Antimicrobial surface grafted thermally responsive PNIPAM-co-ALA nano-gels

Charlotte James, Andrew L. Johnson and A. Toby A. Jenkins

Received (in XXX, XXX) Xth XXXXXXXX 20XX, Accepted Xth XXXXXXXX 20XX
DOI: 10.1039/b000000x

Thermally responsive Poly(N-isopropylacrylamide) co-allylamine (PNIPAM-co-ALA) nano-gels were synthesised and grafted onto non-woven polypropylene. Silver nitrate was incorporated into the nano-gels in their expanded state and their antimicrobial properties tested. Bacterial growth was measured before and after the Lower Critical Solution Temperature. Below the LCST, bacteria grew, above bacterial growth was prevented or retarded.

There is increasing interest in designing films that can respond to their environment, with thermal, pH, ionic-strength and other effects all being probed as potential triggers for changes in a films morphology. One of the most well know systems is poly(N-isopropylacrylamide) (PNIPAM) – sodium acrylate co-polymers which shows a thermally induced morphological change at 34°C.1

Other co-polymers including allylamine (ALA) have been investigated, and it has been shown that the choice of co-polymer allows the LCST (lower critical solution temperature) of such polymer gel systems to be controlled.2 Much of the interest in these systems has been in developing drug delivery vehicles for anti-cancer and other drugs.3

Constructing antimicrobial surfaces using metals such as silver as an antimicrobial has been extensively investigated by many researchers over recent years,4, 5 with many products containing supposed antimicrobial activity due to silver on the market. There is a specific need to produce wound dressings which can fight infection and to this end a number of ‘silver activated’ dressings are on the market including Acticoat from Smith & Nephew and many others. The difficulty is that such dressings are not always effective and there are concerns that releasing silver into a wound, especially a large wound such as a burn might delay healing.6, 7 Once solution to at least some of these problems might be to design a thin film system that can be integrated into a dressing that only releases its antimicrobial payload following stimulation from either bacteria in an infected wound, their secreted virulence factors, change in wound pH or temperature since this would keep the antimicrobial substance such as silver solution or other substance spatially separated from delicate tissue unless it was actually required.8, 9 PNIPAM has been suggested as a matrix for of antibiotics and antimicrobials, but we have not found any discussion of the utilisation of the increased wound temperature as the ‘trigger’ for antimicrobial release using this type of chemistry.10 11

This communication describes the synthesis of thermally responsive PNIPAM-co-ALA nano-gels that have been loaded with silver nitrate and grafted to non-woven polypropylene. This grafting approach is achieved via amide coupling of the free amines in the nano-gels to plasma deposited maleic anhydride.12

The hydrogel nano-gels were synthesised by precipitation polymerisation. N-isopropylacrylamide, allylamine (10% molar ratio), ethylene-glycol diacrylate (cross linker 1% molar ratio) were dissolved in 115 ml deionized water along with 37 mg of sodium dodecyl sulfate (SDS) as a surfactant, freeze thawed three times and bubbled with N₂. Following removal of oxygen sodium persulfate in water was added as an initiator and the solution heated to 70°C for 4 hours. The gels were then purified by dialysis in deionized water for 7 days with a yield of 31%. Silver nitrate was incorporated into the nano-gels by adding to a solution of 50 mmol dm⁻³ silver nitrate, then heating to 50°C and then cooling to 20°C before re-swelling in the silver nitrate solution for 4 hours. The excess silver solution was removed by filter centrifugation 15 minutes at 10,000 rpm then washed in deionized water before a final re-centrifugate. The nano-gel size and LCST was measured using dynamic light scattering (Malvern Instruments), and found to be 220 nm +/- 10 nm below 34°C and 72 nm +/- 12 nm at 37°C and above (figure 1), as the polymer de-swelled with increasing temperature.

In order to attach the nano-gels to non-woven polypropylene and polystyrene Petri dishes, the fabric were washed in isopropanol for 5 minutes and dried before placing in a lab built 13.56 MHz plasma reactor.13 Approximately 100 mg of maleic anhydride powder (Sigma) was degassed by freeze– thaw action under reduced pressure. All plasma reactions were run at 50W, using a pulsed square wave; 1 msec on, 40 msec off in order to create a coherent film, but retain anhydride group functionality in the film. Immediately following formation of the maleic anhydride film (pp-MA) on fabric / polystyrene, the PNIPAM-ALA nano-gels containing silver nitrate were grafted to the pp-MA via amine nucleophilic attack from the AA to the anhydride group on the film, forming amide linkages. The nano-gels were

Fig.1 Diameter of nano-gels containing silver nitrate measured as a function of temperature. LCST at 34.5°C.
left for 24 hours to graft before washing to remove excess material. Tapping mode Atomic Force Microscopy and TEM was used to image the gels on polystyrene pp-MA coated Petri dishes (AFM) and coated copper grids (TEM see ESI). FT-IR was used to follow the surface modification at each stage and shows clearly the maleic anhydride on the fabrics after plasma polymerisation with a peak 1785 cm\(^{-1}\) corresponding to the C=O (ESI). When the nano-gels were grafted, the free amines react with the carbonyl functional groups to form the amide and the peak shifts to 1644 cm\(^{-1}\).

AFM of the unmodified polystyrene dish (figure 2A); pp-MA coated polystyrene dishes (figure 2B); the grafted nano-gels without silver nitrate in their dry state (figure 2 C) and the nano-gels containing silver nitrate (figure 2D) show the morphology of the grafted gels compared with the substrate. The image resolution was limited by the AFM tip diameter of 10 nm. Globular gel structures can be observed on the surface and a change in morphology on adding silver nitrate, probably due to a degree of silver nanoparticle formation within the gel following silver reduction, although this was not quantified. Measurements made in the gels expanded phase at ca. 24\(^\circ\)C.

The antimicrobial properties and the temperature dependant release were tested against two clinically relevant strains of bacteria. S. aureus MSSA476 and P. aeruginosa PAO1 against both the silver nano-gels in solution and the grafted silver nanogels. In each microbiology assay the bacteria was taken from an overnight culture grown in LB media, optical density at 600 nm was measured and the required starting inoculums prepared via dilutions in LB media. The growth of the bacteria in the presence of the silver nano-gels was assessed at two different temperatures one below the LCST at 28\(^\circ\)C and one above the LCST at 37\(^\circ\)C. Starting cultures of 1x10\(^5\) CFU/ml were added to a solution of silver nano-gels and either incubated at 28\(^\circ\)C or 37\(^\circ\)C. Every hour a sample was taken and a dilution series plated out on agar Petri dishes every hour for a period of 5 hours. The plates were incubated over night at 37\(^\circ\)C and the colonies with all measurements performed in triplicate. For both S. aureus and P. aeruginosa there is a reduction in the growth of bacteria in the presence of the silver nano-gels at 37 \(^\circ\)C compared to the control. However at 28\(^\circ\)C there is (virtually) no difference in the growth of bacteria after 5 hours, although the total growth was lower than the control at 37\(^\circ\)C as both S. aureus and P. aeruginosa have evolved to grow most efficiently at this higher temperature (figure 3). Controls were grafted nano-gels which did not contain silver nitrate.

The antimicrobial release trigger here being the fact that infected wounds / skin have a significantly elevated temperature compared...
with healthy surrounding tissue, with a 2010 study of infected leg ulcers showing a mean temperature elevation of 3.6 °C compared with healthy skin.\(^1\)

![Fig.4 Bacterial lawns of P. aeruginosa grown with silver nano-gel containing fabric at 37°C (A) and 28 °C (B). S. aureus at 37°C (C) and 28 °C (D).](image)

These findings suggest that thermal triggering may have utility in designing infection responsive wound dressings, and that the utility of well-known PNIPAM based polymers may be even greater than previously considered.

**Acknowledgements**

We would like to acknowledge the support of the European Commission 7\(^{th}\) framework programme in funding this work Grant #211346. We also thank John Mitchels, University of Bath MAS microscopy and analysis suite for help with AFM.

**Notes and references**

\(^a\) Address, Department of Chemistry, University of Bath, Bath, BA2 7AY United Kingdom, Fax: +441225386231; Tel: +441225386118; E-mail: a.t.a.jenkins@bath.ac.uk

\(^\dagger\) Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/


13. Polypropylene non-woven were nappy liners purchased from Boots the Chemist™, U.K.