Cartilage Engineering From Autologous Fat For Oro-Facial Deformity Reconstruction - Focus On The Nose

12-month progress report submitted to BAOMS

Mr Atheer Ujam
PhD student

Primary supervisor: Professor Patrizia Ferretti
UCL Great Ormond Street Institute of Child Health

Introduction

The nose has a fundamentally important role in facial appearance and breathing, therefore congenital or acquired nasal deformity can have an overwhelming affect on the patient's well-being. Patients often require extensive, repeated surgical procedures commonly using autologous cartilage and bone grafts that result in additional morbidity. Rib cartilage is widely used for this purpose as it provides sufficient volume of cartilage to reconstruct all aspects of the nose (dorsal graft, columella, septum), but is far from ideal. Morbidity at the chest donor site includes:

1. Scarring
2. Pain
3. Chest wall deformity
4. Risk of pneumothorax

Bioengineering cartilage tissue using autologous stem cells would allow surgeons to avoid such invasive procedures and eliminate donor site morbidity. We will exploit the differentiation potential of paediatric adipose tissue-derived stem cells (pADSCs) and develop conditions for generating mature cartilage from pADSCs.

This project aims to engineer a cartilage-like product that can be inserted into paediatric patients with nasal deformity as a result of tumour resection, post traumatic or congenital deformity. This novel product will reconstruct normal function and aesthetics of the nose by utilizing stem cell technology to generate missing nasal cartilage. This product will replace current surgical techniques that use costochondral rib grafts to replace missing or deformed nasal cartilage. 3D printing technology will be used to fabricate the nasal product.

Specific Aims are:

- To exploit the chondrogenic capability of human adipose tissue derived stem cells (ADSCs) for autologous cartilage engineering.

- To develop a novel custom-made product using 3D printing to reconstruct nasal septum.

Specifically, during this grant award I have done the following:

1. Isolated and expanded new ADSC lines.
2. Investigated the behaviour of ADSC in 3D using micromass models to determine cartilage maturation and their extracellular matrix composition.
3. Assessed the level of maturation of chondrogenically induced ADSCs in different matrices to select conditions for scaling up cartilage production.
4. Developed a biodegradable product design using 3D printing.

1. Isolation and expansion of new ADSC lines

In the last 12 months, I have established three new stem cell lines isolated from adipose tissue provided by my clinical academic supervisor Mr Bulstrode (Fig.1). These are collected from consented patients with ethical approval. Derived stem cells were grown and differentiated following protocols used routinely in the Ferretti laboratory (Fig.2).

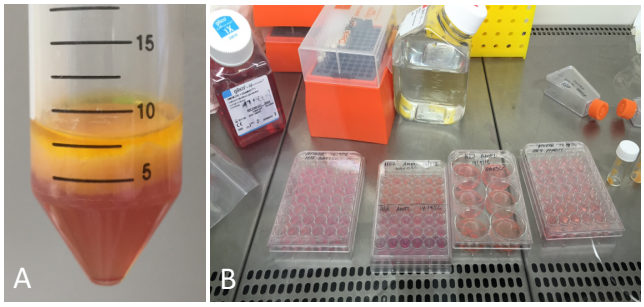

Figure 1. (A) Lipoaspirate washed before seeding onto culture plates. (B) Tissue samples seeded into wells and covered with media to allow cell isolation and

I have demonstrated reproducibility and low patient-patient variation in cell behaviour through tri-lineage staining quantification. Cells are plated onto 24 well plates and once confluent are differentiated using either chondrogenic, osteogenic or adipogenic media.

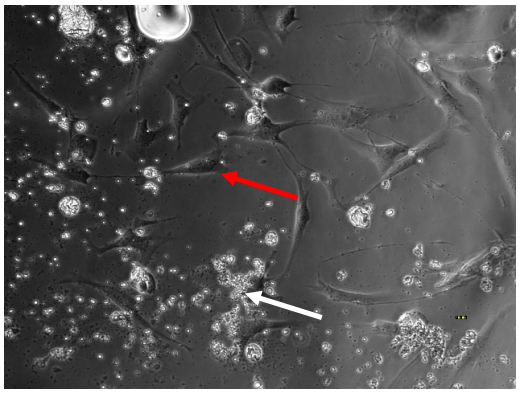

Figure 2. ADSC (red arrow) can be seen migrating away from adipose tissue (white arrow).

At 3 weeks, the experiment is terminated and the cells are fixed with 4% paraformaldehyde. The wells are then stained using established protocols and then examined under the microscope to confirm differentiation has taken place (Fig.3).

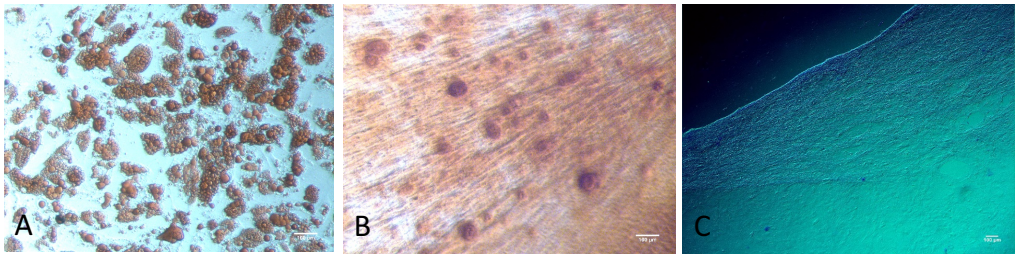

Figure 3. Trilineage differentiation experiment. (A) Oil Red O stain for adipogenic differentiation, (B) Alizarin red for osteogenic differentiation and (C) Alcian blue staining for chondrogenic differentiation.

2. Investigating the behaviour of ADSC in 3D using micromass models.

Chondrogenic differentiation of ADSC on monolayer cultures to generate primitive cartilage is well established and published. The challenge in cartilage regenerative techniques is to develop cartilage in 3D and that is mature enough with favourable mechanical properties that will allow clinical use. Micromass studies provide a model to investigate the behaviour of ADSC in 3D and in particular how extracellular matrix is deposited and its composition.

Micromasses were cultured for 6 weeks ($1x10^5$ cells) in both chondrogenic differentiation media and normal control media. All micromasses were fixed with 4% paraformaldehyde and kept at 4° cold room (Fig 4).

Initial experiments were run to identify cell nucleus using hoischt nuclear staining (Fig 5).

Following this, the micromasses were investigated using immunocytochemical staining to identify both intracellular and extracellular proteins – collagen 2 and vimentin. This enabled more detailed investigation into the 3D structure of micromasses and if indeed cartilage growth was taking place.

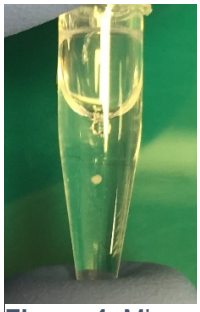

Figure 4. Micromass appearance. Approx diameter is 600μm.

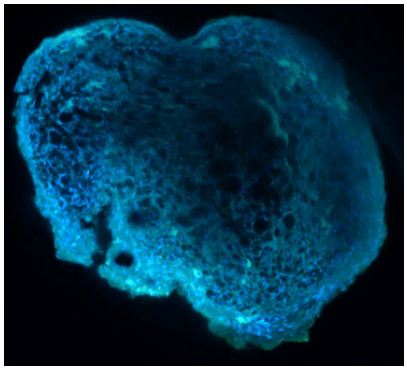

Figure 5. Confocal image. Undifferentiated micromass demonstrating hoescht nuclear staining.

These experiments demonstrated collagen II within the extracellular spaces, and although this was a preliminary investigation, it supports the evidence that cartilage formation is taking place. These initial results provide exciting clues to the behaviour of chondrogenically differentiated ADSC in 3D form.

4. Developed a biodegradable product design using 3D printing

Several potentially suitable materials will be printed (eg PLA, PCL) and seeded with ADSC. Cell viability and expansion will be investigated as well their biomechanical properties. The final product will have biomechanical properties similar to native nasal septal cartilage.