Project Details	
Project Code	MRCIIAR26Br Hammond
Title	Microplastics, big problems: dissecting effects on the immune system and skeletal repair.
Research Theme	IIAR
Project Type	Wet lab
Summary	There is a growing awareness that micro- and nanoplastics, which are increasingly prevalent in our environment, pose risks to human health. This project focuses on the role of microplastics on the innate immune system; particularly neutrophils, which are the body's first line of defense and which are known to engulf microplastics. Our team has data showing that neutrophils change their behaviour when exposed to microplastics. We have also identified a novel pro-reparative role for neurophils in stabilising fractures. In this project we aim to identify how microplastics impact skeletal health in development, injury and regeneration using zebrafish as a model.
Description	While we outline an exemplar project, these is scope for the student to tailor the project to some degree as outlined (in the section on student personalisation) below, therefore the objectives could slightly change following the project reading and proposal stage. In this project we will establish the effects of microplastics on skeletal development, homeostasis and repair and on the cells of the innate immune system, with a particular focus on neutrophils. In this project we combine in vitro assays for neutrophil activation, with in vivo work in the zebrafish model and novel coculture techniques established by Hammond and Amulic labs in which we can culture human neutrophils on zebrafish scales (which are miniature bone organs). Co-supervisor Borko Amulic has established that microplastics induce transcriptional changes in human neutrophils, changing multiple inflammatory genes. Similar effects have been demonstrated in the zebrafish model, in which adult zebrafish treated with microplastics (polyethelene and polystyrenes) show changes to inflammatory cytokines. In vitro assays performed in skeletal cells (osteoblasts) show that microplastics can inhibit osteoblast maturation, can induce cellular stress and therefore impact ability of the cells to form bone. Our lab have shown that neutrophils play a key role in mediating fracture repair, and we and our collaborators have identified pathways which could mediate neutrophil to osteoblast interactions. In this project we will bring these findings together to test how microplastics affect skeletal repair using the genetically tractable zebrafish model. Zebrafish offer excellent opportunities to dissect cellular responses to environmental pollutants as they are optically translucent and have reporter lines for many cell types of interest allowing us to interrogate the whole system. Some of the key objectives are to: Using larvae, establish whether mode of delivery (injection, incubation or ingestion) affect microplastic uptake and effects on a) neutrophils and b) skeletal cells (osteoblasts, osteoclasts and chondrocytes) to be done in conjunction with Dr Jon Ball and Matthew Winter in Exeter.

	To test whether acute delivery of microplastics has same effects as chronic delivery on fracture repair. We will test: - Recruitment of neutrophils to site of injury (dynamic imaging of transgenic reporters) - Degree of ROS production and NETosis at site of injury (live imaging of dyes such as CellRox and of transgenic reproters) - Reverse migration/resolution of immune response - Osteoblast recruitment (using transgenic reporters) - Bone formation (live alizarin red and calcein staining) - Quality of bone repair (microCT, nanoindentation, histology, atomic force microscopy) To dissect whether the primary effect on skeletal outcomes is driven by neutrophils or skeletal cells by using coculture systems (human neutrophils on fish bone) by: - Incubating human neutrophils with microplastics then exposing them to skeletal injury on untreated fish scales - Incubating untreated human neutrophils with injured fish scales treated with microplastics prior to injury. For both assays we will measure neutrophil migration, activation and skeletal repair. The project as a whole will give the student a highly desirable cross disciplinary skill set, and should be highly publishable
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