Final Report

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Title of Project: Thrombotic nanodisks to block abnormal blood vessels in brain

Progress Report 1 (25/5/2019)

We are currently in the middle of performing this work. The first half of this year has been dedicated to preparing the nanodisks in preparation for labelling and optimising the approach to labelling with the lys-lys conjugation technique. We are currently establishing the optimal number of phosphatidylserine-binding Annexin V particles to attach to the nanoparticles (the avidity of the nanodisks). This involves altering the ratio of AnV/NP during the conjugation step and testing each conjugate batch for relative binding over static cell cultures that have been irradiated, then subsequently higher high flow using our established parallel-plate flow system. We compare binding between irradiated and non-irradiated cells. This allows us to determine the best labelling ratio that can provide high binding affinity but also provides high levels of specificity (that is, bind irradiated cells but not non-irradiated cells). The level of specificity is a factor of both surface expression levels but also conjugate dose and avidity (the labelling ratio), so we must establish this first. Once this is established we can then add the effector thrombin to the NPs and run in the parallel-flow system in the presence of blood, which will be done in the second half of the year.

Final Report (20/1/2019)

The first half of the year saw the preparation of the nanodisks. We had some delays due to our nanoparticle expert being on maternity leave for 6 months, however two batches of particles were prepared with average diameters of 250 and 500 nm. The particles showed upconversion fluorescence characteristics as expected (see Figures). We aimed to create the particles with functionalised amino groups on the surface to allow lys-lys conjugation of the targeting ligand using a type of click chemistry. This approach allows nanoparticle to ligand binding without nanoparticle-nanoparticle or ligand-ligand self-association. We initially aimed to use a small protein annexin V for targeting phosphatidylserine as the ligand however this was not successful and we found that we could not assess the level of annexin V binding very easily to determine where the labelling was failing. In the second half of the year we attempted to bind an antibody directed to another radiation target (alpha-b-crystallin) to the nanoparticles, as these antibodies could be more easily assessed for completion of the attachment reactions. Unfortunately despite various protocol

modifications and attempts this was not successful and we could not successfully demonstrate attachment to the irradiated endothelial layers. We also found that the nanoparticle fluorescence was not as high as expected and the confocal microscopy used for particle microscopy was very challenging.

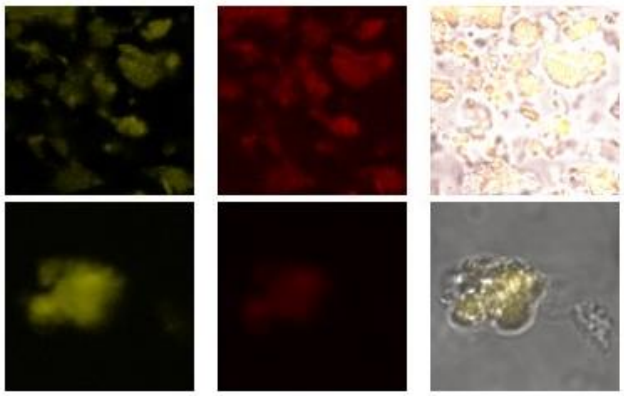

Figure: Top row - The large 500 nm upconversion nanoparticles were suspended in agarose and excited with a 980nm laser. Fluorescence was emitted at lower wavelengths. Bottom row – Attempts to bind nanoparticles to cells after conjugation were unsuccessful. Some nanoparticle uptake into an apoptotic (dying cell) was observed however binding to the cell layers was not achieved.

Hypothesis vs Findings/ Unanswered Questions

The aim was to label nanodisk particles with antibodies and thrombin to target irradiated endothelial cells and compare their binding and thrombotic activity to simple antibody-thrombin constructs that we had tested previously. The nanodisks were created and showed the typical upconversion fluorescence but despite various attempts, labelling of the modified nanoparticles with the antibody was not successfully achieved. One reason for this may have been incomplete or ineffective modification of the nanoparticle with an amine coat to allow antibody binding with a click chemistry approach.

What these research outcomes mean

While overall the project did not reach the expected end at this stage, it did allow construction of the nanoparticles and their initial testing in this approach; experience and training was gained in nanoparticle identification using the confocal microscope. As this work is highly novel, this funding has allowed our first experimentation with these nanoparticles and investigation of the best approaches for labelling. We could not have begun this investigation without the support of the Brain Foundation. Future work will aim to use different surface modifications of the nanoparticle coating during preparation so that a more reliable approach for antibody and thrombin conjugation may be used.