# Summary

My work was focussed on the development of a drug-free, antibacterial, multilayer honey-mimetic wound dressing, composed of: (1) a loaded antibacterial reactive oxygen species (ROS)-producing, injectable hydrogel, (2) an antibacterial glucose oxidase (GO) surface-functionalized electrospun polyurethane fibre patch protecting the hydrogel layer, and (3) a glucose-loaded hydrogel on top of the electrospun fibre wound dressing. Assembled all together, these materials would be applied on the wound site to facilitate healing and prevent bacterial infections.

In the development of the Layer (1) a wound dressing hydrogel, an *in situ* quick forming, water absorbing hydrogel was developed, mimicking the antibacterial mechanism of honey. Within honey, antibacterial ROS such as hydrogen peroxide ($H_2O_2$) are continuously formed from the reaction of GO enzyme and glucose. The hydrogel was prepared by the Michael addition thiol-ene click chemistry between a synthesised hyperbranched polyethylene glycol diacrylate (HB PEGDA) and a commercially available thiolated hyaluronic acid (HA-SH). This reaction occurs rapidly (63 s) in a physiological pH environment and causes a quick sol to gel transition of the mixed solutions. This HB PEGDA (10 w/w%)/HA-SH (1w/w%) 10-1.0 hydrogel containing 250 U/L glucose oxidase and 2.5 w/w% glucose produced 9.1±0.9 mM $H_2O_2$ in static conditions after 24 hours and displayed cytocompatibility when tested in the presence of NIH/3T3 mouse fibroblast cells for 24 hours. Antibacterial ability was also measured against relevant gram-positive bacteria and gram-negative bacteria: *E. coli, S. aureus, P. aeurginosa*, MRSA, MRSE, etc where it had a higher antibacterial response to gram-positive bacteria due to their lack a cell wall.

Layer (2) was made from Lubrizol Tecophilic SP60D60, a commercially available medical-grade thermoplastic polyurethane (TPU). This was coated with a polyDOPA (PDA) film to facilitate immobilization of GO enzyme as it grafts itself on PDA's quinone functional groups. PDA coating by spontaneous oxidation of L-dihydroxyphenylalanine (DOPA) and its self-polymerisation generally requires at least 7 hours to get a well-distributed layer. To reduce oxidation/polymerisation time in PDA-coating a novel protocol was developed by adding an oxidizing enzyme, 1.4 U/mL lactoperoxidase (LPO) and reduced the coating time to 3 hours. Glucose oxidase was successfully grafted into TPU-PDA fibre patches (with and without the assistance of LPO). One of the modified fibre patches of interest, TPU-PDA-GO-0-3 had an enzyme activity of 7.9±1.9 U/g glucose oxidase.

For the top glucose-replenishing hydrogel (Layer 3), the material was prepared from UV-cured gelatin methacrylate (GelMA) loaded with 10 w/v glucose. GelMA was synthesized by following the protocol by Nichol, et al., 2010. GelMA was dissolved at a concentration of 15 w/v% into a PBS solution at pH 7.4 that contained 10 w/v% glucose and 1 w/v% Irgacure 2959. Hydrogels were formed by UV crlosslinking under a UV lamp with an intensity of 4 $mW/cm^2$ at a wavelength of 365 nm. As a bilayer material glucose-loaded GelMA when used with TPU-PDA-GO samples (treated with different coating times and oxidizing enzymes) were tested as cytocompatible in the presence of NIH/3T3 mouse fibroblast cells for 24 hours.

The highest overall cytocompatibility of the assembled trilayer product is 58.3±8.5% with the use of TPU-PDA-GO-0-3 as Layer 2. The obtained cytocompatibility for the multilayer was not considerably ideal, thus it is likely that the GO/glucose concentrations in HB PEGDA/HA-SH 10-1.0 have to be reduced when used with the TPU-PDA-GO fibre patch with glucose-loaded GelMA. An alternative would be to apply the TPU-PDA-GO system to protect the *in situ* HB PEGDA/HA-SH 10-1.0 wound dressing from exposure and once the glucose from the HB PEGDA/HA-SH 10.1 hydrogel has almost depleted itself, the glucose-loaded GelMA hydrogel will be applied as an alternative glucose source.

Despite this, the potential of drug-free GO enzyme/glucose ROS-producing systems to act on various bacterial strains, particularly gram-positive antibiotic-resistance ones, should be explored more in-depth in future studies especially given concerns on the increasing antimicrobial resistance of bacteria. Further optimization of the overall $H_2O_2$ output is required and can be performed in future studies.