

University of Nottingham

nmRC CASE STUDY

ANTIBIOTIC VANCOMYCIN INDUCES COMPLEXATION AND AGGREGATION OF MUCINS

nmRC_CS_13







The antibiotic vancomycin induces complexation and aggregation of mucins

Environmental-SEM Case Study



- Orally-administered vancomycin is the antibiotic of choice to treat pseudomembranous enterocolitis in the gastrointestinal tract.
- However, the risk of vancomycin-resistant enterococcal infection or colonization is significantly associated with oral vancomycin.
- Hence, there is a need to explore vancomycin-gastrointestinal interactions in order to explain the poor absorption from the antibiotic in the intestine. In this investigation, gastrointestinal mucins were used, as a way of models (a) HOTEN HOTE CHONE (b)



(a) vancomycin and (b) mucin structure



- In order to investigate these interactions, we need a technique capable of generate highquality imaging to appraise fine details of mucin-vancomycin aggregates
- The technique must permit the manipulation and control of environmental parameters (such as temperature and humidity), which is highly important when analysing biological samples
- Here, we use the technique of environmental scanning electron microscopy (ESEM) to visualise the large supramolecular complexes resulting from the interactions of vancomycin with <u>pig gastric mucin</u> (PGM), <u>pig intestinal mucin</u> (PIM) and <u>bovine submaxillary mucin</u> (BSM) under controlled dehydration in the ESEM sample chamber.



Environmental SEM (ESEM) allows examination of fully hydrated 'wet' samples and of poorly conductive uncoated materials, all of which cannot be imaged in the high vacuum conditions of a conventional SEM.

- High performance imaging in three modes: High Vacuum, Low
 Vacuum and ESEM
- Water vapour, air and nitrogen ESEM imaging modes for hydrated or non-coated samples
- Deben in-situ Microtest tensile-compression stage MTEST200VT with loading up to 200N and Peltier temperature range -20°C to 160°C
- Variable vapour pressures with peltier based temperature control for relative **humidity cycling**/adjustment sample freeze thaw cycling
- Peltier cooling stage for sample and humidity (gas pressure) control



FEI Quanta 650 ESEM available at the Nanoscale and Microscale Research Centre (nmRC), University of Nottingham



ANALYTICAL ULTRACENTRIFUGATION

University of Nottingham Nanoscale and Microscale Research Centre

Sedimentation velocity



AUC rotor and cell showing the centerpiece containing the solution and solvent. The centrifugal force causes migration of molecules in the solution causing changes in the concentration gradient, or in other words – fringe displacement.

The fringe concentration is plotted against radial position as a function of time, which is used to derive the sedimentation coefficient.







PGM-VANCOYCIN

50



ESEM micrographs for: (a) Vancomycin; (b) Pig gastric mucins; (c,d) Complexes resulted from their interaction. Small globular grains forming the supramolecular aggregate as shown in panel (d), a magnification of lower left panel (c).



PIM-VANCOYCIN

250



ESEM micrographs for: (a) Vancomycin; (b) Pig intestinal mucins; (c,d) Complexes resulted from their interaction with vancomycin. Small globular grains forming the supramolecular aggregate as shown in panel (d), a magnification of lower left panel (c).



BSM-VANCOYCIN

40



ESEM micrographs for (**a**) Vancomycin; (**b**) Bovine submaxillary mucins; (**c,d**) Complexes resulted from the interaction with vancomycin Small globular grains forming the supramolecular aggregate as shown in panel (d), a magnification of lower left panel (c).



Universitu of

Nottingham

| CHINA | MALAYSIA

- ESEM is a powerful technique capable for imaging fully hydrated 'wet' samples that allows the control of temperature and humidity levels to collect highresolution images
- The use of the ESEM helped to demonstrate for the first time complexation/depletion interactions for model mucin systems with vancomycin, providing the basis for further study on how orally administered vancomycin might relate to glycopeptide transit in humans
- Analytical ultracentrifugation (AUC) provides a powerful, matrix-free method for the characterization of the heterogeneity, molecular weight (molar mass) distributions, and interactions of macromolecules such as mucins
- The use of SV-AUC was crucial in demonstrating the formation of complexes between vancomycin and the different types of mucins found at different locations along the oral and gastrointestinal canal



For more details on the work showcased in this case study see the following publications:

V. Dinu, Y. Lu, N. Weston, R. Lithgo, H. Coupe, G. Channell, G.G. Adams, A. Torcello Gómez, C. Sabater, A. Mackie, C. Parmenter, I. Fisk, M.K. Phillips-Jones, S.E. Harding, The antibiotic vancomycin induces complexation and aggregation of gastrointestinal and submaxillary mucins, Sci Rep 10(1) (2020) 960.

"Super-sticky molecules versus Superbugs. Notts TV, 1st broadcast" Jan 28th 2020 at 6pm [Video]. Youtube:

https://www.youtube.com/watch?v=qwAQoYJ0Lyw&list=PLN1ANYZrllJ10BB3zwZHQ1pPTr9lLoga3



University of Nottingham Nanoscale and Microscale Research Centre



- We hope the information provided in this case study is of interest.
- If you wish to get in touch with us to discuss any of the information provided, raise a query/concern or provide feedback then please use any of the methods listed below:

nmRC Commercial Services Nanoscale & Microscale Research Centre University Park Nottingham NG7 2RD

Telephone:+44(0)115 951 5046Email: nmcs@nottingham.ac.ukFax:+44 (0)115 846 7969Website:www.nottingham.ac.uk/nmrc-commercial