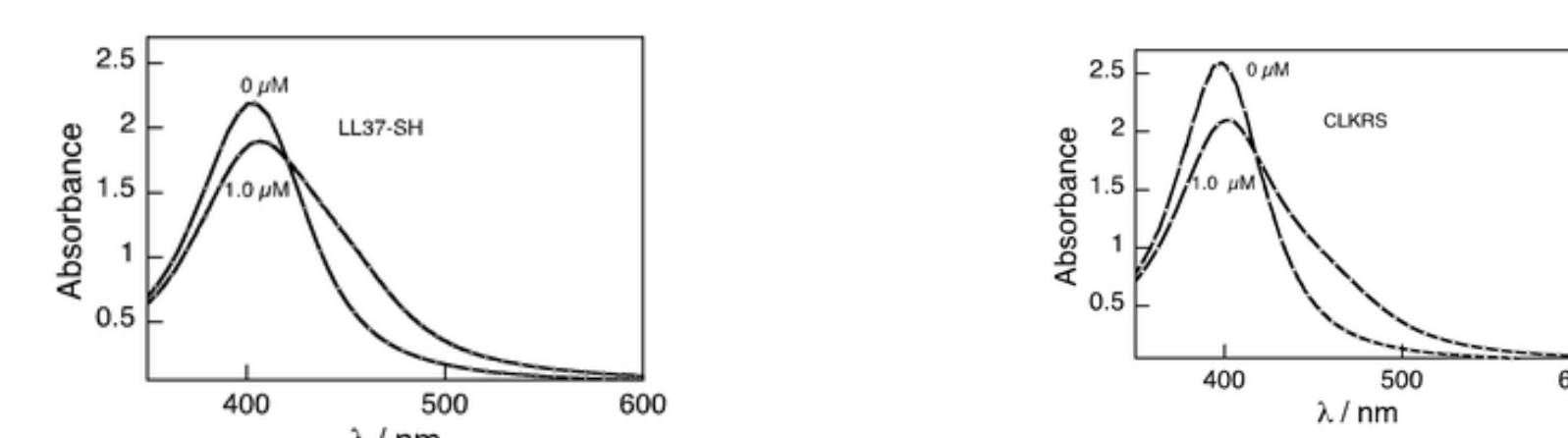


Figure 9. Absorption spectra for 1 μM and control concentrations of LL37-SH (left) and CLKRS (right) peptide + AgNP solution.

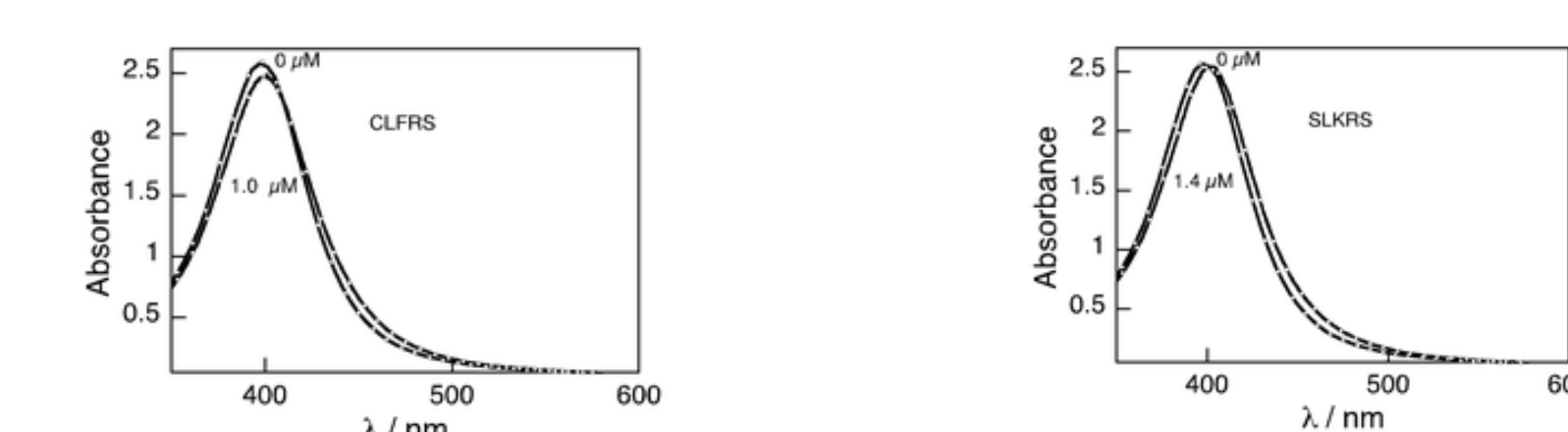


Figure 9. Absorption spectra for 1 μM, 1.4 μM and control concentrations of CLKRS (left) and SLKRS (right) peptide + AgNP solution.

## Conclusion

Hydrogels containing both green and blue AgNPs were successfully produced. However, due to stability issues of the green AgNPs, the green hydrogels failed to reach an adequate intensity of green colour and presented a more brittle material. This is likely due to the degradation of the nanoparticles sometime during the production process.

The mechanical properties of the blue hydrogel were promising as they closely matched a collagen + water control. As the unshaped yellow AgNPs and the green AgNPs altered the mechanical properties, this result for the blue AgNPs is promising. The denaturation temperature was slightly raised for the viable blue hydrogel and the wash tests revealed that the nanoparticles do not leak into solution over time. Overall, these results are encouraging and shows that the material has potential for clinical application.

The results of the peptide + AgNP absorption tests serve to confirm current theory of what makes an effective capping agent. Theoretically, a cysteine residue forms a covalent bond at the surface of the nanoparticle with a silver atom while a lysine residue competes for bonding sites as it too can form a covalent bond. These potential bonding interactions help the peptide to displace the citrate that originally covers the surface of the nanoparticles. Thus it is expected that residues with a cysteine (C) and lysine (K) residues will interact strongly with the AgNPs and cause a shift in the absorbance spectrum. Indeed the LL37-SH human antimicrobial peptide (which contains many lysine residues) and CLKRS (two C/K residues) display strong binding affinity by affecting the spectrum. Meanwhile, the SLKRS and CLFRS peptides (each only containing 1 C/K residue) failed to significantly affect the absorption spectrum.

## Future Work

Work is ongoing on the antimicrobial characteristics against *Pseudomonas aeruginosa* of the AgNP hydrogel as well as the effect on cell growth of HUVECs cells seeded on the hydrogel. If these are found to be favourable, subcutaneous implants in mice model will be also carried out. Furthermore, additional work on synthesizing different shapes and colours of AgNPs needs to be done. Optimization of the capping agent and general improvement of the AgNP stability are potential areas of future work as well.

## Acknowledgments

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