RAFT for Biomedical Applications

The aim of this project was to develop technology platforms based on the **Reversible Addition-Fragmentation chain Transfer (RAFT)** process to prepare polymers for use in biomedical applications. The project is divided into three distinct sub-projects, two of which are with external collaborators, with the following research objectives.

- ***Surface Modification (Cochlear and OBI)***. To develop polymer coatings that modify the surface of implantable materials, to enhance biocompatibility.
- ***Conjugation of bioactives (CSIRO)***. To develop a polymeric scaffold for targeted drug delivery, through conjugation of both a cytotoxic small molecule drug and an antibody fragment.
- ***University of Washington***. To develop a technology for the preparation of polymers that act as a pro-drug for the delivery of therapeutic agents.

Surface Modification (Cochlear and OBI):

A method to modify the surface of implants has been developed with the aim to modulate the foreign body response that is observed upon implantation through the development of novel high-density RAFT-based polymer coatings. These coatings have been demonstrated to provide a highly biocompatible surface modification method for a broad range of polymeric substrate materials. This chemistry has been successfully translated onto cochlear implants and 3D printed polymers.
In vitro experiments using high-density RAFT-based coatings have shown excellent reduction of non-specific protein adsorption and mammalian cell attachment on a range of relevant polymeric substrate materials. In vivo experiments using subcutaneous implantation and 3D printed implants showed that, while high density RAFT-based graft polymer coatings were able to modulate the extent of capsule formation, this was not sufficient for the prevention of capsule formation.

Further experiments using the novel high-density RAFT-based polymer coatings developed in this project have led to highly effective spatial control over the cellular response. This result provides access to implants with high resolution spatially controlled surface chemistry that is non-cell adhesive in one region of the implant and tissue adhesive in a different region.
Importantly, the project has led to new insights into the factors that influence the foreign body response. This will influence the design of future animal experiments and devices at Cochlear and at the O'Brien Institute. By communicating these results in published and forthcoming research publications, these results will also be accessible to the entire research community. Furthermore, the project has led to the development of outstanding relationships between Cochlear and CSIRO as well as the O'Brien Institute and CSIRO and a deep understanding of the clinical issues on the one hand and what biomaterials science can contribute to solving these on the other. Based on the relationships formed in this project, and based on the value of these interactions, the partners are currently pursuing further opportunities for joint projects.

Conjugation of bioactives (CSIRO):

We have prepared a range of homopolymers and copolymers that are known to meet all the requirements for use in a biological setting (high MW, low polydispersity, neutral, water soluble, stable at 37 °C, cheap and easy to prepare, no cell toxicity and not elicit an immune response) and have conjugated each polymer to a therapeutic antibody fragment, through a stable covalent bond. We optimised the linker chemistry that was required to conjugate these polymers to an antibody fragment, giving rise to receptor-targeted polymer conjugates.

Two ADMET (absorption, distribution, metabolism, excretion, toxicity) studies were performed to assess the pharmacokinetics of a range of polymers and their antibody fragment-polymer conjugates in animal models. The ADMET profile of both the polymers alone and the polymer-antibody fragment conjugates, show residence times that are on par with similarly sized PEG-antibody fragment constructs. Within the antibody fragment-polymer conjugates assessed, differences in the elimination phase half-lives of these conjugates, due to the different polymers, have provided us with interesting and unexpected results.

Guided by these previous results we have entered into a final study that involves the use of a RAFT polymer to act as a scaffold for drug conjugation, where the polymer acts as a carrier for the drug. The drug-loaded polymer contains a functional group and will be conjugated to an antibody fragment. By adding a targeting agent to the polymer (the antibody fragment), the conjugate should give rise to an increase in tumour targeting by receptor-mediated delivery.
We have prepared cytotoxic drug-loaded polymers, with an appropriate functional group for conjugation to antibody fragments. The cytotoxic drugs are attached to the polymer via cleavable linkers such that the free cytotoxic can be released at the site of action. These drug-loaded polymer-antibody fragment conjugates will be submitted to an anti-cancer efficacy animal model.

University of Washington:

This project involves delivery of a polymeric-drug conjugate that carries an antimicrobial drug (ciprofloxacin) as a prodrug monomer, copolymerised into a carrier hydrophilic polymer. This prodrug pulmonary delivery vehicle construct is delivered via aerosol into the lung to reach the pulmonary alveoli where it targets macrophages for polymer uptake. The polymer-prodrug constructs are designed to release the antimicrobial drug within the macrophage endosomal environment. Polymerisable prodrug monomers provide an attractive route by which drug conjugates can be prepared directly without the need for post polymerization conjugation reactions. In this strategy the therapeutic agent is linked to a polymerisable group via a hydrolytic, or by an enzymatically cleavable, linkage.

A range of copolymers and block copolymers have been prepared that contain the antimicrobial agent, ciprofloxacin, through the synthesis of ciprofloxacin-containing RAFT monomers.
Release kinetics of the antimicrobial ciprofloxacin, have been measured by HPLC, in buffer and serum as well as in the presence of enzymes known to metabolise drugs. These studies have demonstrated that free ciprofloxacin is produced with varying rates depending on the linker between ciprofloxacin and the polymer.

Cell studies have been performed on these ciprofloxacin-containing polymers in order to understand and probe the toxicity and antimicrobial efficacy of the different polymeric structures and the different linkers installed to release ciprofloxacin. Cell studies have demonstrated that these polymers have a very good antimicrobial efficacy, across a number of structures.
In vivo studies of the ciprofloxacin-containing polymers will be reported in due course.