Osteo-chip: An *in vitro* model to recapitulate the human osteoarthritic joint

Background

Osteoarthritis (OA) is the most common musculoskeletal disease affecting nine million individuals in the UK alone. A chronic disease, OA develops with a pathology characterised by cartilage loss, synovial inflammation, subchondral bone sclerosis and cyst formation, and osteophytosis. This pathology causes pain and a loss of a range of movements and ultimately results in joint failure (Dieppe *et al.,* 2005).

Factors contributing to the development of OA include inflammation, trauma, ageing, obesity and genetic disposition (Heidari *et al.,* 2011). Treatment for OA targets three main areas: pain relief, restoration of function and prevention of disease progression. The development of new therapies not only requires an understanding of the pathophysiology of OA, but also the associated biomechanical, inflammatory, genetic and environmental risk factors (Hunter, 2011).

In the investigation of treatments for OA, a range of humanised mouse, rat and guinea pig models have been developed to study different combinations of output measures such as pain, synovitis and cartilage degeneration, but these currently are not standardised making it difficult to compare results between studies. There is inter-laboratory variability even when the same models are used, making reproducibility of findings difficult (Vincent *et al.,* 2012). Differences in animal and human physiology, particularly with regard to the immune system can impact on the translation of preclinical findings to the clinic. In addition, the load bearing mechanics of joints in animal models which are quadrupeds presents further potential hurdles to translation.

Animal models of OA make it possible to study the whole joint structure with the majority of models focusing on the knee joint. These fall into two main categories: those that model painful behaviour (typically intra-articular monosodiumiodoacetate injection (MIA) and surgical models) and those that model disease progression through cartilage degradation (spontaneous and surgical models). Models recapitulating other aspects of the disease are less well developed – for example, there is a scarcity of papers published on bone changes in OA models and changes in other tissues including, the synovium, capsule, joint adipose tissue (infrapatellar fat pad), muscles, ligaments and tendons.

A typical surgical model of joint disruption is carried out for 12 weeks following surgery (with mice operated on at ten weeks of age) and thus a minimum of six months per experiment is required (Miller *et al.,* 2012). Joint damage over the duration of the experiment can be extensive, leading to ambulatory deficiencies in the animals and in addition, the rodents experience high levels of chronic pain associated with the disease.

The welfare concerns, long duration and significant costs of running these whole animal models, along with their limited translation to the human disease, means that academic and industry researchers are seeking higher throughput *in vitro* models for both chemical and surgical induction (as assessed by histology or biomarkers).

This CRACK IT Challenge aims to develop an advanced *in vitro* model of the human osteoarthritic joint that will:

- Reduce the number of animals used in preclinical OA drug development and academic research by providing an alternative to the animal models.

- Improve the predictivity of preclinical modelling to humans through more extensive use of human tissues and/or cells.

- Provide a robust and reliable tool for development of potential disease modifying OA drugs.

3Rs benefits

Arthritis is an active area of preclinical study with a range of animal models used in academia and industry. No model replicates the human condition precisely, either in terms of pathogenic mechanism or response to treatment, and there is no consensus as to which model best recapitulates the human disease. Often researchers will run multiple models at the same time to assess reproducibility, therefore increasing the number of animals used (Ashraf *et al.,* 2014). Moreover, if an OA model is being used to study pain or test analgesics, the experiment may require researchers to not administer post-operative analgesics, resulting in pain which may last for up to ten days (Kelly *et al.,* 2013).

Chemical and surgical induction of OA in animals (MIA, meniscal transection (MNX), disruption of the medial meniscus (DMM) and anterior cruciate ligament transection (ACLT)) causes sustained and chronic pain, which progressively worsens with study length, typically 14-28 days for MIA injection in rats (Kelly *et al.,* 2013), but up to 16 weeks for DMM surgery in mice (Miller *et al.,* 2012). This ongoing pain and concomitant joint destruction significantly impairs animal movement and can have negative effects on other normal behaviours, such as feeding and nest building.

Although spontaneous models of OA do not involve any post-operative pain, there is significant variability in the development and severity of disease and models have to be used for significantly longer periods for example up to three years for Dunkin Hartley guinea pigs (McDougall *et al.,* 2009) and 12 months for STR/ort mice (Kyostio-Moore *et al.,* 2011).

Studies for preclinical arthritis models often require up to five groups per study with at least two treatment arms, using up to 40 animals per study. For a pharmaceutical company carrying out these types of studies, both in-house and outsourced, the total numbers of animals used per year can be in excess of 700 animals.

If solved, this Challenge will significantly reduce the number of animal models used in the study of OA and provide more human-relevant information such as novel biomarkers that may be used to refine any animal studies that are still needed through using earlier and more humane endpoints.

Need for collaboration

To solve this Challenge collaboration between scientists from different disciplines will be required including, but not limited to, expertise in:

- Tissue engineering.
- iPS cells/cell lines/human tissue.
- Tissue banking- research ethics, regulatory and translational pathways.
- Bioengineering- scaffolds, fluid dynamics/flow, micro-physiological devices.
- Biology and disease expertise - the immune system, OA, joints, biomarkers and pain expertise.

Overall aim

An *in vitro* model to recapitulate the human osteoarthritic joint that will:

- Provide a device based on a human tissue or multiple human cell type co-culture system for research and drug development in OA.

- Recapitulate the human disease being able to model early and late stage disease if possible.

- Be amenable to drug discovery and development studies and provide mechanistic insight into disease development (including pain development through biomarker detection). The device should be medium throughput and compatible with standard equipment and measurement platforms (e.g. microscopy, biochemical analysis, FACS, robotics).

Key deliverables

A device combining relevant multiple cell types (e.g. chondrocytes, synovial fibroblasts, subchondral bone, infrapatellar fat pad and immune cells) which give appropriate responses to stimuli (e.g. mechanical and chemical), mimicking human OA.

Phase 1

- Development of a cell culture platform that produces a mixed stable cell culture of cell types that represent the key components of the human joint. These should include:

    - Synoviocytes – type I and type II
    - Osteoblasts
    - Osteoclasts
    - Chondrocytes/cartilage or cartilage-like matrix
    - Adipocytes
    - Immune cells.

- Demonstration of cell phenotype stability and viability for at least (72 hours) as indicated by appropriate biomarkers/readouts.

- Robust plans to deliver Phase 2 of the Challenge including commercialisation and dissemination.

Phase 2

Essential:

Development of an *in vitro* human OA model that:

- Recapitules the (3D architecture and) physiology of the OA joint evidenced by histological evaluation.

- Provides measurable cartilage matrix and inflammatory responses as evidenced by:

    - Cartilage degradation and regeneration readouts
    - Cytokine readouts
    - Cell activation markers (e.g. immune cell phenotype).

- Provides physiological responses to stimuli and disease states that act as measures of efficacy and toxicity for new treatments (including both small molecules and biologics).

- Achieves a throughput level that permits the screening of ten candidates or more per week.

- Delivers demonstrable improvements on the current *in vivo* and *in vitro* models.

- Improves biological relevance, as evidenced through data demonstrating predictivity.

- Guarantees a robust and ethical supply of source cell material.

- Provides mechanistic insight into:

- Disease progression
- Drug mechanism of action
- Toxicity.

Desirable:

- The ability to model diseased and healthy states.
- A flow system containing synovial fluid or an equivalent.
- The addition of shear stresses and forces to mimic mechanical movement of the joint.
- Measures of pain (biomarkers and/or electrophysiological).

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Sponsors will provide:

- Expertise in OA and *in vitro* models including specifications for an *in vitro* model which is fit for purpose for drug testing in an industry setting.
- A reference training compound set and associated *in vitro* and *in vivo* preclinical and clinical data as available.
- Reagents and appropriate controls.
- Analytical support.
- In-house testing using the system to test transferability and reproducibility of the *in vitro* model.

Duration

Phase 1: six months, Phase 2: Up to three years

Budget

Phase 1: £100k, Phase 2: £1million

Co-funded with Arthritis Research UK and the Engineering and Physical Sciences Research Council.

Sponsors

GlaxoSmithKline.

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