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## Publisher Citation

Gbyli R, Zito C, Amoako KA. In vitro cytocompatibility of antibacterial levels of polymer nitric oxide release. Eng Press. 2018; 2(1): 66-72. <http://publishingpress.info/engineering-press/>

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## Research Article

# In vitro cytocompatibility of antibacterial levels of polymer nitric oxide release

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Received: June 25<sup>th</sup>, 2018

Accepted: June 28<sup>th</sup>, 2018

Published: July 4<sup>th</sup>, 2018

## Citation

Gbyli R, Zito C, Amoako KA. In vitro cytocompatibility of antibacterial levels of polymer nitric oxide release. *Eng Press*. 2018; 2(1): 66-72. doi: 10.28964/EngPress-2-113

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## ABSTRACT

Although the antibacterial property of nitric oxide (NO) has been well documented in gram positive and gram negative bacteria cultures, its cytotoxic effects are not completely clear. To limit potential *in vivo* cytotoxicity, our group recently investigated the effects of a range of NO fluxes on *S. epidermidis* and *S. aureus* to determine a minimum effective NO level. In this study, we report the antibacterial function of this minimum NO level also on gram negative *Pseudomonas aeruginosa* as well as its cytocompatibility effects on lung and kidney cells. Standardized bacterial cultures were treated with NO releasing PDMS substrates followed by plating, 24hr incubation, and colony analyses. Cytocompatibility or cell viability was conducted on WI-38 human lung fibroblasts and HEK-293 human embryonic kidney cells after their exposure to NO *in vitro*. NO flux of  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> significantly reduced *P. aeruginosa* growth compared to controls and PDMS-treated samples (p value < 0.0001). No significant differences was seen between control and cells treated at this flux (total moles delivered in 24hrs =  $0.76 \pm 0.18$ ), but a significant reduction was observed at  $45.1 \pm 2.55 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> (total moles delivered =  $1.6 \pm 0.09$ ). The results suggest that at the lower NO flux level *pseudomonas aeruginosa* growth is significantly inhibited while maintaining cell viability.

**Keywords:** functional biomaterials, bactericidal surfaces, bacterial infection, communicable medical materials, nitric oxide

## INTRODUCTION

Nitric oxide is a multifaceted biological molecule with many physiological functions including acting as an antibacterial agent<sup>1-4</sup> and a promoter of cell proliferation.<sup>5, 6</sup> However, it can be harmful to cells when delivered at levels incompatible with normal cellular function.<sup>7</sup> A crucial objective pertaining to functional materials formulated to deliver NO is to ensure inconsequential cell toxicity effects if any occurs at all. It is therefore critical that biomedical applications that incorporate these properties for developing functional and bioinspired biomaterials aim for the compatible concentrations.

At high and bactericidal concentrations, NO exerts bactericidal effects through many mechanisms via NO itself or its byproducts.<sup>8, 9-11</sup> The interaction of NO with superoxide (O<sub>2</sub><sup>-</sup>) within or outside microbial cell yield reactive nitrogen species (RNOS), that are known to induce nitrosative and oxidative stresses to disrupt microbe's membranes through lipid peroxidation<sup>9</sup> as well as induce DNA damage. Moreover, NO can nitrosate cyteine and tyrosine leading to dysfunction of many key bacterial enzymes, proteins, and cell membrane adhesion proteins that mediate cell-substrate attachment.<sup>5</sup> Accordingly the exploitation of these properties have been pursued using several materials to deliver various levels of NO to bacteria cultures. While the research outcomes do show antibacterial activity, the exact therapeutic concentration of NO remain unclear. Nonetheless, such exact or range of bactericidal concentration(s) must also be cytocompatible to ensure selective elimination of bacteria after they are released by functional materials which may serve as surfaces of blood/tissue communicable devices.

Simultaneously, the interaction of high NO and its byproducts with cells, although bactericidal, can be detrimental to cells due to incompatibility and lead to many biological dysfunctions.<sup>12-17</sup>

Many cellular and tissue functions including DNA alterations and subsequent cell anti-proliferation activity<sup>18, 19</sup> can be impaired.

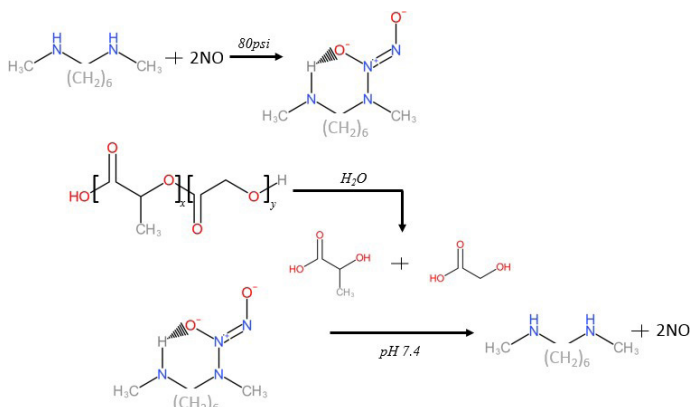
Therefore, studies that have investigated striking a balance between effective bactericidal activity and cytocompatibility using an optimal dose(s) of NO are being pursued for more conclusive data. Data from experiments conducted *in vitro* under conditions of NO gas flow<sup>20-24</sup> and *in vivo* using material-assisted NO delivery report the effects of different ranges of NO concentration on different compatibility outcomes.<sup>25</sup> In this study we investigate the effects of a wide range of NO fluxes (surface area and time normalized NO release), from low, protective, and proliferative fluxes to high levels, on cytocompatibility of lung and kidney cells *in vitro*. This evaluation method is important for the effective application of NO releasing polymers as coatings on implantable medical devices.

## MATERIALS AND METHODS

### Preparation of NO donor compounds for modification of PDMS substrates

Conjugation of NO to secondary amine linear polymers (dimethylhexanediamine or DMHD) to form diazeniumdiolated DMHD or DMHD/N<sub>2</sub>O<sub>2</sub>, which was then incorporated into polydimethylsiloxane (PDMS) along with poly(lactic-co-glycolic acid) or PLGA is shown in (Scheme 1).<sup>26, 27</sup> Hydrolysis of PLGA aids the catalysis of NO from preformed NO donor compounds embedded within the PDMS polymer.

As previously described,<sup>28</sup> synthesis of NO releasing PDMS substrates, as model materials for biological testing, was achieved via polymerization of a two-part silicone rubber (R21-2615, NuSil, CA). A 1:1 ratio of resin A (PDMS oligomer) and resin B (PDMS oligomer with platinum initiator) mixed in organic solvent (Mineral Spirit, Sigma-Aldrich, MO) was cured overnight at room temperature into 1mm thick base layers. 100  $\mu$ l mixtures of THF solvent containing PDMS resin, 15 wt% of DMHD/N<sub>2</sub>O<sub>2</sub>, and 15 or 25 wt% of PLGA (Evonik, NJ) were



**Scheme 1:** Reaction of nitric oxide (NO) with linear dimethylhexanediamine to form diazeniumdiolate, and the subsequent release of the NO stimulated by hydrolysis of poly(lactide-glycolide acid) and physiological conditions.

then casted atop a PDMS-only base layer, cured for 24hrs and followed by top casting 100  $\mu$ l of PDMS solution and curing for another 24hrs.

### Characterization of nitric oxide (NO) release

Real time NO release from modified PDMS and controls samples were quantified by chemiluminescence method using a GE 280i nitric oxide analyzer ((NOA), GE Instruments, CO). To measure NO release, samples were immersed in 10 ml PBS (pH 7.4) at 37°C inside the NOA reaction vessel. Nitrogen was used as a carrier gas to transport NO from the reaction vessel to the chemiluminescence detection chamber and NO flux from modified PDMS samples was calculated as the quotient of the products of NO concentration detected by NOA (ppb or ppm) and NOA calibration factor (mol/ppb\*sec), and sample surface area (cm<sup>2</sup>) and duration NO detection (sec or min).

### In vitro antibacterial test

*Pseudomonas aeruginosa* derived from ATCC 9027 was purchased from Microbiologics, and was cultured using Nutrient agar (NA) plates at 35°C. Overnight Bacterial cultures solutions were adjusted to make a final cell concentration at ~108 CFU/ml. Each NO releasing or control PDMS substrates was placed into a sterile 15-mL tube with 2 mL of the bacterial culture. The tubes were incubated at 37°C for 24 h while shaking (140 rpm). After incubation, the substrates were removed aseptically and bacterial culture was diluted in PBS buffer. 1  $\mu$ L of each dilution was streaked onto NA plates for viable bacterial counting. The NCBI, Image J software was used for colony count. PDMS substrates and levofloxacin, 6mm paper disks saturated with 5  $\mu$ g of levofloxacin (Hardy Diagnostics Inc), were used as additional controls.

### In vitro cytocompatibility test

To determine the effect of the application of NO releasing PDMS on healthy cells, two human cell lines were exposed to NO release from PDMS and cell viabilities were quantified.

### Cell culture

WI-38 human lung fibroblasts and HEK-293 human embryonic kidney cell lines were purchased from ATCC. WI-38 cells were maintained in MEM media (Sigma-Aldrich, Saint Louis MO) supplemented with 10% FBS (Gemini Biosciences, Denver CO), 1% Sodium Pyruvate, 1% l- glutamine, and 1% Penicillin/Streptomycin. HEK-293 cells were cultured in DMEM high glucose media supplemented with 10% FBS (Gemini Biosciences, Denver CO), 1% Sodium Pyruvate, and 1% Penicillin/Streptomycin. Both cell lines were maintained at 37°C, 5% CO<sub>2</sub>.

### Cell viability

MTT assay of WI-38 and HEK 293 treated with NO releasing PDMS-WI-38 and HEK293 cells were treated with a range of

PDMS disks that release different NO fluxes (Table 1). Cells were incubated in treatment for 24 hours. MTT dye (5 mg/mL) was added directly to media for an incubation period of two and half hours at 37°C. Immediately after incubation, media was aspirated and replaced with MTT solubilization buffer (0.04 M HCl in isopropanol). Cells were shaken at room temperature in solubilization buffer for at least ten minutes and then absorbance at a wavelength of 570 nm was detected using biotech microplate reader. Lower absorbance correlated to decreasing cell viability and viability was quantified as percent of control (absorbance from untreated cells).

### Statistical Analysis

Data are expressed as mean  $\pm$  SD (standard deviation of the mean). Comparison of results were analyzed by a comparison of means using Student's t-test or one way ANOVA. Values of  $p < 0.05$  were considered statistically significant for all tests.

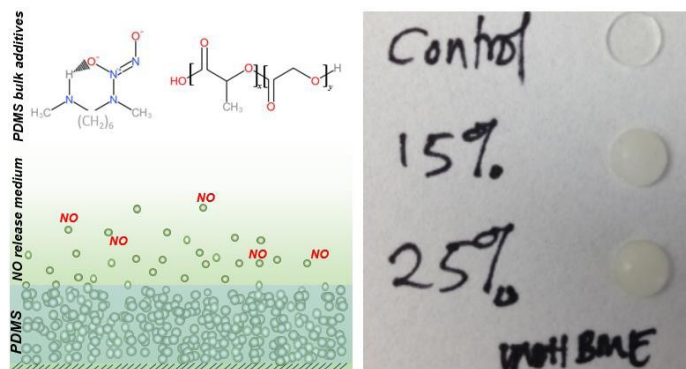
## RESULTS AND DISCUSSION

### Prepared samples and their nitric oxide release

Bulk composition of modified PDMS with stimulated NO release illustration (left) and representative samples for biological testing (right) are shown in Scheme 2. Control PDMS samples unmodified with any additives, top, were transparent while NO releasing samples, bottom two, appeared opaque due to the inclusion of DMHD/N<sub>2</sub>O<sub>2</sub> and PLGA. The samples measured 6.35 mm ID and 1 mm in thickness.

### Nitric oxide release from modified PDMS

To conveniently deliver different levels of NO to *P. aeruginosa*, WI-38 human lung fibroblasts, and HEK-293 human embryonic kidney cell, 15wt% DMHD/N<sub>2</sub>O<sub>2</sub> + 15wt% 5050DLG1A were stored at room temperature for various times before using them to treat bacteria and cells. The levels of NO released from PDMS as measured by chemiluminescence in terms of flux and total moles, and projected NO released into culture medium per



**Scheme 2:** Illustration of NO release from PDMS aided by its bulk composition of diazeniumdiolate (DMHD/N<sub>2</sub>O<sub>2</sub>) and polylactideglycolide, (left) and formulated samples showing PDMS (control) and PDMS-DMHD/N<sub>2</sub>O<sub>2</sub> composites containing 15% and 25% of DMHD/N<sub>2</sub>O<sub>2</sub>.

minute is shown in (Table 1). All three levels of NO flux listed have been investigated on *S. aureus* and *S. epidermidis* strains in our previous study so in this work NO flux levels including the minimum effective flux were tested on *P. aeruginosa* and cells.

### Effect of nitric oxide on bacteria and cells

The effect of  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> flux of NO on *P. aeruginosa* is shown in (Figure 1). It can be seen in the left panel that qualitatively, there were no differences in colony growths between control (untreated bacterial culture streaks) and PDMS (PDMS-treated culture streaks) groups. Significantly less colonies were observed in the NO releasing PDMS group where the colonies were sparsely spotted on the plates. Colony counts in the NO releasing PDMS group ( $54 \pm 7$ )  $\times 2 \times 10^4$  CFU/mL was significantly different from count in no-treatment control ( $607 \pm 52$ )  $\times 2 \times 10^4$  CFU/mL and PDMS-treated control ( $602 \pm 28$ )  $\times 2 \times 10^4$  CFU/mL groups,  $p < 0.001$ . The response of bacteria growth to NO release at ( $45.1 \pm 2.546 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>), Figure 2, shows a significantly ( $p < 0.001$ ) lower colony counts of  $80 \pm 69 \times 2 \times 10^5$  CFU/mL compared to  $625 \pm 75 \times 2 \times 10^5$  CFU/mL and  $700 \pm 71 \times 2 \times 10^5$  CFU/mL in the untreated control and PDMS-treated control groups respectively. Approximately 1 log/ 90% reduction in colony counts was observed at either level of NO flux while a higher reduction was seen in the Levofloxacin antibiotic positive control group, although at a level, ( $9 \pm 2$ )  $\times 2 \times 10^5$  CFU/mL, not significantly different compared to NO groups ( $p > 0.05$ ).

Viabilities of WI-38 and HEK 293 cells responded differently to treatment with NO at various fluxes. At the low NO flux level of  $0.48 \pm 0.10 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>, the morphologies of WI-38 and HEK-293 cells show no qualitative differences between their control and NO treatment groups. See (Figure 3). The adherent and spreading behavior of WI-38 controls can also be seen in the treatment group and the quantitative analysis of their viability showed no significant differences between controls [untreated cells and PDMS-treated cells ( $95 \pm 10$  %)] compared to the NO treatment group ( $117 \pm 25$ %) ( $p > 0.05$ ). The NO treatment group actually seem to improve in viability after the 24hr incubation period supporting the cell proliferative property of NO at low concentrations. In the HEK-293 cells, no observable difference in culture morphology was apparent, and no differences in viability was present between the controls and NO treatment groups ( $p > 0.05$ ).

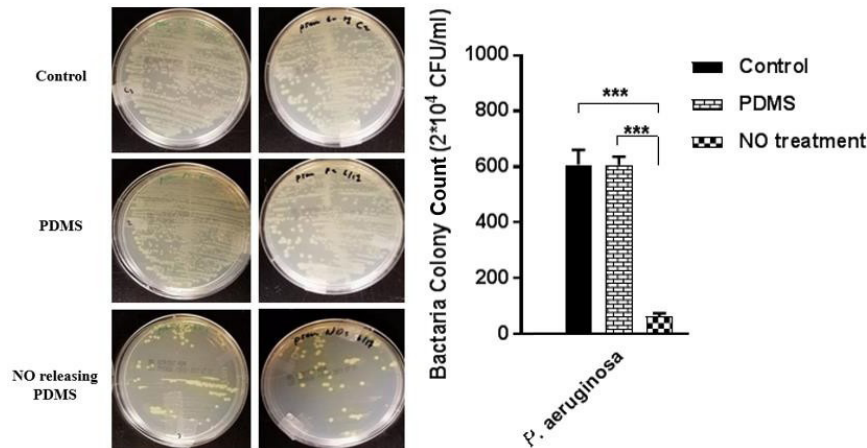
When the WI-38 cells were evaluated for their viability at  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>, (Figure 4), again no statistically significant differences were observed among untreated control, PDMS-treated control ( $90 \pm 12$  %), and NO treatment groups ( $84 \pm 15$  %) ( $p > 0.05$ ). Similarly, qualitative analysis of W1-38 cell culture, the only cell line evaluated here, showed no dissimilar adherent and spreading behavior between controls and the NO treatment groups.

Finally, treatment of cells with the highest NO flux tested

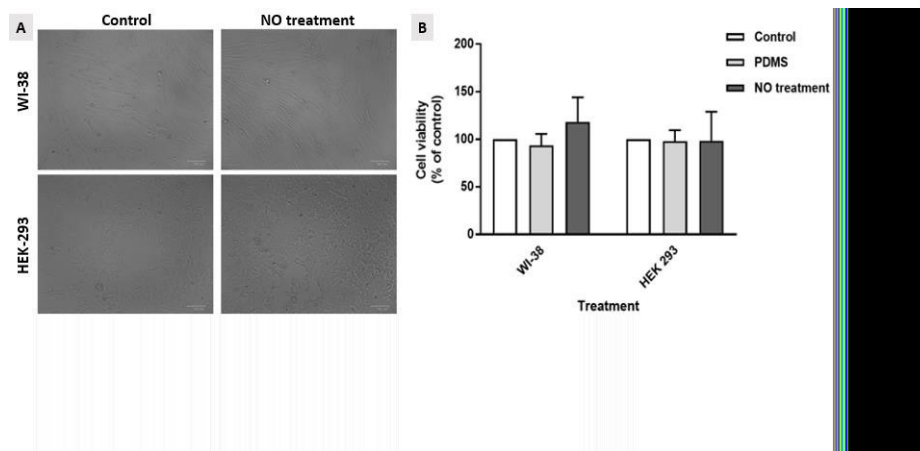


PDMS Active Layer Composition	Average NO flux (x 10 <sup>-10</sup> mol/min/cm <sup>2</sup> )	Average total NO (moles)	Average moles delivered to culture/minute
15wt% DMHD/N <sub>2</sub> O <sub>2</sub> +15wt% 5050DLG1A (24hr cured)	45.10±2.55	1.60x10 <sup>00</sup> ± 0.89x10 <sup>-01</sup>	1.16x10 <sup>-03</sup> ± 6.69x10 <sup>-05</sup>
15wt% DMHD/N <sub>2</sub> O <sub>2</sub> +15 wt%5050DLG1A (24hr cured + 24hr storage)	21.18±5.31	7.60 x10 <sup>-01</sup> ± 1.77 x10 <sup>-01</sup>	5.47 x10 <sup>-04</sup> ± 1.28 x10 <sup>-04</sup>
5wt% DMHD/N <sub>2</sub> O <sub>2</sub> +15wt% 5050DLG1A (24hr cured + 48hr storage)	0.48±0.10	1.84 x10 <sup>-02</sup> ± 3.64 x10 <sup>-03</sup>	1.29x10 <sup>-05</sup> ± 2.58 x10 <sup>-06</sup>

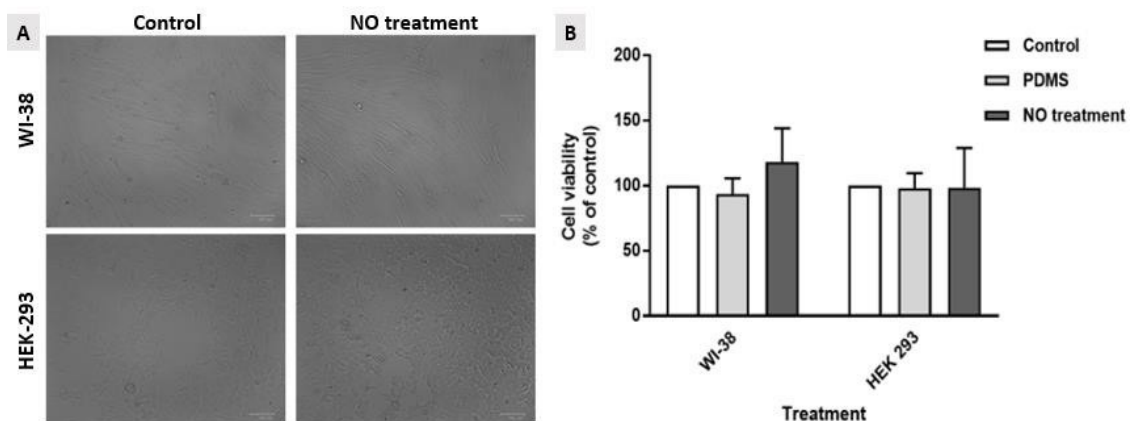
**Table 1:** Averages of nitric oxide fluxes and averages of total nitric oxide moles released from NO releasing PDMS substrates used for in vitro testing on bacterial cells growth



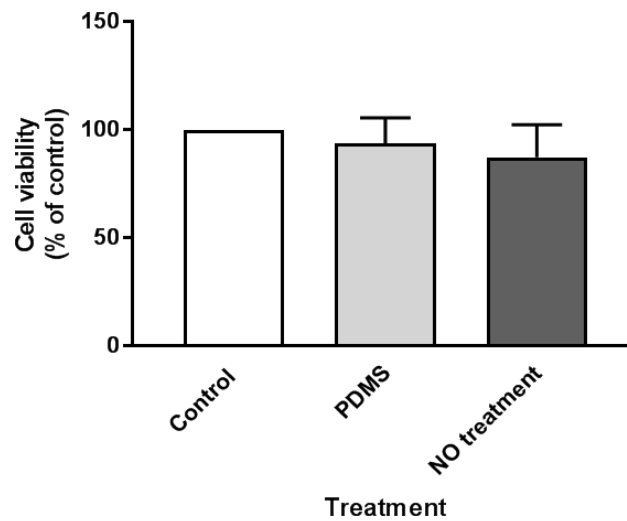
**Figure 1:** Antibacterial effect of  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> NO flux. Representative images of 1:10 dilution streaks of *P. aeruginosa* after 24 hours of NO treatment in comparison to control and PDMS alone (left). Quantitative analysis of streak plates, (n=12 per group), of *P. aeruginosa* shows significant reduction in colony count after NO treatment. Three asterisk denotes statistical significance (P value < 0.001)



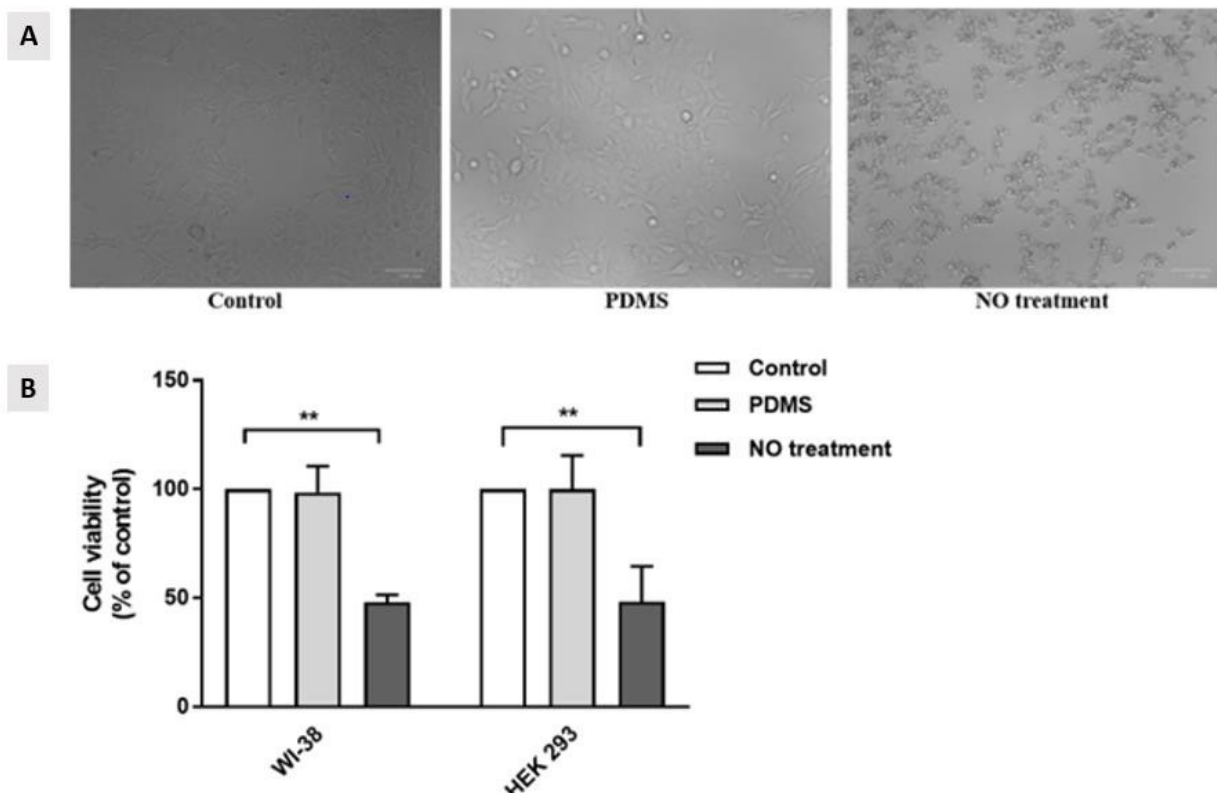
**Figure 2:** The effect of NO flux ( $45.1 \pm 2.546 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>) on *P. aeruginosa* growth in nutrient broth. Representative images of 1:100 dilution streaks of *P. aeruginosa* after 24 hours of NO treatment (left) and colony counts (right) showing significant reduction in bacteria growth in the NO releasing group (\*\*p < 0.001)



**Figure 3:** The effect of low NO flux on cell viability. (A) Representative images of both cell lines with NO treatment. (B) NO flux at  $0.48 \pm 0.10 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> promotes the growth in WI-38 cells.



**Figure 4:** The effect of bactericidal NO flux on WI-38 human lung fibroblasts viability. No significant difference was found between groups. It shows no significant difference between control and cells treated with average NO flux of  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>, average total NO moles delivered over 24 hours was  $0.76 \pm 0.177$  mole.



**Figure 5A:** The Effect of high NO dosage on cell viability. Representative images of HEK-293 cells with NO treatment in comparison to controls. **(5B)** Significant reduction in cell viability after treatment with higher dose of nitric oxide ( $45.1 \pm 2.546 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>). For WI-38 cells (p value =0.0023) and for HEK-293 (p value =0.0027).

( $45.10 \pm 2.55 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>), a level which showed the highest reduction in *P. aeruginosa* growth although not significantly different from the  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> flux effect, affected viabilities as anticipated. It can be seen in Figure 5A that compared to untreated control WI-38 culture, cell viabilities after PDMS control and NO treatments were  $94 \pm 13\%$  ( $p > 0.05$ ) and  $45 \pm 07\%$  ( $p < 0.05$ ) respectively. With HEK-293 cells, viabilities were  $100 \pm 21\%$  ( $p > 0.05$ ) in PDMS control and  $45 \pm 18\%$  ( $p < 0.05$ ) in NO treatment compared to untreated controls. The morphology of HEK-293 cells elected

for qualitative analysis of the effect of NO treatment revealed non-spreading and non-adherent properties. This was unlike the cell adhesion phenomenon seen in untreated and PDMS treated controls. The image in (Figure 5B) suggests the effect of such high level of NO flux can impair the spreading and adhesion of cells onto surfaces. Perhaps such levels significantly interact with cell adhesion molecules (CAMs) on cell membranes which are involved in cell adhesion, and may interfere with important CAMs properties such as maintaining tissue structure, function, and cell growth.

## CONCLUSION

This study investigated the cytocompatibility of antibacterial property of nitric oxide at different concentrations to determine effective antibacterial level of NO that could also be safe to healthy cells. PDMS biomaterials formulated to release different levels of NO was characterized for their total levels of released NO, effect on gram positive (not included in this study) and gram negative bacteria growth as well as for an effective minimum flux with antibacterial yet cytocompatible properties. The effects of the NO fluxes, including the effective minimum, on lung and kidney cells viabilities were also determined. The results from this work suggest that marginal antibacterial benefits are gained at fluxes greater than  $21.18 \pm 5.31 \times 10^{-10}$  mol/ min/ cm<sup>2</sup> and most importantly, cell viabilities at this NO level are seemingly not affected. The largest NO flux tested,  $45.10 \pm 2.55 \times 10^{-10}$  mol/ min/ cm<sup>2</sup>, however had a negative effect on the viabilities of both cell lines.

Further studies including long term antibacterial NO effects on cytocompatibility using additional standard intracellular markers to evaluate cell function and the antibacterial functionalization of implantable devices associated with risk of infection are needed to fully understand NO effects and to ensure safe and effective applications.

## ACKNOWLEDGEMENTS

This research is supported in part by NASA CT Grants Consortium (Award No. NNX15AI12H), University of New Haven's University Research Scholar grant, and the Tagliatela College of Engineering's faculty and student research support. The authors would like to thank the department of cellular and molecular biology for access to their microbiology labs and instrumentation.

## REFERENCES

- Ignarro LJ. Nitric Oxide. A Novel Signal Transduction Mechanism for Transcellular Communication. *Hypertension*. 1990; 16(5): 477-483.
- Fang FC. Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. *J Clin Invest*. 1997; 99(12): 2818-2825. doi: 10.1172/JCI119473 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC508130/>
- Green SJ. Nitric oxide in mucosal immunity. *Nature Medicine*. 1995; 1(6): 515-517. doi: 10.1038/nm0695-515 <https://www.nature.com/articles/nm0695-515>
- Hezel MP, Weitzberg E. The Oral microbiome and nitric oxide homeostasis. *Oral Dis*. 2015; 21(1): 7-16. doi: 10.1111/odi.12157
- Charville GW, Hetrick EM, Geer CB, Schoenfisch MH. Reduced bacterial adhesion to fibrinogen-coated substrates via nitric oxide release. *Biomaterials*. 2008; 29(30): 4039-4044. doi: 10.1016/j.biomaterials.2008.07.005
- Shami, PJ, Moore JO, Gockerman JP, Hathorn JW, Misukonis MA, et al. Nitric oxide modulation of the growth and differentiation of freshly isolated acute non-lymphocytic leukemia cells. *Leuk Res*. 1995; 19(8): 527-33. Website. <https://www.ncbi.nlm.nih.gov/pubmed/7658698> Accessed August, 1995.
- Hibbs JB, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: A cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun*. 1998; 157(1): 87-94. Website. <https://www.ncbi.nlm.nih.gov/pubmed/3196352> Accessed November, 1998.
- Burgner D, Rockett K, Kwiatkowski D. Nitric oxide and infectious diseases. *Arch Dis Child*. 1999; 81(2): 185-188. Website. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1718000/> Accessed August, 1999.
- Schairer DO, Chouake JS, Nosanchuk JD, Friedman AJ. The potential of nitric oxide releasing therapies as antimicrobial agents. *Virulence*. 2012; 3(3): 271-279. doi: 10.4161/viru.20328 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3442839/>
- Thomas DD, Ridnour LA, Isenberg JS, et al. The Chemical Biology of Nitric Oxide. Implications in Cellular Signaling. *Free Radic Biol Med*. 2008; 45(1): 18-31. doi: 10.1016/j.freeradbiomed.2008.03.020
- Habib S, Ali A. Biochemistry of Nitric Oxide. *Indian J Clin Biochem*. 2011; 26(1): 3-17. doi: 10.1007/s12291-011-0108-4
- Glenn TG, Thelma YY, Chan, Pak HC. Nitric Oxide- and Superoxide-Mediated Toxicity in Cerebral Endothelial Cells. *J Pharmacol Exp Ther*. 1997; 282(3): 1600-1607. Website. <https://www.ncbi.nlm.nih.gov/pubmed/9316877> Accessed September, 1997.
- Castro L, Rodriguez M, Radi R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. *J Biol Chem*. 1994; 269: 29409-29415. Website. <https://www.ncbi.nlm.nih.gov/pubmed/7961920> Accessed November, 1994.
- Dimmeler S, Lottspeich F, Brüne B. Nitric oxide causes ADP-ribosylation and inhibition of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem*. 1992; 267: 16771-16774. Website. <http://www.jbc.org/content/267/24/16771.abstract> Accessed August, 1992.
- Hausladen A, Fridovich I. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. *J Biol Chem*. 1994; 269(47): 29405-29408. Website. <https://www.ncbi.nlm.nih.gov/pubmed/7961919> Accessed November, 1994.
- Mohr S, Stamler JS, Brune B. Mechanism of covalent modification of glyceraldehyde-3-phosphate dehydrogenase at its active site thiol by nitric oxide, peroxynitrite and related nitrosating agents. *FEBS Lett*. 1994; 348(3): 223-227. Website. <https://www.ncbi.nlm.nih.gov/pubmed/8034046> Accessed July, 1994.

17. Wink D, Kasprzak K, Maragos C, Elespuru R, Misra M, et al. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science*. 1991; 254(5034): 1001-1003. Website. <https://www.ncbi.nlm.nih.gov/pubmed/1948068> Accessed November, 1991.
18. Arroyo PL, Hatch-Pigott V, Mower HF, Cooney RV. Mutagenicity of nitric oxide and its inhibition by antioxidants. *Mutat. Res*. 1992; 281(3): 193-202. Website. <https://www.ncbi.nlm.nih.gov/pubmed/1371842> Accessed March, 1992.
19. Isomura K, Chikahira M, Teranishi K, Hamada K. Induction of mutations and chromosome aberrations in lung cells following in vivo exposure of rats to nitrogen oxides. *Mutat. Res*. 1984; 136(2): 119-125. Website. <https://www.ncbi.nlm.nih.gov/pubmed/6717477> Accessed May, 1994.
20. Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, et al. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA*. 1992; 89(7): 3030-3034. Website. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC48797/> Accessed April, 1992.
21. Ghaffari A, Miller CC, McMullin B, Ghahary A. Potential application of gaseous nitric oxide as a topical antimicrobial agent. *Nitric Oxide*. 2006; 14(1): 21-9. doi: [10.1016/j.niox.2005.08.003](https://doi.org/10.1016/j.niox.2005.08.003)
22. Clutton-Brock J. Two cases of poisoning by contamination of nitrous oxide with higher oxides of nitrogen during anaesthesia. *Br J Anaesth*. 1967; 39(5): 388-392. Website. <https://www.ncbi.nlm.nih.gov/pubmed/6025380> Accessed MaY, 1967.
23. Greenbaum R, Bay J, Hargreaves MD, Kain ML, Kelman GR, et al. Effects of higher oxides of nitrogen on the anesthetized dog. *Br J Anaesth*. 1967; 39(5): 393-404.
24. Lehnert BE, Archuleta DC, Ellis T, Session WS, Lehnert NM, et al. Lung injury following exposure of rats to relatively high mass concentrations of nitrogen dioxide. *Toxicology*. 1994; 89(3): 239-277. doi: [10.1016/0300-483X\(94\)90100-7](https://doi.org/10.1016/0300-483X(94)90100-7)
25. Joshua DN, Friedman JM, Martinez LR, Han G. *Journal of Investigative Dermatology*. 2009; 129: 2463-2469. doi: [10.1038/jid.2009.95](https://doi.org/10.1038/jid.2009.95)
26. Gbyli R, Mercaldi A, Sundaram H, Amoako KA. Achieving Totally Local Anticoagulation on Blood Contacting Devices. *Adv. Mater. Interfaces*. 2017; 1700954. doi: [10.1002/admi.201700954](https://doi.org/10.1002/admi.201700954)
27. K Amoako, R Gbyli. Improving the hemocompatibility of biomedical polymers Hemocompatibility of Biomaterials for Clinical Applications. 2018; 223-252.
28. Amoako KA, Archangeli C, Major TC, Meyerhoff ME, Annich GM, et al. Thromboresistance Characterization of Extruded Nitric Oxide Releasing Silicone Catheters. *ASAIO J*. 2012; 58(3): 238-246. doi: [10.1097/MAT.0b013e31824abed5](https://doi.org/10.1097/MAT.0b013e31824abed5)