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Stem cells and scaffolds for skeletal muscle reconstruction with focus on repair of anal sphincter insufficiency

With this project we aim at developing tools for reconstructing small and large skeletal muscle defects with focus on anal sphincter insufficiency by using skeletal muscle stem cells and scaffolds. Skeletal muscle has a remarkable ability to fully regenerate after an injury, but in cases with a larger portion of the muscle missing e.g. due to trauma or surgery, there is need for a scaffold to guide the cell proliferation, migration and tissue formation. However, for reconstruction of smaller muscle defect, e.g. those seen in the anal and urethral sphincters due to injuries during delivery and leading to anal incontinence and stress incontinence, transplantation of autologous muscle stem cells could become a potential treatment. However the use of muscle stem cells in regenerative medicine is in its very early phase. Good primary cell isolation procedures has to be developed according to GMP (Good Manufacturing Practice) and considerations of an optimal state of the stem cells for transplantation must be made. The use of biodegradable scaffolds are exellent for cell delivery purpose and provide support for 3-D growth of cell and tissue formation.

Specific Aims

We will work towards establishing a method for isolating and culturing human myoblasts in accordance to GMP regulations in preparation for transplantation in human muscle disorders. This part of the project will be conducted together with the Department of Gynecology and Obstetrics and in collaboration with The Fertility Clinic, Odense University Hospital.

For in vitro studies, human skeletal muscle cultures will be established by two methods; cell dissociation and explant and muscle cultures and explants will be cultured on different types of biodegradable compliant scaffolds. Different culture conditions will be used to study cell attachment, growth, differentiation and tissue formation.

A combination of human myoblasts and scaffolds will be used for implantation studies in SCID mice. The purpose of this part of the project is to test survival of myogenic cells under different conditions in vivo and thus to develop methods for reconstructing skeletal muscle lesions using scaffolds.

Methods

In order to find the best conditions for culturing human myoblasts in a three dimensional structure, we will use the injectable and noninjectable scaffolds with and without coating of extracellular matrix (ECM), VEGF and NGF. Primary human myoblasts will established by two technicques, 1) dissociation of the muscle tissue with trypsin, followed by collection of cells, 2) small cubes of muscle tissue will be placed directly on the scaffolds which will facilitate outgrowth of cells from the tissue and onto the scaffold. Myoblast cultures will be seeded and cultured in conditions promoting proliferation and differentiation and At different time points during proliferation and differentiation, the scaffolds containing cells will be harvested and studied using scanning electron microscopy (SEM) and immuno fluorescence. The scaffolds will be stained for the proliferation marker Ki67 and myogenic markers Pax7, MyoD, NCAM, Desmin, Myogenin and Myosin and results will be evaluated with confocal microscopy. Using these markers we will study the proliferation and myogenic differentiation potential of the cells on scaffolds.

In vivo implantation studies of myogenic stem cells and scaffolds will be made in SCID mice to avoid immune response against human myoblasts. A large wedge of skeletal muscle tissue will be removed by longitudinal cutting from the core of the TA muscle in all the mice and the implantation studies will be made according to the following

Group 1: Mice will receive injectable and noninjectable scaffolds containing human myoblasts Group 2: Mice will receive empty biodegradable scaffold Group 3: Mice will receive human myoblasts Group 4: Mice will receive no implantation material

The regeneration process will be followed for three weeks and mice will be sacrificed at day 1, 2, 4, 6, 8, 10, 12, 15, 18 and 21 after induction of lesion. The isolated muscle will be embedded in paraffin and sections will be immunostained to study proliferation (Ki67), myogenic regeneration (Pax7, NCAM, Desmin, MyoD, Myogenin and Myosin), inflammation (F4/80 (macrophage marker), CD45 (general immune cell marker), connective tissue formation (collagens, tenascin C), and vascularisation, (VEGF, CD31, CD34), combined with a marker specific for human cells (Human Nuclear Antigen). The number of human cells including human muscle fiber nuclei will be studied using morphometric analyses (Cast, VisioPharm).

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