Background to the project: Sonodynamic therapy (SDT), in which tumours are treated with a sensitiser and exposed to relatively low intensity ultrasound resulting in the generation of cytotoxic reactive oxygen species (ROS), offers a minimally-invasive, stimulus-responsive means of treating deep-seated lesions [1]. Similar to photodynamic therapy (PDT) in that the stimulus and sensitiser are harmless, SDT offers the advantage of providing access to lesions that would otherwise be inaccessible to PDT. Since SDT requires a relatively harmless stimulus and a relatively harmless sensitiser it offers very significant advantage over existing site-directed, stimulus responsive ablative therapeutic approaches including stereotactic radiological-, microwave ablative- and high intensity focussed ultrasound-based approaches. In attempts to enhance the latter approaches, particularly with respect to treating off-target, metastatic disease, it has been suggested that inclusion of immunoadjuvants in treatment protocols can enhance therapeutic efficacy [2, 3]. The underlying hypothesis to be tested in this proposal is that NPs can be formulated to co-incorporate a sonosensitiser and an immunoadjuvant.

Objectives of the research project:
Objective 1: Formulation of nanoparticles.
Nanoparticles will be prepared using a poly(lactic-co-glycolic acid) (PLGA)-based platform that we have already developed in our laboratory [4]. Currently this platform co-harboura a sensitising agent together with a nIR imaging agent and has been shown to be an effective sensitiser platform for SDT. In this part of the project we will explore the possibility of co-incorporating adjuvants such as glycated chitosan [3], MPL [5], CpG oligonucleotide [6] and poly (I:C) and modified poly (I:C) [7]. An imaging capability may also be incorporated and this could involve the use of nIR dyes as described previously [4].

Objective 2: Physical characterisation of nanoparticles.
The physical characteristics of formulations, including size, charge, shape, optical characteristics (if an imaging agent is incorporated), stability, payload content and payload release in the presence and absence of a stimulus (ultrasound).

Objective 3: Assess and optimise particle response to ultrasound.
In this part of the project the response of formulations to ultrasound will be assessed, initially in terms of SDT-mediated generation of reactive oxygen species (ROS) and subsequently in terms of ultrasound-mediated cytotoxicity. This will involve identification of optimised ultrasound parameters including
frequency, pulse repetition frequency, treatment duration and power density to enhance the overall ultrasound-mediated cytotoxic effect.

**Methods to be used:**

**Objective 1:** Our original approach has used a solvent diffusion-based approach to the formulation of PLGA-based nanoparticles [4]. Experimentation will involve selection of the appropriate solvent mixtures to facilitate co-solubilisation and subsequent precipitation of the matrix together with the relevant payloads.

**Objective 2:** Physical characterisation will involve the use of dynamic light scattering to assess the size and charge of nanoparticles. Size and shape will be determined using electron microscopy. If a nIR dye is employed as a fluorescence imaging agent, then UV/Vis and fluorescence spectrophotometry will be employed to determine the optical characteristics of the nanoparticles. Payload release will be determined using dialysis-based approaches and payload detection will be performed using UV/Vis spectrophotometry for detection of sensitiser or imaging agent and HPLC for detection of adjuvant release. Stability studies with respect to payload retention in various media including biological fluids such as plasma will be performed in a similar manner.

**Objective 3:** This part of the project will involve determining the response of formulations to ultrasound in terms of (i) generating ROS and (ii) delivering cytotoxic effects in vitro. In the former, colorimetric or fluorogenic ROS traps will be employed to quantify ROS generation during exposure to ultrasound in cell free systems. In the latter, various cell lines will be employed as targets (in both 2D and 3D environments) to determine and optimise ultrasound-mediated cytotoxicity. The latter will be determined using conventional cell viability assays (e.g. MTT, FDA, clonogenic assays).

**Skills required of applicant:** Basic laboratory skills. Experience in tissue culture would be a benefit but not a necessity. Willingness to learn.

**References:**


