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TITLE: A Novel Urinary Catheter with Tailorable Bactericidal Behavior

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CONTRACTING ORGANIZATION: LONDON HEALTH SCIENCES CENTRE RESEARCH
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14. ABSTRACT

This is a project being conducted at two sites. Our US collaborator's role is the development of novel biomaterials that will be used to manufacture urinary catheters. Our portion of the project is the evaluation of these urinary catheters first in vitro, and then in an in vivo animal model. We have established animal protocols and written ethics in place to be able to undertake this work once we receive the materials from our research collaborator.

15. SUBJECT TERMS
None listed

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1. **INTRODUCTION:**

Catheter-associated urinary tract infections (CAUTIs) are the most common nosocomial infection globally accounting for roughly 40% of all reported healthcare-associated infections (HAIs). Developing more biocompatible and infection-resistant urinary catheter materials may help to reduce the morbidity and mortality associated with CAUTIs. We believe that the proposed research has the potential to significantly reduce costs (product and care) and improve outcomes for military and civilian populations. In this project there are six specific aims that will be evaluated to test the silicone composite materials that employ a novel antimicrobial ion exchange (AM-IE) resin system. THREE of the aims are to be carried out at Iasis Molecular Sciences and are intended to optimize device configurations. FIVE specific aims to be carried out by ourselves (Razvi & Burton) are intended to evaluate the the killing potential of the material against various uropathogens *in vitro* and *in vivo* performance of these devices in a rabbit model.

2. **KEYWORDS:**

CAUTI, urinary tract infection, catheter, infection, silicone

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Our contribution to the project is to test the novel polymers that have been developed using *in vitro* and *in vivo* evaluations (Aims 4-8).

Specific Aim 1 – To synthesize coating polymers, identify an optimal lubricious coating, and optimize antiseptic loading
Specific Aim 2: Synthesis of AM-IE resins, fabrication, and testing of ten composite silicone test articles
Specific Aim 3: In Vitro characterization of six AM-IE silicone composites with antiseptic lubricious coating

_____OUR AIMS START HERE_____

Specific Aim 4: Determination of *in vitro* efficacy of the candidate composites
Specific Aim 5: *In vivo* rabbit ureteral stent model
Specific Aim 6: *In vivo* rabbit urethral catheter model
Specific Aim 7: Histopathological evaluation of kidney, bladder and ureter tissue samples
Specific Aim 8: Reporting and results evaluation for future clinical evaluations

_____

- **What was accomplished under these goals?**

To date, we have successfully screened the test material a selection of common uropathogens *in vitro* in order to assess killing potential.

Specific Aim 4: Determination of *in vitro* efficacy of the candidate composites

The test material was screened against a selection of uropathogenic bacteria in order to assess killing potential. The bacteria that were screened included *Escherichia coli* 67, 28927 and WE6933, *Pseudomonas aeruginosa* AK1 and PA01, *Staphylococcus epidermidis* 3399 and 35984, *S. aureus* Newman, *Klebsiella pneumonia* 280, *Proteus mirabilis* 296 and *Enterococcus faecalis* 33186. These genera of bacteria were selected as they are implicated with causing complicated UTI due to implantation with either a stent of catheter.

Uropathogenic bacteria were grown overnight at 37°C in tryptic soy broth (TSB). These bacteria were then added to 1 mL of artificial urine (AU) in 1 1.5 mL microfuge tube at a concentration of $1x10^7$ cfu per mL. A 1 cm section of either control silicone tubing, test material at a concentration of 0.75% silver or 1.5% silver was aseptically placed in the AU, carefully making sure it didn't stick to the sides of the tube. These tubes were then incubated at 37°C, with samples being taken 2, 4, 6, 8, 24 and 48 hours post infection to measure cfu.

The results from these screening experiments can be seen in Figure 1. Both the 0.75% and 1.5% material demonstrated potent killing against most of the uropathogens screened. While the time taken to achieve a notable knockdown in bacterial numbers differed based on both bacterial genera and strain, in most instances there was a notable decline at every time point and complete killing by the 24 hours post infection. The major exception to this was the *E. faecalis* 33186, which did not see any decrease in cell number until 24 hours post infection and complete kill until 48 hours post infection in the 1.5% test group. This is not completely unexpected, as *E. faecalis* is known to be resistant to many antimicrobial agents, so it is to be expected that it also demonstrated resilience to the bactericidal potential of silver.

Using a peristaltic pump, we have also assayed the killing potential of the material under flow conditions. The schematic for this experiment is outlined in Figure 2. These experiments were carried out as this system more accurately represents the fluid and material interaction that would occur *in vivo*. Uropathogenic bacteria (*E. coli* 67, *E. faecalis* 33186 or *P. aeruginosa* AK1) were grown overnight at 37°C in TSB. These bacteria were then added to 250 mL of AU in a 1 L bottle at a concentration of $1x10^5$ cfu per mL. A 45 cm section of either control silicone tubing, test material at a concentration of 0.75% silver or 1.5% silver was attached either side of peristaltic pump tubing, which was fixed over the rollers of a peristaltic pump and set with a flow rate of 5 rpm (0.5 mL per minute). These bottles were then incubated in a 37°C water bath and samples were taken 2, 4, 6, 8, 24 and 48 hours post infection to measure cfu.

The results from the flow experiments can be seen in Figure 3. While the test material killed both the *E. coli* 67 and *P. aeruginosa* AK1 at 4 and 2 hours post infection respectively, the *E. faecalis* 33186 was more resilient to the treatment, maintaining the initial inoculum level until 48 hours post infection where it declined 2 logs. However, these results did mimic those seen in the static killing assays shown in Figure 1, which does suggest that the killing potential in AU in vitro is similar regardless of the system used to test the material.

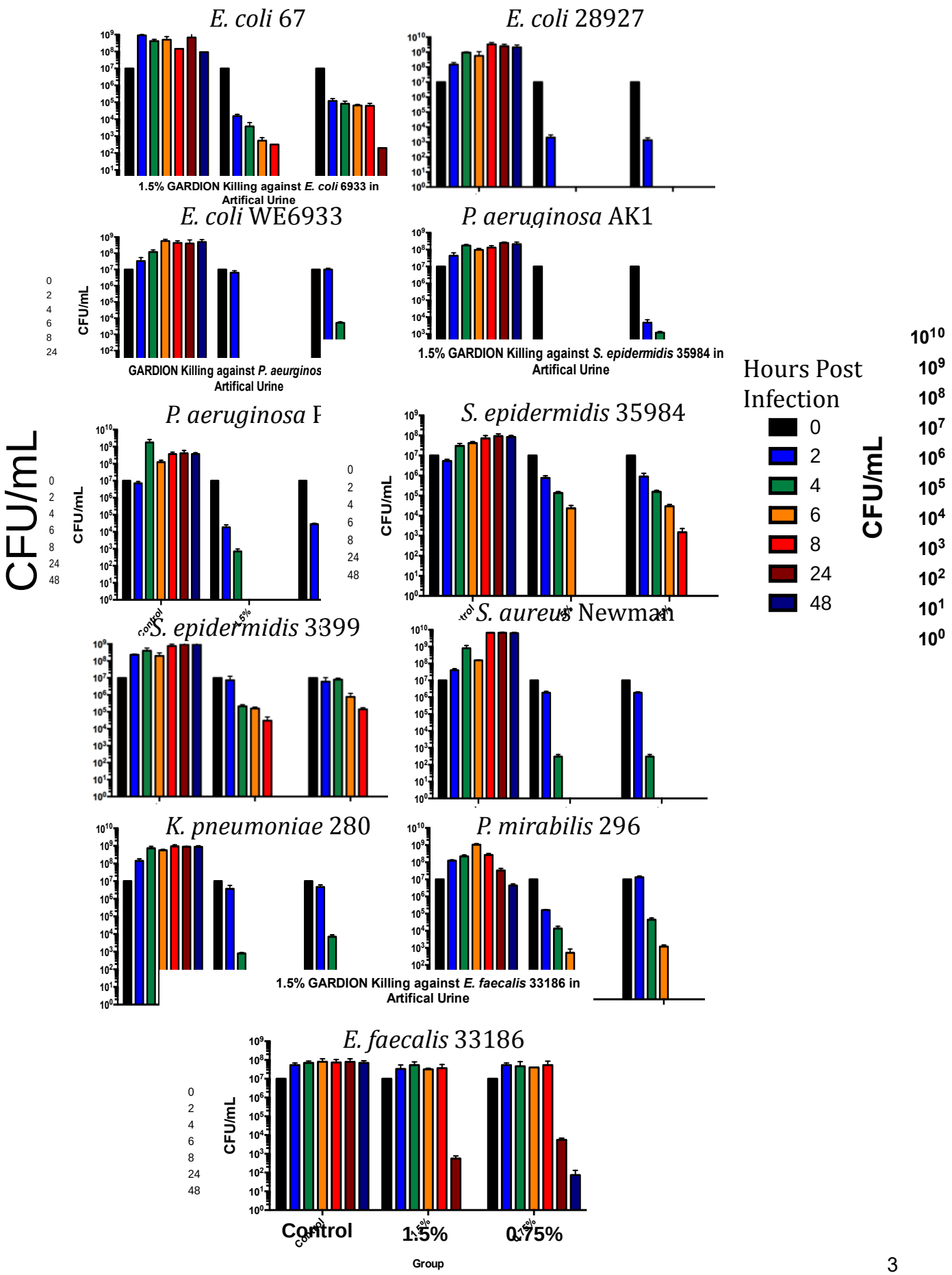
E. coli 67
E. coli 28927
1.5% GARDION Killing against E. coli 6933 in Artifical Urine
E. coli WE6933
P. aeruginosa AK1
GARDION Killing against P. aeurginos Artifical Urine
1.5% GARDION Killing against S. epidermidis 35984 in Artifical Urine
P. aeruginosa
S. epidermidis 35984
S. epidermidis 3399
S. aureus Newman
K. pneumoniae 280
P. mirabilis 296
1.5% GARDION Killing against E. faecalis 33186 in Artifical Urine
E. faecalis 33186
CFU/mL
Hours Post Infection
0
2
4
6
8
24
48
Control
1.5%
0.75%
Group

Figure 1: Test material demonstrates strong killing potential against uropathogens *in vitro*. A selection of uropathogenic bacteria were tested against either control material or test material at either 1.5% or 0.75% silver to assess killing potential. CFU were counted at 2, 4, 6, 8, 24 and 48 hours post infection.

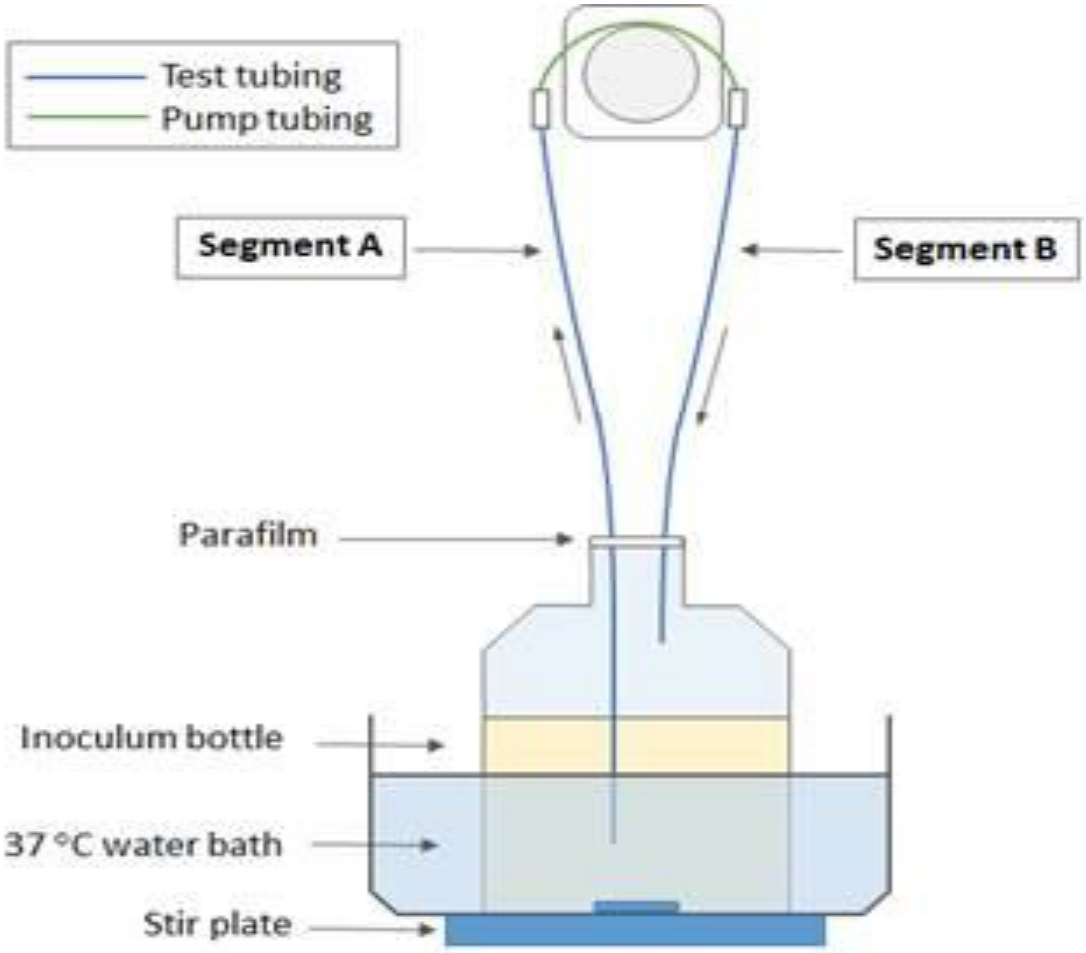

Figure 2: Schematic of in vitro continuous flow system. An inoculum bottle with 250 mL of AU is sat in a water bath on top of a magnetic stirrer and inoculated with $1x10^5$ of an uropathogen to be screened. Two tubing sections 45 cm in length as attached either side of peristaltic pump tubing and feed into the flask, with the input line (Segment A) being submerged in the media and the output line (Segment B) is left suspended above the liquid. As the peristaltic pump is in motion, media containing bacteria is drawn up through Segment A and drips back into the bottle via Segment B

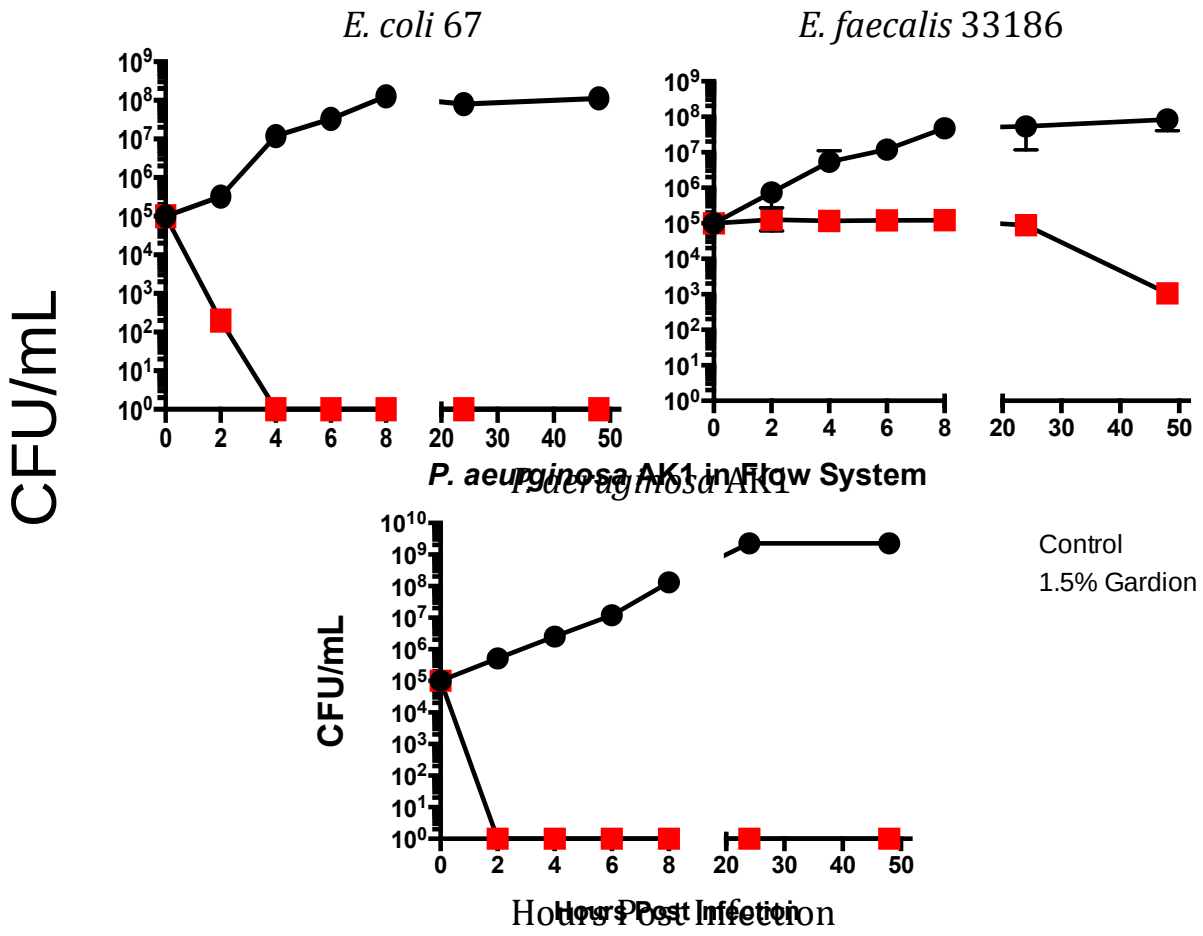

Figure 3: Test material demonstrates strong killing potential against uropathogens in *in vitro* continuous flow system. A selection of uropathogenic bacteria were tested against either control material or test material at either 1.5% silver to assess killing potential. CFU were counted at 2, 4, 6, 8, 24 and 48 hours post infection.

In addition to the bactericidal assays, we also tested the cytotoxic potential of the test material *in vitro*. The material was tested against HEK293 and T24 cell lines, which are derived from human kidney and bladder cells respectively and these were selected as these would be the two organs that the material would be associated with the most if used in a patient.

Both cell lines were grown to high confluency in 10% Foetal Bovine Serum (FBS) Dulbecco's Modified Eagle Media (DMEM) with 4 mM L-Glutamine and a Penicillin/Streptomycin antibiotic mix (10,000 I.U./mL and 10,000 µL/mL) and incubated at 37°C with 5% CO_2. The cells were then re-seeded at $1x10^6$ cells per mL per well of a 6 well tissue culture plate and allowed 24 hours to re-adhere. Following this, a 1 cm^2 section of material (either control silicone, 0.75% material with and without coating and 1.5% material with and without coating) was placed gently in the middle of the well and plate was incubated again for 24 hours. Material was then removed and cells were incubated with Alamar Blue reagent for 4 hours to assess cell viability. The Alamar Blue reagent is reduced by living cells, which causes a colour change that can be detected using a plate reader.

The results for this experiment can be seen in Figure 4. There does not appear to be a difference in the percentage reduction of Alamar Blue in any of the test groups when compared to both the control material and the cells only (no material) control, which suggests that the test material is not exerting any direct cytotoxic effects on the cells.

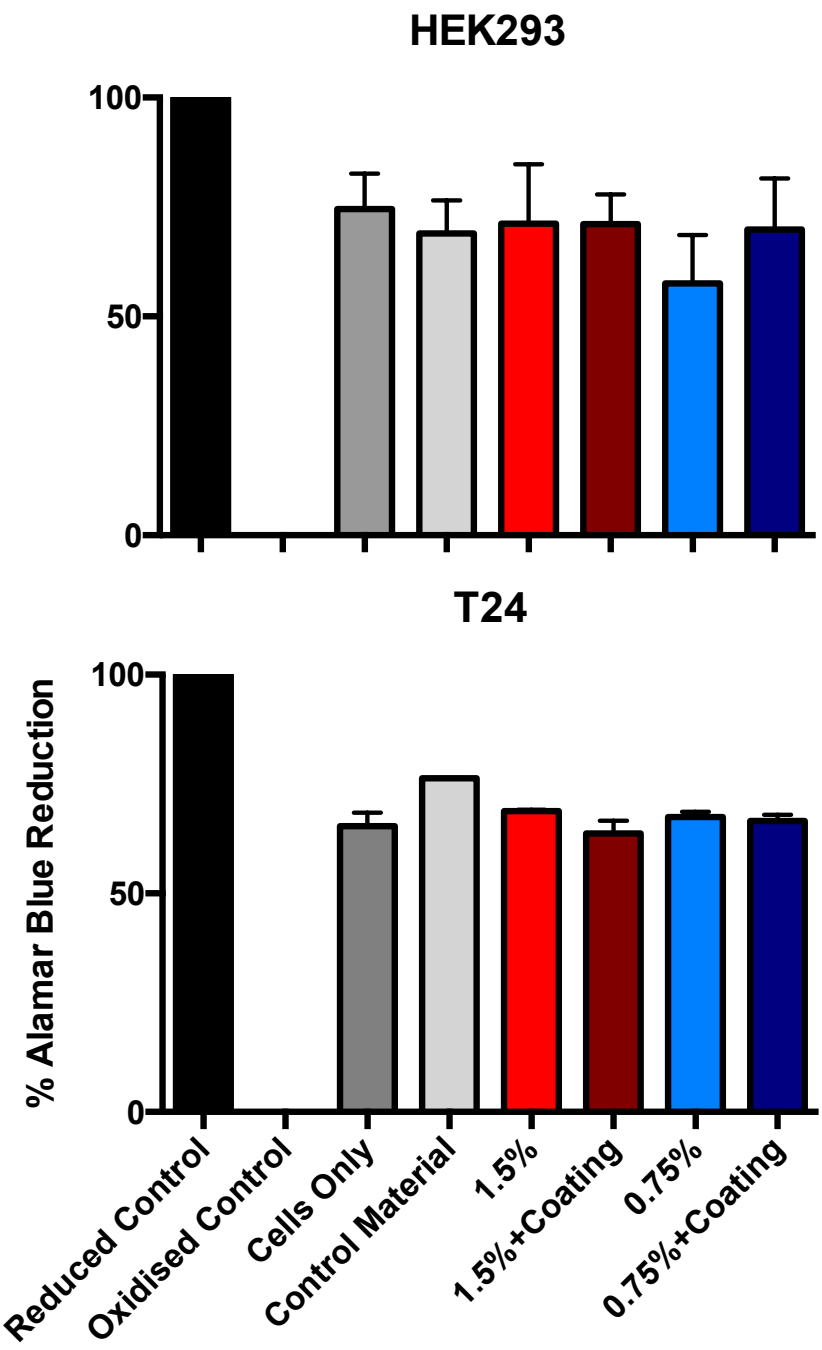

Figure 4: **d kidney cell lines.** HEK 293 and T24 cells 24 hours after being treated with either control material, 1.5% silver material, 1.5% silver material and coating, 0.75% material and 0.75% material and coating or no material.

Specific Aim 5: *In vivo* rabbit ureteral stent model
Specific Aim 6: *In vivo* rabbit urethral catheter model
Specific Aim 7: Histopathological evaluation of kidney, bladder and ureter tissue samples

As of the writing of this report, the Animal Use Protocol for this study has undergone final approval by Westerns Animal Care Committee. The *in vivo* experimentation will be carried out over the coming months and these 3 aims will be completed before the next reporting period.

- **What opportunities for training and professional development has the project provided?**

NA

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

We will commence the animal studies in the coming months and forsee the study completed well before the end of next year's reporting period.

4. **IMPACT**

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to Report.

- **What was the impact on other disciplines?**

Nothing to Report.

- **What was the impact on technology transfer?**

Nothing to Report.

- **What was the impact on society beyond science and technology?**

Nothing to Report.

5. **CHANGES/PROBLEMS:**
    - **Changes in approach and reasons for change**
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
    - **Changes that had a significant impact on expenditures**
    - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    - **Significant changes in use or care of human subjects**
    - **Significant changes in use or care of vertebrate animals.**
    - **Significant changes in use of biohazards and/or select agents**

Nothing to Report.

6. **PRODUCTS**
    - *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
        - **Publications, conference papers, and presentations**
        - **Journal publications.**
        - **Books or other non-periodical, one-time publications.**
        - **Other publications, conference papers, and presentations.**

Nothing to Report.

- **Website(s) or other Internet site(s)**
- **Technologies or techniques**
- **Inventions, patent applications, and/or licenses**
- **Other Products**

Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	*Liam Paul Brennan*
Project Role:	*Post Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*8*
Contribution to Project:	*Dr Brennan has carried out all the in vitro experiments outlined in this report. He has also written and submitted the animal use protocols for the in vivo portion of the study and will be responsible for coordinating and carrying that out*
Funding Support:	*This award*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

No changes to report.

- **What other organizations were involved as partners?**

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

9. APPENDICES

Nothing to Report.