SOCIETY FOR NATURAL SCIENCES STUDENT JOURNAL

A reservoir for pathogens: modifying nasogastric tube surface topography to produce anti-adherence properties

Jane Wilson

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

Supervisor: Prof. Charles Brealey

Abstract

Nasogastric tubes (NGTs) are essential medical devices widely employed in both acute and chronic care settings. Yet, their prolonged use is frequently associated with microbial colonisation and infection, contributing to patient mortality and increasing healthcare costs. This study explored novel surface modification of NGTs as a means to mitigate infection, improving patient outcomes. The investigation evaluated the anti-adherence potential of zwitterionic compounds—dimethylsulfoniopropionate (DMSP) and glycine betaine—as well as the antimicrobial properties of copper sulphate ($CuSO_4$). Fabricated NGT-mimicking samples were coated with these zwitterions and compared to copper sulphate ($CuSO_4$)–treated discs as an antimicrobial reference. While neither zwitterion produced measurable anti-adhesive effects under current experimental conditions, copper discs yielded clear antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. Future research should focus on optimising zwitterion formulation, concentration, and coating technique, and evaluating dual-action approaches integrating anti-adhesive zwitterions with selective antimicrobial agents such as endolysins. From both clinical and economic perspectives, this interdisciplinary innovation may yield far-reaching impacts by improving patient care whilst alleviating the burden of infection-related complications.

Keywords: nasogastric tube, zwitterion, DMSP, glycine betaine, copper sulphate, biofilm, endolysin.

1. Introduction

Nasogastric tubes (NGTs) are routinely employed in care settings to deliver enteral nutrition, relieve gastric distension, and facilitate gastric lavage or sampling (Sigmon and An, 2022). Despite their ubiquity, long-term NGT use is strongly linked to microbial colonisation and device-associated abrasion and downstream infections, prolonging hospital stays and elevating risks of mortality (Ozen *et al.*, 2022). NGTs, typically composed of polyvinyl chloride (PVC) or polyurethane, traverse the nasal cavity and oropharynx—regions heavily populated with commensal and opportunistic microorganisms (Vongbhavit *et al.*, 2022). Once inserted, the NGT provides a continuous surface upon which biofilms rapidly establish, serving as reservoirs for pathogenic species including *S. aureus* and *E. coli* (Petersen, Greisen and Krogfelt, 2016).

To mitigate infection risks, current management strategies rely on frequent tube replacement or systemic antibiotic therapy, both of which enhance cost, patient discomfort, and accelerate the global crisis of antimicrobial resistance (Miller and Arias, 2024). In 2020, the NHS spent an estimated £2.1 billion on healthcare-associated infections alone, evidencing the need for preventive rather than reactive solutions to infection (Guest *et al.*, 2020). Modifying the surface properties of NGTs offers a promising strategy to limit bacterial adhesion and biofilm formation without increasing antimicrobial selection pressure (Desrousseaux *et al.*, 2013).

Zwitterions—molecules bearing balanced positive and negative charges—have recently attracted attention for their anti-fouling and anti-adherent capabilities. These compounds form a tightly bound hydration layer on material surfaces, limiting bacterial attachment (Colilla, Izquierdo-Barba and Vallet-

Regí, 2018; Li *et al.*, 2021) (Figure 1). Glycine betaine, a naturally occurring osmolyte, and dimethylsulfoniopropionate (DMSP), a marine-derived organosulfur compound, were selected for this investigation based on evidence of reduced microbial adhesion in marine and biomedical contexts (Rewak-Soroczyńska *et al.*, 2019; Saha *et al.*, 2012).

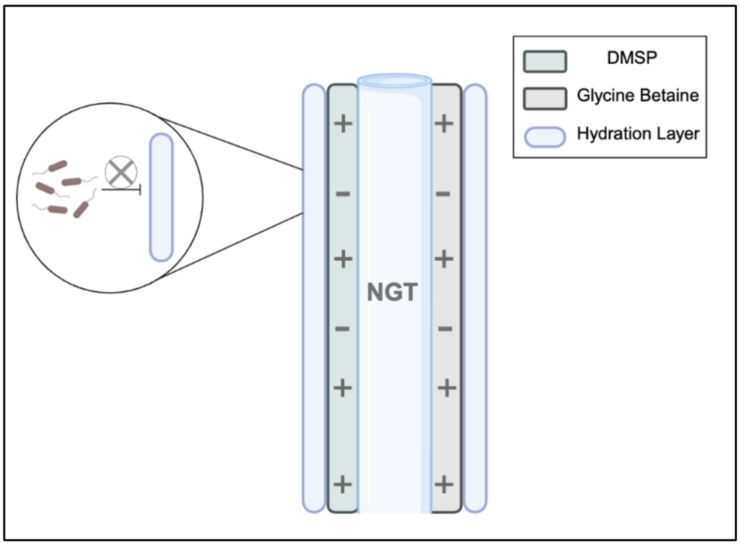

Figure 1 Zwitterionic coatings promote the formation of a hydration layer, impeding bacterial adherence and biofilm formation on NGT surfaces.

Copper is ubiquitously recognised for its potent antimicrobial properties (Vincent, Hartemann and Engels-Deutch, 2016). Notably, when applied to medical surfaces, copper ions generate reactive oxygen species (ROS), disrupting microbial membranes and enzymes and yielding broad-spectrum bactericidal effects (Benhalima *et al.*, 2019). Although copper was not the principal focus of this study, $CuSO_4$-soaked discs were included as a comparator to verify assay sensitivity and benchmark antimicrobial efficacy.

An initial aim of this investigation lay in fabricating NGT-mimicking surfaces using 3D printing and a medical grade resin. Fabricated surfaces were subsequently coated with DMSP and glycine betaine zwitterion solutions. Following 3D printing and coating procedures, bacterial adherence and biofilm formation of three clinically relevant pathogenic strains were evaluated on coated and uncoated control surfaces. Subsequent aims involved quantitatively measuring the anti-adherence performance of DMSP and glycine betaine-coated surfaces through zones of inhibition assays.

A summary of objectives:

Formulate and apply DMSP and glycine betaine coatings onto NGT analogues.

Evaluate the anti-adherence potential of named zwitterion coatings against *E. coli*, *S. aureus*, and *E. faecalis* using diffusion assays.

Compare results with $CuSO_4$-treated controls.

It was hypothesized that DMSP would demonstrate greater anti-adherence efficacy than glycine betaine when employed as a zwitterion coating on NGT-mimicking surfaces. This hypothesis was guided by prior research promoting DMSP's superior ability to interfere with bacterial communication and biofilm formation, particularly in marine and environmental bacteria (Johnson *et al.*, 2016). By contrast, although literature supports glycine betaine's ability to inhibit bacterial adhesion, the zwitterion is concomitantly associated with enhanced bacterial stress tolerance and biofilm resilience is certain species (Xia *et al.*, 2024). Finally, consistent with findings in literature, CuSO4 treatment was expected to yield antimicrobial effects against all bacterial strains assessed (*S. aureus, E. coli* and *E. faecalis*) (Vincent, Hartemann and Engels-Deutch, 2016).

2. Materials and Methods

2.1 Study Overview

This study involved interdisciplinary collaboration between engineering, chemistry, and biological sciences. NGT-mimicking samples were fabricated using 3D printing, coated with zwitterionic compounds (DMSP and glycine betaine), and evaluated for bacterial anti-adherence properties. A $CuSO_4$ disk assay served as a positive antimicrobial control.

2.2 Fabrication of NGT-Mimicking Samples

A custom "spatula" design incorporating detachable diffusion-disc analogues was created using SolidWorks CAD software. Samples were printed on a Stratasys Origin One printer using BASF Ultracur3D® ST45 resin, selected for high surface fidelity and ease of post-processing. After printing, samples were cleaned in isopropanol, air-dried, and UV-cured to ensure structural integrity.

2.3 Preparation of Zwitterion Coatings

Two zwitterionic coating solutions were prepared:

- **Glycine betaine (GB)**: 1.172 g dissolved in 5 mL distilled water and 5 mL of 70% ethanol.
- **DMSP**: 0.134 g dissolved in 0.5 mL distilled water and 0.5 mL of 70% ethanol.

Although ethanol facilitated dissolution, its potential to disrupt the zwitterion hydration layers is discussed later as a limitation. Sterile spatulas were submerged in each solution for 10 seconds, air-dried under a fume hood, and stored aseptically until testing.

2.4 Copper Disk Preparation

As a comparator, sterile paper discs were soaked in 1 M $CuSO_4$ for 30 minutes and air-dried before use.

2.5 Bacteriology Assays

Assays were conducted using *E. coli* CFT073, *S. aureus* USA300, and *E. faecalis* OGRF1. Each strain was cultured overnight in appropriate media at 37°C. Agar plates (LB with horse blood) were inoculated with 100 µL of bacterial suspension and spread evenly. Four coated or control discs were placed per plate and incubated at 37°C for 24 hours. Antimicrobial or anti-adherent activity was measured as the diameter (mm) of visible inhibition zones using an electronic calliper.

2.6 Statistical Analysis

Mean inhibition zone diameters ($n = 4$) were analysed using one-way ANOVA with Tukey's post-hoc test in Excel. Data were expressed as mean ± standard error of the mean (SEM), with $P < 0.05$ considered significant.

---

3. Results

3.1 3D Printing and Sample Preparation

Seventy samples were fabricated, of which 48 (68.6%) met design specifications with acceptable dimensional fidelity. Mean print time was 32 minutes per batch, and total resin use was approximately 233 mL. The method provided a reproducible platform for downstream coating and bacterial testing.

3.2 Anti-Adherence Potential of Zwitterionic Coatings

Neither DMSP nor glycine betaine coatings produced visible inhibition zones against any of the three bacterial species tested, suggesting an absence of measurable anti-adherent or antimicrobial effects under current assay conditions (Figure 2).

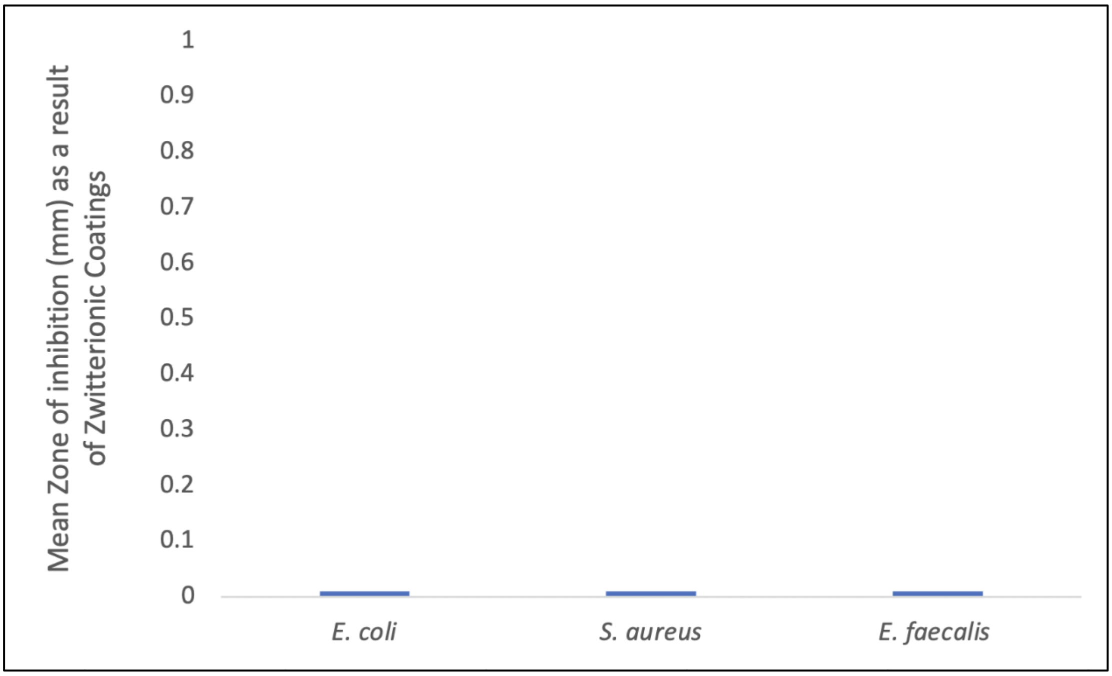

Figure 2 DMSP and glycine betaine fail to produce anti-adherence properties. Insignificant anti-adherence properties of the zwitterions; the treatments failed to produce a visible zone of inhibition.

3.3 Antimicrobial Effects of Copper Controls

In contrast, $CuSO_4$-treated discs displayed clear antimicrobial activity (Figure 3). *E. faecalis* exhibited the highest susceptibility with a mean inhibition zone of 11.35 ± 0.5 mm, followed by *S. aureus* (10.6 ± 0.6 mm) and *E. coli* (6.45 ± 0.9 mm). One-way ANOVA confirmed significant differences among

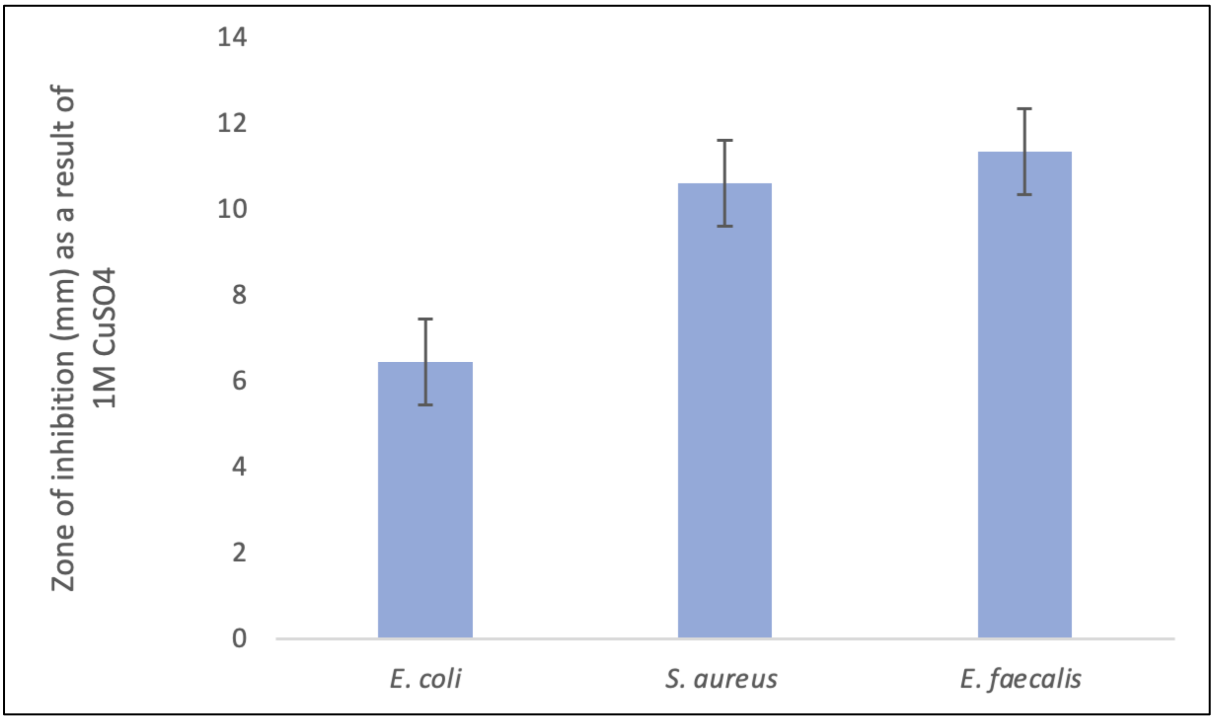

Figure 3. CuSO4 treatment yields antimicrobial effects against three pathogenic strains. Mean zones of inhibition (mm) following 1M CuSO4 treatment against *E. coli*, *S. aureus* and *E. faecalis*. Error bars are based on SEM. Data are presented as ± SEM, n = 4. One-Way ANOVA with Tukey post- hoc analysis was performed, where $*P < .05$. Full ANOVA outputs and replicate data

treatments (P = 0.00117), with post-hoc analysis identifying *E. coli* as significantly less susceptible than the other two species.

These results confirm the validity of the assay and benchmark antimicrobial activity, whilst simultaneously demonstrating that under the tested conditions, DMSP and glycine betaine coatings did not produce measurable bacterial inhibition.

4. Discussion

4.1 Summary of Findings

This study investigated whether zwitterionic compounds DMSP and glycine betaine could reduce bacterial adhesion on NGT-mimicking surfaces. While neither compound demonstrated detectable anti-adherence or antimicrobial activity in diffusion assays, $CuSO_4$-treated discs exhibited strong, broad-spectrum antimicrobial effects. The findings validate the assay and highlight the contrast between well-established inorganic antimicrobials and emerging zwitterionic approaches.

Although the results did not support the original hypothesis, several experimental and material factors likely contributed to the observed outcomes. These include resin composition, coating formulation, and the limitations of agar-based screening in evaluating surface-level interactions.

4.2 Experimental Limitations

The use of BASF Ultracur3D® ST45—a non-medical-grade resin—limited translational relevance. Future iterations should employ a biocompatible material such as LOCTITE® 3D MED412TM, which more closely resembles the polymer composition of clinical NGTs and provides a realistic substrate for coating adhesion (Turnage, 2022).

Zwitterion formulation was also suboptimal. Ethanol, included to aid solubility, likely disrupted the hydration layer fundamental to zwitterion anti-fouling behaviour (Level *et al.*, 2020; Pałecz, 2005). Future studies should eliminate ethanol and test higher zwitterion concentrations to enhance surface coverage. Additionally, other zwitterions—such as trimethylamine N-oxide—may offer superior hydrophilicity and bacterial repellence (Lee *et al.*, 2025).

Assay design represented another limitation. Zone-of-inhibition tests primarily assess diffusible antimicrobial agents, not surface-bound anti-adhesive effects. More appropriate quantitative assays include colony-forming unit (CFU) enumeration and crystal-violet biofilm staining, which directly measure bacterial adherence and biomass (Axelsson *et al.*, 2024; Kamimura *et al.*, 2022). Incorporating these would provide more relevant insights into zwitterion performance.

4.3 Toward a Dual-Action Coating

While zwitterions alone did not inhibit bacterial growth, their potential synergy with established antimicrobial agents remains promising. A "two-pronged" NGT surface design combining a zwitterion's anti-adhesive hydration layer with a controlled antimicrobial component could offer both passive and active protection (Figure 4).

Copper coatings, already used on urinary catheters, demonstrate strong bactericidal efficacy (Ding *et al.*, 2024). However, concerns about cytotoxicity and microbiome disruption motivate investigation of biologically selective alternatives. Bacteriophage-derived endolysins—enzymes that degrade bacterial cell walls—provide such selectivity, targeting pathogens like *S. aureus* while sparing commensals known to support skin health and wound healing (Wilkinson *et al.*, 2024). Integrating an endolysin with a zwitterion layer could yield a biocompatible dual-action surface that prevents adhesion and selectively eliminates pathogens.

4.4 Future Directions

Future work should prioritise optimising zwitterion formulation, exploring polymeric variants, and establishing reproducible coating methods. Notably, researchers advocate nanoimprint lithography as a means to induce surface charges that may enhance zwitterion and endolysin binding to NGTs (Jeoung *et al.*, 2015; Lan and Ding, 2010).

Furthermore, modified NGTs should undergo deformability studies. That is, NGT flexibility and integrity following aforementioned surface modulations must be assessed, ensuring clinical transferability and safety. Formal deformability studies should be accompanied by optical microscopy to assess NGT surface conditions post-deformation, verifying the integrity of coatings.

Advances in organoid technology now allow ex vivo testing of nasal or oropharyngeal models that closely replicate native microbiota (Rajan *et al.*, 2022; Svensson *et al.*, 2025). Such systems could evaluate coated NGTs under physiologically relevant conditions before progressing to clinical trials.

Finally, incorporating soothing biocompatible coatings—such as Aloe vera extracts—may reduce tissue irritation, enhancing both patient comfort and compliance (Hekmatpou *et al.*, 2019).

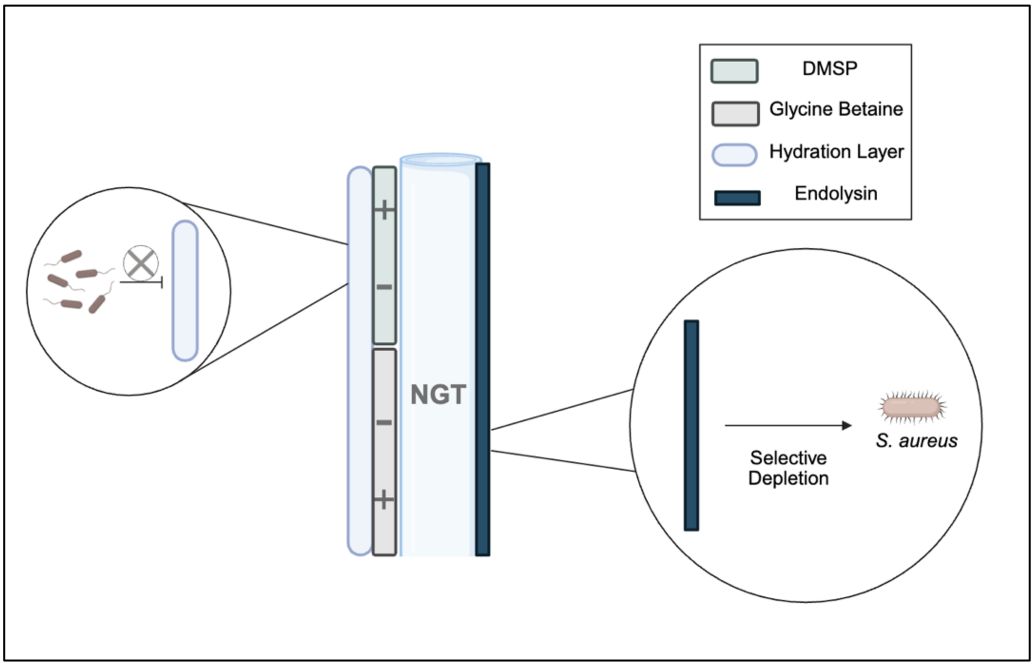

Figure 4. Dual-action coating combining zwitterions and selectively depleting endolysin.

5. Conclusion

This investigation underscores the immense potential of NGT surface modulation as a novel and clinically impactful strategy to mitigate infection and save lives. By integrating the strengths of biomedical research and engineering, this interdisciplinary work was grounded in a singular, patient-centred goal: to enhance the safety and comfort of long-term NGT users. Although results failed to support the anti-adherence properties of zwitterions DMSP and glycine betaine under current experimental conditions, their future efficacy should not be precluded —particularly with the proposed experimental refinements. The investigation promotes future exploration of dual-action coatings combining zwitterions and endolysins as an alternative to copper. Endolysins offer selective pathogen depletion whilst supporting a stable skin microbiome, an outcome that is clinically significant when considering NGT-induced skin abrasion or ulceration. From a broader perspective, the economic and market potential for modified NGTs is clear; reducing device-associated infections may mitigate downstream healthcare costs, for example. Driven by a commitment to enhance patient wellbeing, this investigation provides a novel foundation for continuing NGT surface modifications and device development.

References

Axelsson, C., Nilson, B. and Rehnstam-Holm, A.-S. (2024) Efficient absorbance-based assay for rapid antibiotic susceptibility testing of enterobacterales, *Antibiotics*, 13(9), p.852.

Benhalima, L., Amri, S., Bensouilah, M. and Ouzrout, R. (2019) Antibacterial effect of copper sulfate against multi-drug resistant nosocomial pathogens isolated from clinical samples, *Pakistan Journal of Medical Sciences*, 35(5).

Colilla, M., Izquierdo-Barba, I. and Vallet-Regí, M. (2018) The role of zwitterionic materials in the fight against proteins and bacteria, *Medicines*, 5(4), p.125.

Desrousseaux, C., Sautou, V., Descamps, S. and Traoré, O. (2013) Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation, *Journal of Hospital Infection*, 85(2), pp.87–93.

Ding, R., Xu, H., Zhang, J., Cai, Z., Peng, P., Zhang, Y. and Li, P. (2024) Copper-phenolic coating constructed on silicone urinary catheters to prevent catheter-associated infections, *Materials Today Communications*, 39, pp.109162–109162.

Guest, J.F., Keating, T., Gould, D. and Wigglesworth, N. (2020) Modelling the annual NHS costs and outcomes attributable to healthcare-associated infections in England, *BMJ Open*, 10(1).

Hekmatpou, D., Mehrabi, F., Rahzani, K. and Aminiyan, A. (2019) The effect of aloe vera clinical trials on prevention and healing of skin wound: a systematic review, *Iran J Med Sci,* 44(1), pp.1-9.

Jeoung, E., Duncan, B., Wang, L.-S., Saha, K., Subramani, C., Wang, P., Yeh, Y.-C., Kushida, T., Engel, Y., Barnes, M.D. and Rotello, V.M. (2015) Fabrication of robust protein films using nanoimprint lithography, *Advanced Materials*, 27(40), pp.6251–6255.

Johnson, W.M., Kido Soule, M.C. and Kujawinski, E.B. (2016) Evidence for quorum sensing and differential metabolite production by a marine bacterium in response to DMSP, *The ISME Journal*, 10(9), pp.2304–2316.

Kamimura, R., Kanematsu, H., Ogawa, A., Kogo, T., Miura, H., Kawai, R., Hirai, N., Kato, T., Yoshitake, M. and Barry, D.M. (2022) Quantitative analyses of biofilm by using crystal violet staining and optical reflection, *Materials*, 15(19), p.6727.

Lan H and Ding Y (2010) *Nanoimprint Lithography*. [online] Available at: http://dx.doi.org/10.5772/8189 [Accessed 20 March 2024].

Lee, S.-H., Yoo, S., Kim, S.H., Kim, Y.-M., Han, S.I. and Lee, H. (2025) Nature-inspired surface modification strategies for implantable devices, *Materials Today Bio*, 31, pp.101615–101615.

Level, G., Zhang, J., Brown, J., Hammond, O., Hannigan, B., Stella, L., Nockemann, P. and Marijana Blesic (2020) Multicharge zwitterionic molecules: hydration, kosmotropicity and anti-fouling potential, *Journal of colloid and interface science*, 562, pp.391–399.

Li, W., Thian, E. S., Wang, M., Wang, Z. and Ren, L. (2021) Surface design for antibacterial materials: from fundamentals to advanced strategies, *Adv Sci (Weinh),* 8(19), pp. e2100368.

Miller, W.R. and Arias, C.A. (2024) ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics, *Nature Reviews Microbiology*, 22(22), pp.1–19.

Ozen, N., Sis Celik, A., Terzioglu, F., Ozen, V., Ozmen, O., Kose, S., Tosun, B., Dogan, N., Ardic, B., Atabeyoglu Cimen, B., Kilic, D. and Uslu, H. (2022) Prevention of microbial colonization of feeding tubes in the intensive care unit, *Nursing in Critical Care*, 28(6).

Pałecz B (2005) Thermodynamics of interactions between zwitterions of several L-α-amino acids and ethanol in aqueous solution, *Thermochimica Acta*, 435(1), pp. 99–101.

Petersen, S.M., Greisen, G. and Krogfelt, K.A. (2016) Nasogastric feeding tubes from a neonatal department yield high concentrations of potentially pathogenic bacteria— even 1 d after insertion, *Pediatric Research*, 80(3), pp.395–400.

Rajan, A., Weaver, A.M., Aloisio, G.M., Jelinski, J., Johnson, H.L., Venable, S.F., McBride, T., Aideyan, L., Piedra, F.-A., Ye, X., Melicoff-Portillo, E., Malli, Zeng, X.-L., Mancini, M.A., Fabio Stossi, Maresso, A.W., Kotkar, S.A., Estes, M.K., Blutt, S. and Vasanthi Avadhanula (2022) The human nose organoid respiratory virus model: an *ex vivo* human challenge model to study respiratory syncytial virus (RSV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis and evaluate therapeutics, *mBio*, 13(1).

Rewak-Soroczyńska, J., Paluch, E., Siebert, A., Szałkiewicz, K. and Obłąk, E. (2019) Biological activity of glycine and alanine derivatives of quaternary ammonium salts (QASs) against micro-organisms, *Letters in Applied Microbiology*, 69(3).

Saha, M., Rempt, M., Gebser, B., Grueneberg, J., Pohnert, G. and Weinberger, F. (2012) Dimethylsulphopropionate (DMSP) and proline from the surface of the brown alga Fucus vesiculosus inhibit bacterial attachment, *Biofouling*, 28(6), pp.593–604.

Sigmon, D. F. and An, J. (2024) Nasogastric tube, *StatPearls*. Treasure Island (FL).

Svensson, L., Nordgren, J., Lundkvist, Å. and Hagbom, M. (2025) Recent advances in nose and lung organoid models for respiratory viral research, *Viruses*, 17(3), p.349.

Turnage, L. (2022) *Loctite MED412 Loctite Additive Manufacturing*. [online] Available at: https://www.loctiteam.com/med412-biocompatible/ [Accessed 20 April 2025].

Vincent, M., Hartemann, P. and Engels-Deutsch, M. (2016) Antimicrobial applications of copper, *International Journal of Hygiene and Environmental Health*, 219(7), pp.585–591.

Vongbhavit, K., Salinero, L.K., Kalanetra, K.M., Masarweh, C., Yu, A., Taft, D.H., Mills, D.A. and Underwood, M.A. (2022) A comparison of bacterial colonization between nasogastric and orogastric enteral feeding tubes in infants in the neonatal intensive care unit, *Journal of Perinatology*, 42(11), pp.1446–1452.

Wilkinson, H.N., Stafford, A.R., Rudden, M., Rocha, N.D.C., Kidd, A.S., Iveson, S., Bell, A.L., Hart, J., Duarte, A., Frieling, J., Janssen, F., Röhrig, C., de Rooij, B., Ekhart, P.F. and Hardman, M.J. (2024) Selective depletion of staphylococcus aureus restores the skin microbiome and accelerates tissue repair after injury, *Journal of Investigative Dermatology*, 144(8), pp.1865-1876.e3.

Xia, Y., Jiang, X., Guo, S., Wang, Y., Mu, Y. and Shen, J. (2024) Glycine betaine modulates extracellular polymeric substances to enhance microbial salinity tolerance, *Environmental Science and Ecotechnology*, 20, p.100406.

Acknowledgements

Many thanks to my dissertation advisor, Professor Charles Brearley, for your time and support in conducting this project.

Thank you to my parents, sister, and partner for your unwavering support.