Antimicrobial reverse thermal gel for surgical applications

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Statement of Purpose: A surgical site infection (SSI) is defined as an infection that is developed during a surgical procedure or up to 30 days after. In the US, 2-5% of patients undergoing surgery suffer from SSIs. These patients have 2-11 times higher risk of death [1]. A main aspect of SSI prevention is the use of an antiseptic-impregnated plastic surgical incision drape. However, these drapes present numerous pitfalls. Amongst the most severe, are that the placement process of the drapes is time consuming, they often do not remain well-attached and they only provide temporary protection. Polymers functionalized with quaternary ammonium groups have shown high inherent antimicrobial properties. The antimicrobial activity is due to the disruption of the negatively charged cell wall or membrane. We developed a polymer-based antimicrobial surgical coating that can act as a surgical incision drape. This polymer is specifically designed to possess a reverse thermal gelling property to ease the application process. It can be sprayed onto the skin and turns into a thin adhesive gel when it comes into contact with the body and can then be removed by washing with soap/water at low temperatures (less than 20°C).

Methods: Poly(serinol hexamethylene urea) (PSHU) was synthesized using urea, N-Boc-serinol and hexamethylene diisocyanate. Next, the Boc groups in PSHU were removed to de-protect the secondary amines (-NH2) on PSHU. PNIPAAm Poly(N-isopropylacrylamide) was then conjugated to PSHU to produce PSHU-NIPAAm. Finally, we quaternized the remaining -NH2 groups using 1-bromohexane to obtain *N*-quaternized PSHU-NIPAAm (Quat-PSHU-NIPAAm). The percent conjugation of PNIPAAm was controlled by varying the molar amount of each reactant. The antibacterial property of the Quat-PSHU-NIPAAm was evaluated against *Staphylococcus aureus.* Both 2D and 3D (mixed) experiments were performed. First, 200 µl of 10% (wt/v) antimicrobial polymer in lysogeny broth (LB) were added to each well in a 24-well plate and placed in a 37°C incubator to promote the solution-to-physical gel transition. Subsequently, 500 µl of bacterial suspension at a concentration of 1×10^4 colony forming units (CFU)/ml were added on the gel and cultured at 37°C for 24 hr along with a negative control (500 µl of the bacterial suspension). The antibacterial activity was determined by measuring the optical density of the bacterial suspension at 610 nm using a microplate reader (SYNERGY Mx, BioTek). The % increase in bacteria growth was calculated by comparing optical densities of the bacterial suspensions before and after incubation. The numbers were normalized to the control group. To confirm the antibacterial activity, all bacterial cells were recovered from each sample and mixed with 500 µl of LB in a 24-well plate, cultured for 24hr and the antibacterial activity was determined by the method previously described. An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was performed to assess polymer cytotoxicity on smooth muscle cells (BPASMCs L2 Control). Tests were performed after a 1, 3, and 5-day culture period following the supplier's instructions. Results were obtained using a microplate reader and an absorbance of 570 nm.

Results:

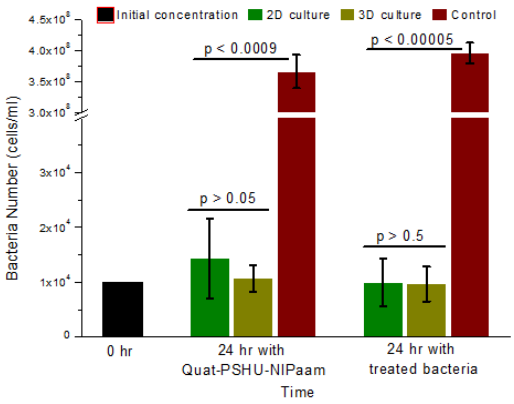

Figure 1. *Antibacterial activity of Quat-PSHU-NIPAAm*
The 2D and 3D cultures that were exposed to polymer showed no increase in the bacteria cell number. As can be observed on the graph,* (p>0.1) and # (p<0.001) represent statistical difference with 0 hr sample. The results obtained from the 24 hour culture with polymer treated bacteria showed no bacterial increase indicating that all cells were killed by the polymer.

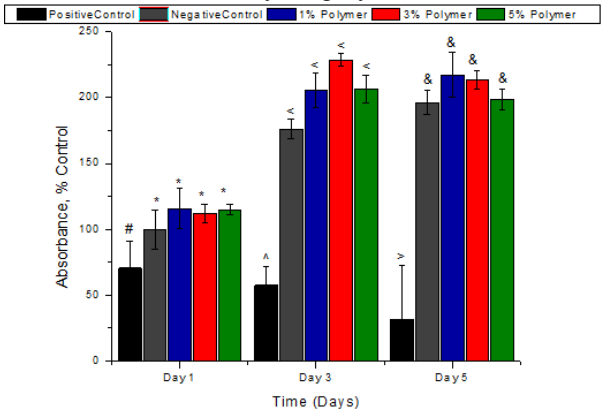

Figure 2. *The copolymer was non-cytotoxic against SMCs as tested by MTT mitochondrial activity assay*
Positive control samples (5% DMSO in culture media) were significantly different #, ˆ, > (p<0.000001) to the experimental samples. Data were normalized to day 1 negative control (media and cells).

Conclusions: We observed that the Quat-PSHU-NIPAAm showed promising antibacterial activity and the polymer concentration range tested showed no smooth muscle cell cytotoxicity and no growth inhibition.

References: 1. Centers for Disease Control and Prevention. at <http://www.cdc.gov/HAI/ssi/ssi.html>