STRATIS: Soft Tissue Regeneration after Traumatic Injury

Overall aim

The aim of this Challenge is to produce a human-relevant and high throughput *in vitro* or *ex vivo* platform that recapitulates the complex structures of skeletal muscle and the pathology of significant injury to them. The platform must offer a model for novel wound therapeutics and approaches to restore form and function after significant soft tissue injury.

Duration

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

Budget

Phase 1: Up to £100k, Phase 2: Up to £800k

Sponsor(s)

Dstl

Co-funders

Dstl and EPSRC

Background

Volumetric muscle loss (VML) is the substantive loss of skeletal muscle and impairment of regenerative capability that can occur as a result of trauma, surgery or degenerative disease. Wounds of this nature are complex, involving skin, muscle and associated structures such as microvasculature, nerves and the extracellular matrix. In addition, volumetric wounds caused by trauma – particularly blast and ballistic injuries – can evolve, resulting in loss of tissue beyond that lost as a result of the primary insult. VML wounds often exhibit sub-optimal healing, with a poor response to reconstructive efforts and resulting in long-term scarring. Current strategies for reconstruction of VML wounds focus on preservation of the tissue that remains, with a return of full function often unachievable. Loss of form and function, particularly in the limbs, manifests as severe permanent deficits that adversely impact on physical and mental rehabilitation.

A variety of clinical regenerative and tissue engineering strategies designed to replace soft tissue are under investigation, including novel materials, either seeded with cells or coated with materials that stimulate endogenous tissue growth (O'Brien, 2011; Zhu *et al.,* 2019). Physiological and functional integration of these constructs is challenging, and it is likely that therapeutic interventions to stimulate the survival and proliferation of existing structures alongside tissue reconstruction will be required. Due to the complex interplay between the structures within soft tissue during healing and regeneration, preclinical models to evaluate reconstructive and regenerative approaches for VML need to include muscle, vasculature and where possible, skin.

In vivo models

In vivo VML models have a full complement of cell types and tissue architecture and allow long-term studies which is critical when looking at regenerative responses. However, there are differences in animal and human pathophysiology associated with wound healing, particularly in rodents, which makes translation to humans difficult (Ansell *et al.,* 2012; Grada *et al.,* 2018). For example, mice have an additional muscle layer that is mostly absent in humans – this enables their skin to move independently of the deeper tissues and allows wound healing *via* contraction rather than the re-epithelialisation that is seen in humans. *In vivo* models are usually not suitable for high throughput screening of therapeutic candidates, and the severity and duration of experiments which involve creating surgical wounds create ethical concerns.

Ex vivo models

Ex vivo approaches including explant and perfused systems using animal and human tissue are being explored for both skin (Mendoza-Garcia *et al.,* 2015; Ternullo *et al.,*2017) and to a lesser extent muscle (Park *et al.,* 2012; Smith and Meyer, 2019). These approaches are advantageous in terms of inclusion of relevant cell types and tissue architecture and are amenable to wounding and the testing of novel therapeutics (Mendoza-Garcia *et al.,* 2015). However, they are limited in the length of time they can be kept physiologically viable.

In vitro models

Commonly-used 2D *in vitro* platforms only model simple wounds such as the scratch test (Cory, 2011) and therefore do not recapitulate the complexity of VML or translate well to the clinic (Ansell *et al.,* 2012; Grada *et al.,* 2018). These models are cheap, high throughput and can include primary human and animal skin or muscle cells but usually only employ a limited number of cell types, lack tissue architecture and do not support functional or mechanical readouts or manipulations (Cory, 2011).

Complex 3D *in vitro* models of skin and muscle have been developed that are amenable to long-term culture, contain multiple cell types and tissue architectures, and retain the ability to support higher throughput studies and functional readouts (Afshar *et al.,* 2020; Matei *et al.,* 2019). The availability of models however, that contain innervated muscle and skin and are applicable to the study of wounding, wound healing and regeneration is limited. The Horizon 2020 MyoChip project aims to

develop an innervated and vascularised muscle-on-a-chip model but it is unclear what functional outcomes will be achieved and whether it will be suitable for wounding and other mechanobiological investigations.

This Challenge aims to build on the emerging capabilities in the area of complex 3D cultures and regenerative medicine to develop a human cell or tissue-derived 3D *in vitro or ex vivo* model of vascularised skeletal muscle, with additional tissue architecture, for example, skin and/or innervation that replicates the mechanical properties of native tissue and is suitable for 'wounding' and the study of subsequent regenerative processes.

3Rs benefits

Models of muscle loss are invasive and often involve surgically exposing the muscle of interest (e.g. tibialis anterior or biceps femoris) and creating a defect using a biopsy punch before closing the wound and allowing the animals to recover from the anaesthesia. These models cause pain and distress, even when analgesics are used, and the welfare of the animal is closely monitored. A typical study for testing engineered tissues and supportive materials and biological therapeutics uses five experimental groups (six to eight rats per group) and a control group (16 rats).

The development of a more complex *in vitro,* or *ex vivo,* model of wounding to muscle could replace a significant number of animal models in the screening and testing of regenerative therapeutics by providing a higher throughput option for tissue engineering strategies using, for example, small molecules or gene therapy candidates. This could improve the early evaluation of approaches and, optimise translation.

The market for advanced wound care products is extensive and expected to grow from $10.8 billion USD in 2019 to $15.56 billion USD by 2027 (Fortune Business Insights, 2020). Within this market, the development of a complex *in vitro* or *ex vivo* model of wounding and wound healing could also be adapted for other acute wounding modalities, for example, burns, ischaemia or chronic wounds, using appropriate tissue or primary cells. This has the potential to replace animals typically used in such studies that involve simple incisional/excisional wounds to more complex crush injuries, involving electroporation, myotoxin administration or ischaemia.

Key deliverables

- The aim of this Challenge to develop a human cell or tissue- derived vascularised *in vitro* or *ex vivo skeletal* muscle construct that recapitulates the architecture of the native tissue and is amenable to wounding and studying wound regeneration. The final model should use human-derived cells or tissue, though animal-derived cells and tissue may be used in development. The model must be amenable to the measurement of changes in function (e.g. muscle contraction) and be compatible with exposure to strain or shear stress for mechanobiological study.

Phase 1 deliverables

- Initial development of a stable *in vitro* human skeletal muscle construct or an *ex vivo* perfused culture system that includes:

    - Cell types that represent the key components of skeletal muscle in 3D. For an *in vitro* model this should be differentiated myotubes and early evidence that the model can support endothelial cells formed into vascular-like networks.

- Basic morphological and functional characterisation of the model:

    - Cell or tissue viability and morphology (e.g. multi-nucleated and striated myotubes).

    - Appropriate cell marker expression (e.g. myoblast determination protein 1, myogenin and myosin heavy chain) with confirmation of differentiation as applicable.

- Demonstration and evidence of cell phenotype stability and viability for at least seven days.

- Applicants should also include robust plans to deliver Phase 2 of the Challenge including commercialisation and dissemination.

Phase 2 deliverables

Essential:

- Establish the ability to create a repeatable wound. This should cause a defect of appropriate size and/or severity such that it does not heal or close spontaneously over the duration of the experiment (at least seven days).

- Extensive functional (e.g. response to electrical and biochemical stimulation) and morphological characterisation of the model in the wounded and unwounded state.

- Evidence that the muscle construct is amenable to the addition of skin layers or other tissue structures in order to investigate the interplay between muscle and other cell types during injury and regeneration.

- Evidence that the model improves on current *in vitro/ ex vivo* systems (e.g. Jones *et al.,* 2018).

- Development of a series of methods to address key outcome measures of wound regeneration and functional output, and validation of these methods against *in vivo* and clinical data where available (e.g. Chao *et al.,* 2019).

- A supporting physical structure for the model (e.g. cell culture plate or ‘chip’) that mimics, as far as is practicable, the physical properties of soft tissue in order that a mechanical load, for example a shock wave, can be applied to the model.

- Assessment of the inter/intra-laboratory reproducibility of the model.

Desirable:

- Evidence the model is physiologically-stable for longer than seven days after wounding.
- Incorporation of a flow system of biologically relevant fluid through the vascular structures.
- The development of methods to investigate the effect of beneficial (e.g. probiotic) or pathogenic contamination.

The Dstl Regenerative Medicine project funds approaches to understand and treat significant soft tissue injury as a result of combat injury including the preservation and proliferation of existing structures (Spear AM *et al.,* 2018). This Challenge has come from the UK Defence Regenerative Medicine research project and, while the model developed under this Challenge will have broad applicability in the wider wound healing research area, concepts of use within the Defence Medical context are outlined below and should be considered by applicants:

- The model could be used to screen potential therapeutic candidates for the preservation of cells and/or tissue and potentiation of regenerative responses early after injury with an assessment of how this affects later therapeutic and reconstructive approaches.
- The model may be used to investigate the specific effects of the energy associated with injurious mechanisms of interest to Defence Medical (e.g. blast and ballistics) on muscle tissue and how this affects wound progression and regenerative responses over time.
- The presence of functional vascular structures within the model could allow facets of the systemic response to severe injury to be overlaid on the model of local injury.

Sponsor in-kind contributions

- Dstl will provide staff time to support the competition and resulting projects.
- Dstl will provide context to the requirement for this model, including the clinical point of view from partners at the Royal Centre for Defence Medicine. Access to stakeholders and end-users can be provided and Dstl will facilitate networking (showcasing of the work, publication, conference attendance etc) as required.
- Where appropriate, Dstl will facilitate engagement with other parts of the regenerative medicine programme, including with projects that might make use of any such model developed under this Challenge.
- Dstl will also provide facilities in which to test the model under relevant injurious mechanisms, or for the purposes of testing inter-laboratory variability, if appropriate.

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