Raman transmission spectroscopy (RaTS) for objective monitoring of rheumatoid arthritis progression in rodent models

Overall aim

The aim of this Challenge is to develop and validate a handheld device for objective monitoring of rheumatoid arthritis (RA) progression in conscious rodents (either restrained or unrestrained).

While it is expected that Raman transmission spectroscopy will be the technology needed to solve the Challenge, applications involving other approaches (such as Surface-enhanced Raman spectroscopy) are also welcome, provided they do not require the use of transgenic animals, do not substantially complicate the study design or create more burden on the experimental animals (e.g. multiple injections of dyes or nanoparticles).

Duration

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

Budget

Phase 1: £100k, Phase 2: £750k

Sponsors

Galvani Bioelectronics, GSK

Co-funders

Engineering and Physical Sciences Research Council (EPSRC)

Background

RA is a chronic inflammatory autoimmune disorder characterised by persistent joint inflammation which leads to cartilage and bone damage (Picerno V *et al*., 2015). Over 400,000 people in the UK have RA (Symmons D *et al.*, 2002), and the associated cost to the UK economy alone is estimated to be £3.8-4.8 billion per year (National Collaborating Centre for Chronic Conditions, 2009). Various pharmacological treatments are being developed to alleviate symptoms and slow disease progression with early, aggressive intervention yielding the best results.

One of the biggest challenges in the study of RA both in humans and animal models is the imaging of the affected joints. Several different imaging approaches are used, each with benefits and limitations.

Indirect *in vivo* measurements

In animal models, the induced joint pain and inflammation in conscious rodents can only be monitored indirectly by detecting paw withdrawal in response to noxious or mechanical stimuli and measurements of joint swelling using callipers or a plethysmometer. Under terminal anaesthesia, more direct measurements are possible, such as drawing synovial fluid from the inflamed joints for biochemical analysis and *in vivo* imaging such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI).

Direct *ex vivo* measurements

More detailed measurements of joint damage are currently performed *ex vivo*.

- Infrared vibrational spectroscopy permits assessment of the loss of structural proteins in cartilage (Croxford AM *et al.*, 2013) and the loss of minerals in the cartilage and bone matrix (Lim NSJ *et al.,* 2011). Two types of infrared vibrational spectroscopy have been applied to *ex vivo* joints: absorption and Raman spectroscopy. Raman spectroscopy has the advantage over the absorption spectroscopy in that it can detect a wider spectrum of biomolecular changes associated with early cartilage degradation (Lim NSJ *et al.*, 2011) and can be used in live animals (de Souza RA *et al*., 2014).

- Ultrasound, X-ray/CT, MRI/ MR spectroscopy and optical coherence tomography, have all shown promising results in calculating volumes of inflammation and bone/cartilage deformation and demineralisation, with some limited insights of the chemical environments. However, they have low sensitivity and specificity in detecting biochemical changes in the inflamed joint, as well as problems with image resolution, high costs, requirement for specialised facilities, low throughput, use of anaesthetics, and complex image analyses to extract arthritis-specific information (Kirwan JR, 1999; Marenzana M, 2015).

- Fluorescence imaging *in vivo* is useful to identify some molecular biomarkers; however, these biomarkers are typically few and limited, and have no specificity to the early cartilage destruction. The technique also requires multiple injections of the fluorescent dyes or quantum dots/rods either systemically or intra-articularly; resulting in heterogeneous tissue distribution (Chen H *et al*., 2014) and clearance (Yong KT *et al.*, 2009; Liu JM *et al.,* 2013). The variability observed in the pharmacokinetics hinders the longitudinal comparison of measurements taken within individual animals.

- Surface-enhanced Raman spectroscopy has a similar limitation to fluorescence imaging, as it requires multiple injections of the metal nanoparticles in the joints of interest. Furthermore, addition of the biocompatible coatings (such as silica or polyethylene glycol) reduces the efficacy of surface plasmon effect (Andreou C *et al*., 2015). Despite these limitations, the metal nanoparticles exhibit good long-term stability during three week *in vivo* measurements (Dinish U *et al.*, 2015) and its higher signal to noise ratio provides a promising alternative to Raman spectroscopy.

The use of Raman transmission spectroscopy for *in vivo* monitoring of RA progression in rodents is currently limited by:

1. Selection of the optimal infrared laser wavelength for minimal amount of absorption in the skin and bone.

2. Optimisation of the optics for maximal amount of light capture by the spectrometer.

3. Advanced analysis of the spectral data using adaptive algorithms.

(1) and (2) can potentially be resolved using advances in lasers and optical detectors. While most of the existing Raman lasers operate in the 700-800 nm wavelength range, minimal amount of absorption in the skin tissue requires the use of lasers in the 1000-1100 nm range (Bashkatov A *et al*., 2005). Recent advances in optics using no-slit and multi-slit spectroscopy provide a considerable increase in light capture (Maher JR *et al.*, 2014; Barnett PD, 2016; Gooding EA *et al.*, 2016).

Improved Raman spectroscopy developed through this CRACK IT Challenge could provide richer data regarding the RA disease progression (such as the hypoxia, cartilage erosion, and bone erosion) as compared to the present X-ray/micro-CT approach that evaluates only the bone erosion evident in the later stages of untreated RA, while the onset of cartilage damage appears in the early stages of disease. This could provide a more sensitive metric for evaluating the therapeutic treatments and provide more relevant data to clinical manifestations of the disease.

3Rs benefits

Induction of RA in animal models causes joint swelling and pain which, although closely monitored and countered by the administration of analgesics, can cause significant pain and distress (Hawkins P *et al.,* 2015). Currently, the collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA) are the most widely used rodent arthritis models in research in academic and industrial laboratories. The CIA model is induced by immunisation with type II collagen (prevalent in articular cartilage), causing inflammation of the joints. The pathogenesis observed in rat CIA makes it more clinically-relevant when compared to the AIA model.

GSK and Galvani Bioelectronics have experience of conducting efficacy studies to evaluate the effect of potential therapies in the rat CIA model, typically performing research on over 300 CIA rodents a year. With over ten of the world's major pharmaceutical companies having active RA programmes (GlobalData, 2017) and a large academic research sector, application of the technology developed through this Challenge could have significant impact on the numbers and welfare of animal models used.

For X-ray and micro-CT evaluation of the bone erosion, animals are evaluated *ex vivo* at 21 days post CIA induction, as the bone changes are not evident at earlier timepoints. Successful delivery of this Challenge would allow the detection of the cartilage and bone damage at earlier timepoints (e.g. at seven and ten days post CIA induction) and subsequently at an additional two to three time-points during administration of anti-arthritic therapy (e.g. at 14, 21 and 28 days). Such improved sensitivity would allow, for the first time, the longitudinal monitoring of a time course of the cartilage and bone healing during the administration of anti-RA treatments. This would also permit within animal assessment which will allow the application of advanced mixed-effects population analyses to further reduce sample size per group.

In summary, Raman compared to X-ray/micro-CT methods will offer these 3Rs benefits:

- Reduced animal numbers (by up to a factor of ten) due to longitudinal measurements and within-animal repeated-measured design.
- Refinement of the animal studies based on shorter study duration and reduced severity of end points (especially shortening the late disease duration) due to higher sensitivity of the cartilage erosion metric during early RA progression and recovery, as compared to the current ability to detect the bone damage no earlier than day 21.
- More data-rich information generated regarding the cartilage and bone damage and healing from each animal, further reducing the numbers needed.

Need for collaboration

The proposed project requires multi-disciplinary expertise in the following fields:

- Infrared lasers
- Advanced optics (e.g. Raman spectroscopy)
- Mechanical engineering
- Advanced signal processing
- Animal models of RA (in Phase 2 only)

Such expertise is unlikely to be found in a single department or a single university/company.

Key deliverables

Phase 1 deliverables

- Initial validation of the selected optical approach.
- *Ex vivo* testing of depth penetration and signal-to-noise in phalangeal and tarsal joints using rodent cadavers.
- Technical specifications (if using the Raman transmission spectroscopy device).
    - laser wavelength in the 800-1100 nm range.
    - no-slit or multi-slit aperture or other approaches for beam forming.
- Minimum performance requirements relative to current state of the art (if using the Raman transmission spectroscopy device).
    - Twice the depth penetration through the bone relative to the 700-800 nm laser.
    - Five times increase in the signal-to-noise relative to slit-based Raman transmission spectroscopy.
- Robust plan for Phase 2.

Phase 2 deliverables

- Development and fabrication of the handheld device suitable for taking quick (< 1 minute) optical measurements.
- Full validation of the handheld device for detecting the optical signal-to-noise in rodent phalangeal and tarsal joints.
- Acute testing under anaesthesia.
- Repeated testing in conscious animals every two days for 14 days prior and after the initiation of the collagen-induced arthritis (CIA).
- Final performance requirements, when comparing the pre-CIA and post-CIA measurements:
    - At least 10% decrease in the amplitude of amide peak (loss of structural proteins in the cartilage) as a marker of cartilage damage, if using the Raman spectroscopy.
    - At least 10% decrease in the phosphate/carbonate peak (loss of structural minerals) as a marker of bone damage, if using the Raman spectroscopy.
    - At least 10% decrease in the cartilage and/or inflammatory markers, if using non-Raman optical detection methods.
- Initiate activities toward device commercialisation.
- Identify the commercial partner for mass fabrication.
- Negotiate the intellectual property licensing rights with the commercial partner.

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

Galvani Bioelectronics and GSK will participate in technical discussions and offer advice on the technology requirements, applications and commercialisation. If the project achieves the targeted technical requirements, the Sponsors will undertake evaluation of prototype devices and validation of the final product to aid the product's commercialisation.

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