- **FINAL PROJECT REPORT**

- ***Date: 30.03.2010***
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- ***Name:***

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- ***Project Title:***

Alternative Cartilage Tissue Engineering Strategies: Smart Scaffolds & Perfusion Bioreactors

Please copy the "Outcome(s)" statement, entered on your application form, in the space below.

The outcome of the study are based on the general objectives of the study. These are:
Controlling scaffold pore architecture to mechanically fix pre-differentiated pellets and provide appropriate scaffold design for high cell density seeding via perfusion using clinically relevant cell sources (i.e. expanded human articular chondrocytes). By increasing chondrogenic potential, the objective of our repair strategy is to increase the patient population able to receive cartilage cell therapy treatment to include those over 55, where incidence of cartilage defects are greatest, and thereby delay the debilitating onset of OA in particular.

Will your work contribute to this outcome(s) in the manner you envisaged? If not, what has changed?

Based on the current results the work will contribute the outcome in the way we sought.

Please copy the "Specific Objective(s)" statement, entered on your application form, in the space below.

Aim 1: Produce 3D Plotted scaffolds with accurately designed 3D pore architectures to allow spherical pellets to be fixed in place (pellet seeding). Thus investigate whether pellet seeding and single cell seeding within designed scaffolds improves the amount and quality of tissue engineered cartilage.
Aim 2: Produce 3D Plotted scaffolds with accurately designed 3D pore architectures to seed chondrocytes at high local cellular density by direct perfusion. Thus investigate whether a high local cell density improves chondrogenic differentiation and cartilage tissue forming capacity.
Aim 3: Test the relevance of gap-junction mediated cell-cell interact-ions on chondrogenic differentiation and tissue forming capacity of de-differentiated adult chondrocytes cultivated in: i) 2D culture system, ii) 3D pellets, and iii) pellets seeded within 3D scaffolds and/or at high local cell density within perfused constructs.

Briefly describe how successful you were in achieving the stated objective(s). If the objective(s) was not achieved, explain why that is the case and describe what you did manage to achieve.

According to the 2 year project timeline the aims must only be partially be completed during the first year of the two year project where CMRF funding was received. We achieved our specific objectives/aims as outlined in the application. The results and progress during one year of the project is summarized below.
Aim 1: According to the optimised procedure of spherical bovine chondrocyte pellets formation in a 96-multiwell format chondrocytes from human origin were assessed on pellet formation and tissue differentiation. of spherical pellets. Manual integration into 3D plotted

scaffolds with specific 1mm fibre spacing could be successfully refined and revealed that the pellet seeding approach results in an increased seeding efficiency (>4-fold) as compared to single cell seeding via spinner flask. The histological assessment of pellet seeded constructs cultured up to 3 weeks revealed cartilage tissue more uniformly distributed compared to constructs single cell seeded by spinner flask. However pellet seeded construct showed lower GAG/DNA quantities and necrotic cell cores were observed in constructs after 3 weeks cultivation. Experimental work is on going to determine whether dynamic cultivation via spinner flask known to reduce critical nutrient diffusion limitations also reduces cell necrosis towards the core of the construct. We achieved our work target regarding Aim 1.

Aim 2: Specific 3D plotted scaffolds with a u-shaped channel design were press-fit integrated into chambers of a direct perfusion system to seed single cells (chondrocytes) into these channel structures. The direct perfusion system, validated and optimised on cell seeding efficiency and seeding uniformity/allocation showed cells allocated at a high density within u-shaped channels with a cell seeding efficiency of 58.0%. Initial post seeding re-differentiation assays with cells of human origin showed no improved differentiation capacity compared to non-channelled scaffold control design. Experiments with other human donors are required to further assess the of seeding allocation/density dependent cartilage tissue differentiation. These results were presented at the annual meeting 2009 of the European Society for Biomaterial (ESB) in Lausanne, Switzerland. We could achieve our defined work target regarding Aim 2.

Aim 3: Specific fluorescent labelling techniques to visualize gap junction mediated dye transfer could be established in 2D cell culture. The Calein-AM dye transfer of neighbouring cells (human chondrocytes) was reduced in the presence of 18a-GCA gap junction blocker. These results indicate that chondrocyte can form gap junction in *in vitro* cell culture. In the presence of 18a-GCA no significant decrease in GAG/DNA produced could be observed in 3D chondrocytes pellets (human cells). However Calcein-AM dye transfer techniques have to be established in 3D cell culture and whether blocking gap junction mediated cell to cell communication decreases the differentiation capacity of chondrocytes. We achieved our defined work target regarding Aim 3.

Please confirm delivery of the outputs listed on your application form. If these outputs were not to be delivered, please explain why.

We confirm that the outputs were delivered as listed in our application.