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CULTURE OF ADIPOSE-DERIVED STEM CELLS IN A CLOSED BIOREACTOR SYSTEM FOR CELL THERAPY

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Adipose-derived stem cells (ASCs) can be isolated from fatty tissue or liposuction. Similar to MSCs isolated from bone marrow, ASCs have multi-lineage potential and can be used as a potential source in regenerative medicine. Additionally, fat tissue is more accessible than bone marrow, and larger volumes can be obtained. For the production of cells for cell therapy in patients, an upgrade to large, clinical scale culture (> 200x10⁶ cells) is necessary. Clinical scale cultures require a reproducible and efficient process. Therefore, a process for culturing of large quantities of ASCs using microcarriers (MCs) was developed.

Adipose tissue, obtained after abdominoplasty, was minced and digested using collagenase I solution in culture medium. Human ASCs were isolated by differential centrifugation and grown in culture medium. ASCs were seeded onto various microcarriers and cell attachment was determined by visual inspection. CellBind MCs were found to be most suitable for ASC culture. ASCs were seeded onto CellBIND MCs in spinner flasks, and fresh MCs were added every other day. Cell growth was determined during 8 days. FACS analysis and a standard differentiation assay was done to confirm multi-lineage potential of the cells.

Visual inspection showed that ASCs had the expected morphology (Fig. 1 A). The differentiation assay confirmed the multi-lineage potential of the ASCs (Fig. 1 B-C). Analysis of spinner flasks culture showed that the ASCs were able to adhere to and proliferate (PDT 46.9 h) on the CellBIND MCs without the formation of large aggregates (Fig. 2).

Based on the results it can be concluded that fatty tissue is an excellent alternative source for the isolation of stem cells. An efficient culture process for cell therapy using microcarriers was established. This culture process will be combined with the Scinus bioreactor, an advanced closed bioreactor, for the production of GMP-compliant cell therapy.

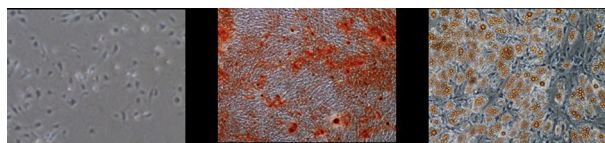


Figure 1. Monolayer culture of primary ACS's (A). Multi-lineage potential was confirmed for adipogenic and osteogenic differentiation using Oil Red O and Alizarin Red staining, respectively.

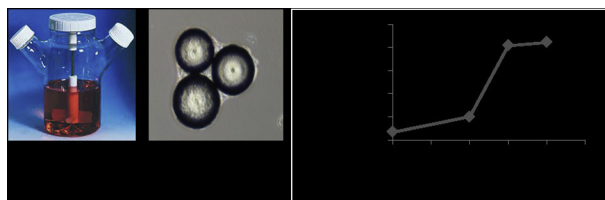


Figure 2. Spinner flask (A) culture of ASCs on CellBind microcarriers (B). Analysis of cell proliferation showed that ASCs can adhere and proliferate on CellBind microcarriers with a population doubling time of 46.9 hours (C).

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INDUCED PLURIPOTENT STEM CELLS EFFECTIVELY ATTENUATES TH2 ALLERGIC REACTION IN MICE

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Background: The prevalence of allergy in children increases gradually in recent years. Many drugs including steroids have been tested for allergy control. However, most of them have only temporary therapeutic effects. Allergic reactions frequently relapse after stop of medication because long term immunologic tolerance is not successfully induced by these medications. Reprogramming induced pluripotent stem cells (iPSCs) from fibroblasts inserted by Oct-4/Sox-2/Klf-4/c-myc has demonstrated their ability for multi-lineage differentiation like embryonic cells. The therapeutic effect of iPSCs on allergic reaction has not been examined. We tried to evaluate the effect of iPSCs injection on Th2 allergic reaction in BALB/c mice.

Methods: Groups of BALB/c mice sensitized by i.p. injection with alum-adsorbed ovalbumin (OVA) twice with a two-week interval. iPSCs or PBS were then intravenously injected after sensitization. Serum allergen-specific antibody levels and cytokine production from cultured spleen cells were examined.

Results: Treatment w iPSC effectively suppressed not only Th2 but also Th1 immune responses, which was characterized by reduction in serum OVA-specific IgE, IgG, IgG1, and IgG2a levels as well as in interleukin-5 and interferon- γ levels in cultured spleen cells. In the same time, regulatory cytokine, IL-10, was enhanced.

Conclusions: iPSC injection once effectively suppressed both Th2 allergic and Th1 inflammatory immune reaction which may be induced by IL-10 related immune tolerance.

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BUILDING BRIDGES FROM RESEARCH TO THERAPY: A ROADMAP FOR THE SUCCESSFUL GENERATION OF CLINICAL-GRADE iPSCS

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In 2007, Dr. Shinya Yamanaka became the first to successfully convert adult human cells to induced pluripotent stem cells (iPSCs). These cells have similar characteristics to embryonic stem cells (ESCs) including the potential to become any cell type in the body. Therefore, it is thought that human iPSCs (hiPSCs) can be utilized as the starting material for the manufacture of cell therapies to treat a multitude of diseases. While human ESCs are limited to allogeneic therapies, hiPSCs can be used for the development of both allogeneic and autologous therapies; the latter having the advantage of using a patient's own cells for the generation of hiPSCs. However, before iPSC-based therapies can become technically and economically viable, several hurdles need to be overcome. From a clinical manufacturing perspective, the challenges are quite different depending on the type of therapy (allogeneic vs. autologous), phase of development (clinical phase I, phase II, phase III and commercial phase), and the clinical indication. Our presentation will expand on these challenges and address the critical steps necessary to enable the use of hiPSC-derived cells in a clinical setting. These fundamental steps include:

- Establishing a tissue sourcing protocol that includes informed consent and donor eligibility
- Developing a robust process that is compliant with current Good Manufacturing Practices (cGMP) regulations for the generation and maintenance of clinical-grade iPSCs
- Defining a strategy for characterization and release testing of hiPSC Master Cell Banks (MCBs)

In addition we will provide a status update on the clinical manufacturing of iPSCs that we are currently working on, as part of a contract Lonza has in place with the National Institutes of Health, USA.

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ISOLATION OF MESENCHYMAL STEM CELLS USING MATRIX METALLOPROTEASES

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Mesenchymal stem cells (MSCs) are known to have the capacity for self-renewal and differentiation into mesenchyme-lineage cell types, including