Proof of Concept

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Title of Study:	**A Fat Chance of Curing Faecal Incontinence**
Aims and Objectives: (max 400 words)	Better long-term outcomes following anterior sphincteroplasty might be achieved by improving the union that occurs between the overlapped muscle. This might be attained through increased mechanical strength of the union with the use of prosthetic meshes or a reduction in tissue ischaemia by increasing vascularization. Given current concerns about prosthetic meshes in pelvic floor surgery, and the high risk of mesh infection in the anodermal region, the preferred strategy is to mitigate the effects of ischaemia on muscle repair. Unprocessed autologous lipoaspirate has been used in other tissues to reduce scarring and increase vascularization, which is thought to relate to resident mesenchymal stem cells in the adipose tissue [1,2]. Large quantities of adipose tissue are accessible from the perianal fat adjacent to the anal sphincter complex and could be readily harvested during anterior sphincteroplasty. We have previously shown purified populations of adipose-derived mesenchymal stem cells attach readily to the surface of TIPS microparticles [3]. Furthermore, TIPS microparticles have been proven to be retained at the site of intermuscular implantation in pre-clinical *in vivo* models. Combining clinically ready TIPS microparticles with an adipose concentration system already in clinical use and applying this to an established surgical procedure could provide a novel and readily translatable biomedical engineering approach to improving continence. It is hypothesised that refined adipose tissue containing mesenchymal stem cells isolated, mixed with TIPS microparticles, implanted and retained between the overlapped sphincter muscle will enhance union between the joined muscle, leading to improved clinical reproducibility and long-term outcomes. The aim of the pilot study is to establish the feasibility of developing a point-of-care product for the isolation, concentration, and targeted delivery of autologous cells from raw human lipoaspirates that can be retained between the overlapped sphincter muscle in order to facilitate quality healing. The objectives of the project are: 1. Devise a simple and efficient protocol for the preparation of refined and concentrated adipose tissue that can be combined TIPS microparticles with minimal sample handling in a closed processing system. 2. Determine whether cells present in the isolated adipose tissue bind to the surface of TIPS microparticles before delivery and if they do, whether the cells display multi-lineage potential in vitro. [1] Huang *et al.* Ann Plast Surg. 2012;69(6):656-62.[2] Pallua *et al.* J Plast Reconstr Aesthet Surg. 2014;67(8):1033-7. [3] Parmar *et al.* Tissue Engineering Part C. 2015;21(4):404-12.

Description of research work: (max 400 words)	The project was divided into 3 work packages: WP1: Instead of using AdiPrep® adipose concentration system to prepare highly refined concentrated adipose tissue from a sample of lipoaspirate, we opted to use a human adipose tissue digestion protocol described by Cytori. The reason for this was simplicity of procedure, access of the components required, and availability of reagents needed for tissue digestion. Surgically resected lipectomy samples were from surgeons at the Royal Free Hospital. The tissue was manually dissected and minced into small pieces for enzyme digestion. Preliminary experiments investigated the mixing of TIPS microparticles with the adipose tissue to determine whether pre-treatment of the microparticles was required to facilitate mixing and cell attachment. The need for this was evident so we used a pre-treatment method established in our lab for this purpose. WP2: Optimized mixing of the stromal vascular fraction with the TIPS particles was investigated using two mixing approaches. Very few cells had attached within 15 minutes incubation, so longer the samples were incubated for longer intervals. This aspect of the technology needs further attention to optimize the surface of the microparticles to improve cell attachment. There are several ways this could be achieved, either through functionalization to attach ligands specific for the cells of interest, or through non-specific physicochemical modification of the polymer. WP3: Due to the limited amount of adipose tissue samples available during the duration of the project we were unable to obtain a sufficient number of cells to perform immunostaining to determine the presence of adipose-derived mesenchymal stem cells attached to the surface of TIPS microparticles. We are currently using commercially available adipose derived stem cells as a surrogate cell type for this purpose and will include lipectomy tissue samples into this study as and when the tissue becomes available.
Key findings:	1. Attachment of stromal vascular faction cells isolated from digested adipose tissue to the surface of TIPS microparticles is technically feasible. 2. Revision of the attachment protocol is required to achieve optimal cell attachment to the particles within a shorter incubation period.
Outputs: e.g. publications, new links etc.	The project has delivered promising preliminary data by demonstrating this novel approach for the handling and delivery of stromal vascular fraction isolated from digested adipose tissue is technically feasible. In doing so, the project has opened up new collaborations with industry (Cytori) and academia, especially between UCL and University of Leeds. The collaboration between Prof Jayne and Dr Day has resulted in the co-supervision of a new PhD student working on a related project at the University of Leeds. This collaboration has also led to the submission of a funding application to the Medical Technologies Innovation Centre IKC POC scheme (Adipose derived regenerative cells (ADRCs) to prevent anastomotic leak following gastrointestinal surgery; [PI D. Jayne].

PROPOSED NEXT STEPS

Follow on funding Strategy:	We intend to validate our preliminary data with further replicates. This will be conducted via the new collaborations established through the PoC project. Once we have a robust data set we plan to apply for further funding. The IMPRESS network as well as a number of internal UCL funding schemes are available that could provide resources needed to obtain further data that will strengthen proposals for a longer term project. Potential sources of longer term funding will be via MRC responsive mode calls, Wellcome Trust Technology Transfer and NIHR i4i Late Stage Development Scheme.
Future research work plan:	We hope to continue working with the IMPRESS network on this project. The technology being investigated offers a tremendous opportunity for a truly interdisciplinary research project involving engineers, biologists, material scientists and industry. We will draw on the wealth of expertise and support available from IMPRESS network to fulfil this opportunity.

We encourage you to use diagrams and figures to illustrate your work and you may also submit additional material such as videos.