Combining Adipose-Derived Stem Cells, Resorbable Scaffolds and Growth Factors: An Overview of Tissue Engineering

George K.B. Sándor, *MD, DDS, PhD, FRCDC, FRCSC, FACS;*
Riitta Suuronen, *MD, DDS, PhD*

Auteur-ressource

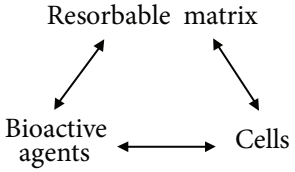
Dr Sándor
Courriel :
george.sandor@utoronto.ca

SOMMAIRE

Il existe une corrélation entre l'adipogénèse, production de tissus adipeux à partir des cellules souches, et l'ostéogénèse, production d'os à partir de ses cellules souches précurseurs. Les cellules souches mésenchymateuses adipocytaires adultes peuvent s'avérer plus tard utiles dans la régénération osseuse et le génie tissulaire. Le présent article examine les relations futures possibles entre les cellules souches adipocytaires autogènes, les facteurs de croissance et les membranes résorbables à des fins d'applications futures en génie tissulaire.

Pour les citations, la version définitive de cet article est la version électronique : www.cda-adc.ca/jcda/vol-74/issue-2/167.html

Tissue engineering involves the vital collaboration between cell biologists, biochemists, material scientists, engineers and clinicians, and an understanding of the complex role of the various components on which it is built. The 3 key components, stem cells, resorbable scaffolds and bioactive molecules such as growth factors, continuously interact with each other, like the 3 points of an equilateral triangle:



The combination of scaffolds and cells is often referred to as a construct. Constructs may be fashioned ex vivo, then implanted into an individual.

Sources of Stem Cells

The source of cells for tissue engineering depends on the structure to be replaced. Human *embryonic* stem cells, pluripotent stem cells isolated from the inner mass of human blastocysts, have great potential because of their capacity for differentiation. However, before human embryonic stem cells can be used clinically for tissue engineering, problems, such as culturing them without exposure to animal proteins as well as avoiding teratoma formation and immune rejection by the recipient's host, must be solved.[1] Currently, autogenous human *adult* stem cells are used clinically. For the tissue engineering of bone, for example, the use of cells with the lowest morbidity during harvesting and those that retain a degree of pluripotentiality are most advantageous.

Mesenchymal stem cells that are useful for bone regeneration can be derived from

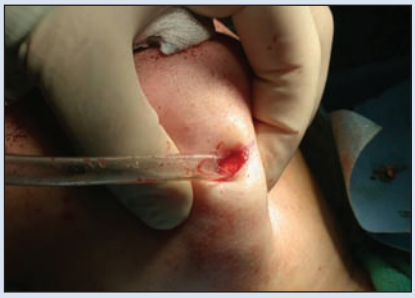


Figure 1: Liposuction is one convenient and minimally morbid way of harvesting adipose-derived stem cells for cell culturing and tissue engineering.

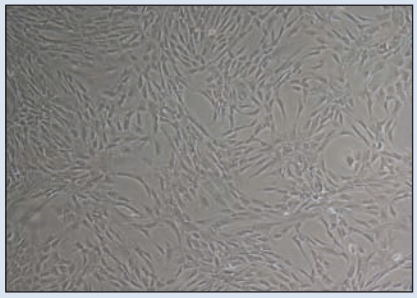


Figure 2: The appearance of adipose-derived stem cells grown in cell culture. These cells can be frozen for storage and later usage.

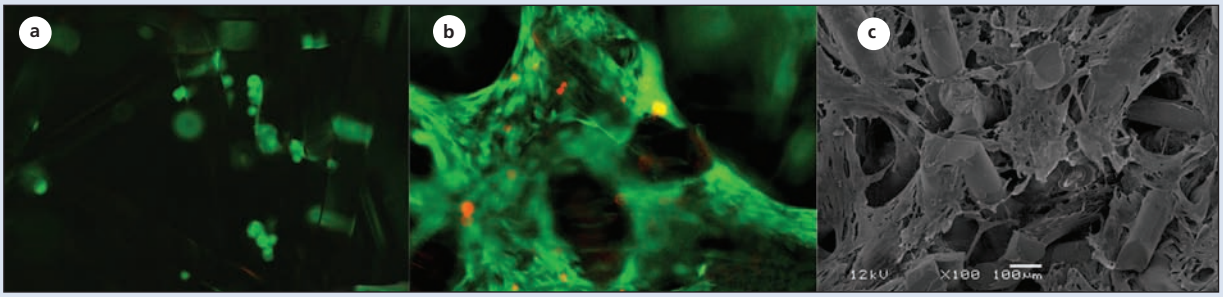


Figure 3: (a) Live and dead staining of adipose stem cells attached to a scaffold of resorbable biomaterial fibres (3-hour time point). Scale 200 µM. Green cells are viable; dead cells are red. **(b)** Live and dead staining of adipose stem cells attached to a scaffold of resorbable biomaterial fibres (2-week time point). Scale 200 µM. Green cells are viable; dead cells are red. **(c)** Scanning electronic miscroscopy image of adipose stem cells attached to a scaffold of resorbable biomaterial fibres (2-week time point). Scale 100 µM.

adipose tissue because of the interdependency between adipogenesis and osteogenesis.[2] Harvesting adipose tissue is not morbid and could be advantageous for some if liposuction were used as the harvesting method (**Figs. 1** and **2**).

A key question about the use of mesenchymal stem cells is whether they can make the transition between 2 differentiated states.[2] Cloned human bone-marrow mesenchymal stem cells are known to be able to differentiate into multiple lineages using adipocyte, chondrocyte and osteoblast pathways.[3,4] Mesenchymal stem-cell clones can sequentially differentiate into adipocytes, dedifferentiate, then transdifferentiate into osteoblasts in vitro.[2,5] Future therapeutic interventions may therefore promote these same cells to transdifferentiate. It is also possible that phenotypic drift may occur as part of this adipose-bone relationship, which could be useful in future therapy.[2]

Stimulating Stem Cells

With the use of the tissue-engineering model, autogenous adipose-derived stem cells could be harvested from a patient having a liposuction procedure. These cells could then be used to seed a resorbable scaffold[6] made with CAD/CAM technology to the precise dimensions of a missing segment of bone. The seeded cells could be stimulated physically with magnetic or galvanic stimulation, ultrasound, hypoxic or hyperoxic gradients,[7] growth factors such as transforming growth factor beta-1 (TGB-1),[8] bone morphogenetic proteins (BMPs)[9] or vascular endothelial growth factor (VEGF),[10] to guide the differentiation and growth of the cells.

Manipulating the Construct

Once the cells have populated the scaffold (**Fig. 3**), the resulting bioimplant or construct could be transplanted into the patient to restore the defect. This ex vivo reconstruction has 1 major obstacle: the vitality of the bioimplant is entirely dependent on the vascularity of the recipient bed. To this end, growth factors such as VEGF could be used to stimulate angiogenesis[10] to help vascularize the construct, while TGB-1 or other BMPs could be used to guide the osteogenic differentiation of the cells.

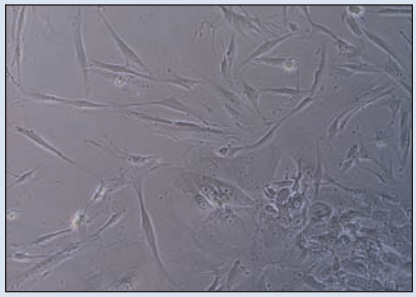


Figure 4: Cementoblasts growing in culture taken from a removed third molar.

Future Directions

Stem cells could also be harvested from suction-trap aspirates of bone during mandibular third molar removal,[11] one of the most common oral surgical procedures done today. Third molar removal also presents some other new opportunities. Removal of the developing third molar follicle can yield follicular cells, cementoblast-like cells and dental pulp stem cells, which can also be cultured and studied (**Fig. 4**). The receptors of these cells can be characterized, an important first step in understanding these cells and their potential future use.

THE AUTHORS

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Dr. Sándor *is professor, Regea Institute for Regenerative Medicine, University of Tampere, Tampere, Finland; clinical director, graduate program in oral and maxillofacial surgery and anesthesia, Mount Sinai Hospital; coordinator, pediatric oral and maxillofacial surgery, The Hospital for Sick Children and Bloorview Kids Rehab; professor of oral and maxillofacial surgery, University of Toronto, Toronto, Ontario; and docent in oral and maxillofacial surgery, University of Oulu, Oulu, Finland.*

Dr. Suuronen *is professor and director, Regea Institute for Regenerative Medicine, Tampere University and University Hospital, Tampere, Finland.*

Corresponding Author: *Professor George K.B. Sándor, The Hospital for Sick Children, S-525, 555 University Ave., Toronto, ON M5G 1X8.*

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About the Regea Institute

The Regea Institute of Regenerative Medicine is an independent centre, affiliated with the University of Tampere in Tampere, Finland. The institute is a national multi-tissue bank, overseeing the handling of tissue for transplantations such as cornea, amniotic membrane and bone.

The institute has successful in vitro fertilization and embryonic stem cell programs. It also has 6 European Union-certified GMP (Good Manufacturing Practice) "clean rooms" that can be used in the production of tissue-engineered products for reconstructive surgery. Regea's divisions include embryonic stem cell, nerve, cardiac, ophthalmologic, mesenchymal stem cell and skin groups.

The institute has undergone tremendous growth since its inception in 2003. Beginning with just 3 employees, there are currently over 70 people working at Regea under the direction of professor Riitta Suuronen. Professor Suuronen has vast experience in the development of resorbable materials used in surgery along with developing techniques vital to cell culturing and cell monitoring.

The city of Tampere — Finland's third largest municipality — provides significant funding for the institute and several other biotechnical companies in the area. Many of these companies are Regea's closest collaborators, including firms that produce unique cell culture monitoring

devices and resorbable scaffold materials important to future surgical reconstruction.

In addition to the funding from the city of Tampere, Regea also benefits from support from the Finnish Funding Agency for Technology and Innovation (TEKES), the Academy of Finland, the University of Tampere, Pirkanmaa Health District, private foundations (competitive grants), private donations and costs recovered from the provision of tissues for transplantation.

In 2006, professor George Sándor of the University of Toronto received a competitive grant from TEKES as part of the Finnish Distinguished Professor Program (FiDiPro). Professor Sándor was awarded the largest grant amount, approximately €2 million, of the 20 successful applicants in the FiDiPro program.

Collaboration between Regea and the Discipline of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Toronto

Given the environment of committed funding within a backdrop of innovative corporate biotechnology at Regea, professors Suuronen and Sándor are embarking upon a unique collaborative research project involving Regea and the University of Toronto's faculty of dentistry. The faculty's discipline of oral and maxillofacial surgery has a history of research related to bone regeneration through its orthobiologics group. The group has also been active in the area of growth factor research as it relates to tissue engineering. The results of these studies have led to the development of novel treatment methods to reconstruct major human mandibular defects without the need to use an otherwise morbid and painful bone graft donor site. The collaboration with Regea will focus on stem cell, growth factor and scaffold interactions, and will involve exchange programs for graduate students at the master's, doctoral and post-doctoral levels.

Strict operating procedures are in place for the handling of tissues and cells in a clean room environment at the Regea Institute.