Biomaterials 29 (2008) 3953–3959

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Human clinical experience with adipose precursor cells seeded on hyaluronic acid-based spongy scaffolds

F.B. Stillaert [a,*], C. Di Bartolo [b], J.A. Hunt [c], N.P. Rhodes [c], E. Tognana [b], S. Monstrey [a], P.N. Blondeel [a]

[a] Department of Plastic and Reconstructive Surgery, University Hospital Gent, De Pintelaan 185, 9000 Gent, Belgium
[b] Fidia Advanced Biopolymers s.r.l., Abano Terme, Padova, Italy
[c] University of Liverpool, UK Centre for Tissue Engineering, Liverpool, United Kingdom

ARTICLE INFO

Article history:
Received 24 March 2008
Accepted 10 June 2008
Available online 17 July 2008

Keywords:
Adipose tissue engineering
Hyaluronic acid
Scaffold

ABSTRACT

Histioconductive approaches to soft-tissue defects use scaffolds seeded with lineage- and tissue-specific progenitors to generate tissue which should reside in equilibrium with adjacent tissue. Scaffolds guide histiogenesis by ensuring cell–cell and cell–matrix interactions. Hyaluronic acid-based (HA) pre-adipocyte-seeded scaffolds were evaluated for their adipo-conductive potential and efficacy in humans. Preadipocytes were isolated from lipoaspirate material and seeded on HA scaffolds. The cellular bio-hybrid (ADIPOGRAFT®) and an acellular control scaffold (HYAFF®11) were implanted subcutaneously. At specific time points (2, 8 and 16 weeks) explants were analyzed histopathologically with immunohistochemistry. No adverse tissue effects occurred. Volume loss and consistent degradation of the HYAFF®11 scaffolds compared to the ADIPOGRAFT® group indicated progressive tissue integration. No consistent histological differences between both groups were observed. By 8 weeks all void spaces within the scaffolds were filled with cells with pronounced matrix deposition in the ADIPOGRAFT® bio-hybrids. Here we show that HA scaffolds were stable cell carriers and had the potential to generate volume-retaining tissue. However, no adipogenic differentiation was observed within the preadipocyte-seeded scaffolds.

1. Introduction

Engineered adipose tissue could overcome the disadvantages associated with the implantation of synthetic materials or autologous soft-tissue grafts. The fascination for adipose tissue as an autologous substitute to approach tissue deficiencies emerges from its intrinsic characteristics. It not only is available in easily accessible subcutaneous depots but is also very manageable in the way tissue defects can be structurally moulded to reconstruct the dimensions of the lost body contour. Adipose tissue is organized into a three-dimensional dynamic connective tissue which hosts a stromal fraction, and a microvascular plexus entwined within a highly organized extracellular matrix (ECM) [1]. This stromal-vascular fraction (SVF) consists of a heterogeneous cell population of which the adipose-derived adult stem (ADAS) cells are of significant interest for potential regenerative applications [2]. These multipotent cells [3] can be harvested through liposuction without altering their viability [4,5]. The objective in engineering adipose tissue is to shape a three-dimensional tissue substitute which prospers by biochemical, physical, and cellular cues and approximates the native tissue equivalent. Histioconductive tissue engineering strategies use additional materials as cell carriers (scaffolds) to create the shape and dimensions of reparative tissues and then functionally and molecularly integrate those bio-hybrids within the surrounding host tissue. Materials that meet fundamental requirements such as controlled degradation, cytotoxicity and immunogenity are needed. Research reports have shown successful and reproducible inoculation and culturing of adipocyte- and preadipocyte-precursor cells (preadipocytes and progenitors, respectively) on synthetic [6,7] or natural [8] prefabricated scaffolds [9] with subsequent differentiation *in vitro* or adipose tissue formation *in vivo* [10]. The scaffolds within these bio-hybrids provided the seeded cells specific attachment or binding sites, shaped the tissue construct and functioned as geometrical environments in which not only cells received their essential cues for a structural cell organization and cellular behaviour but also cellular function was modulated [11,12]. Various scaffold materials have been analyzed in experiments for autotransplantation as well as for *in vitro* or *in vivo* growth and differentiation of preadipocytes [6,9,13,14] but no data extrapolations have been realized yet to clinical applications. In this pilot clinical trial hyaluronic acid-based (HA) scaffolds (HYAFF®11) were used as a cell-carrier material. This naturally occurring polysaccharide is a major component of the ECM in connective tissues [15], plays a prominent role in cellular behaviour

* Corresponding author.
E-mail address: filip.stillaert@ugent.be (F.B. Stillaert).

0142-9612/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2008.06.005

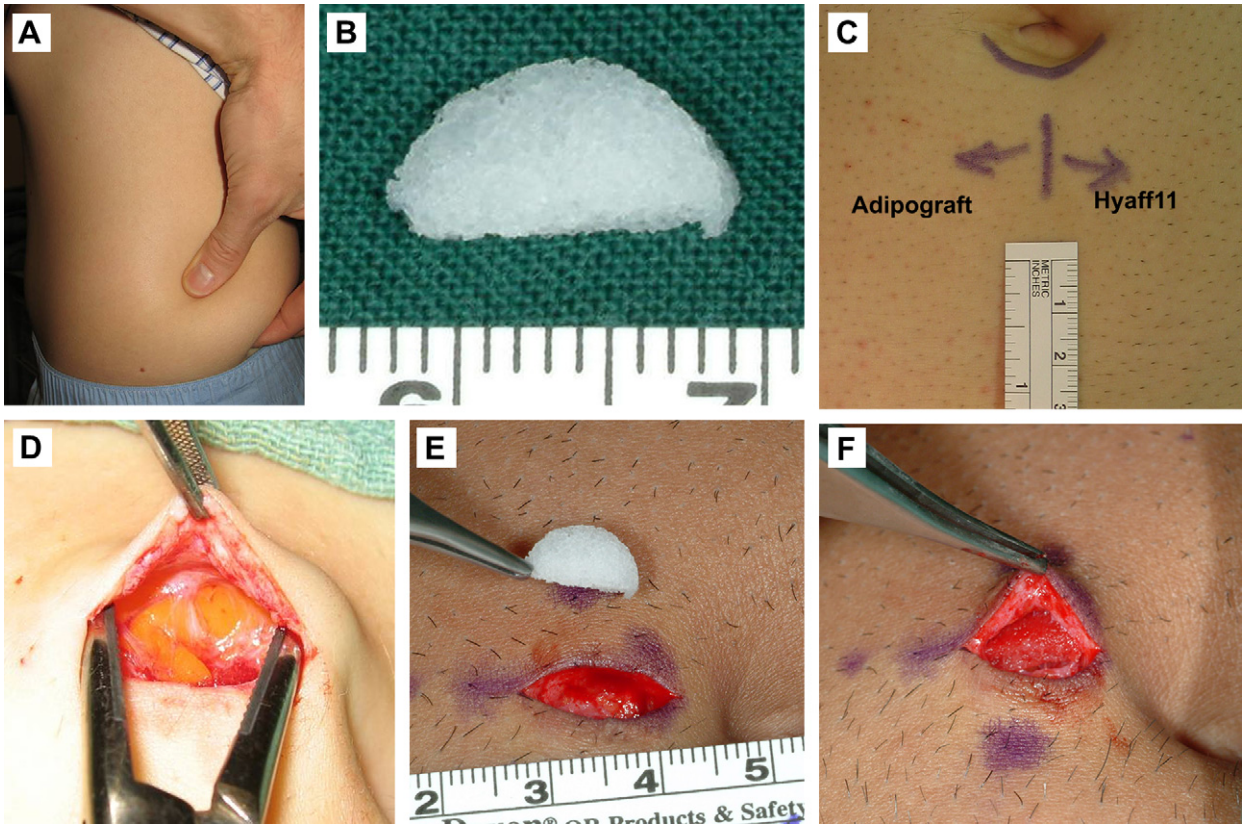

Fig. 1. Lipoaspirate was harvested from the lateral abdominal region under local anesthesia (A). Hemi-cylindrical HYAFF®11 scaffolds were used as cell carriers and implanted in the sub-umbilical area (B and C) with the cell-seeded implant located on the left side in a subcutaneous pocket (D). Scaffolds were easy to handle (E). (F) the cell-seeded scaffold in place.

and exerts an important mechanical role [16]. Being a polysaccharide and not a protein it is potentially less antigenic, which is an important property when considering clinical uses. Its supportive role for precursor cell proliferation and differentiation has been confirmed by several *in vivo* and *in vitro* studies [17]. HYAFF®11 is based on hyaluronic acid modified by esterification which increases the hydrophobicity, the residence time *in vivo* and makes it more resistant to hyaluronidase activity. Previous studies observed the potential to support expansion and differentiation of adipose precursor cells *in vivo* [8]. When implanted in nude mice human preadipocyte-seeded HYAFF®11 sponges showed a higher cell density than collagen constructs after 3 weeks [10]. Halbleib et al. defined the experimental conditions under which human adipocyte precursor cells could be effectively inoculated and cultured on HA scaffolds [9] and provided basic evidence that human preadipocytes can be used to establish adipose tissue engineering techniques to obtain a three-dimensional soft-tissue filler. Their appropriate and well-defined porous microarchitecture not only allows morphogenesis but also provides an environment for matrix deposition and angiogenesis. Based on these promising findings and the straightforward clinical flowchart a clinical trial was setup to evaluate the efficiency of those scaffolds in humans *in vivo*, mimicking a clinical small-volume tissue deficit.

2. Materials and methods

2.1. Lipoaspirate material

All experimental procedures were approved by the Human Research Ethics Committee. Twelve volunteers, aged 20–35 years, were included in this trial according to specific inclusion criteria (average age = 25.5; average BMI = 23.5). Lipoaspirate material was obtained (~5 to 10 cc) through a liposuction procedure under local anaesthesia (Xylocain 1% without adrenalin) with informed consent through the Department of Plastic Surgery of the University Hospital Gent. Lipoaspirate material was aspirated through a Coleman ASP I (∅ = 2.5 mm) liposuction cannula connected to a 10 cc sterile luer-lock disposable syringe. The average aspirated fat volume per volunteer was 8.27 cc. Donor sites were the lower lateral abdominal areas in all cases (Fig. 1A). The sealed luer-lock syringes containing the lipoaspirate were stored in a cooled foamy kit.

2.2. Isolation of human adipose stromal cells

The fat tissue samples were immediately dispatched to Fidia Advanced Biopolymers (FAB) Laboratories (Abano Terme, Italy) for further processing. All samples arrived within 24 h. Lipoaspirate material was washed three times with PBS (vol:vol, 1:1) and digested at 37 °C with a sterile filtered collagenase solution (collagenase Type I 500 U/ml, 8% FCS, 0.02 M HEPES and 0.02 IU–0.02 mg/ml penicillin/streptomycin in Dulbecco's modified eagle medium/Ham's F-12) (vol:vol, 1:1) for 1 h. Prior experiments have demonstrated a cell concentration recovery of 0.15–0.2×10^6 cells/cc of fat after collagenase digestion. The yield of preadipocyte cells from 1 g adipose tissue was in the range of $2–3 \times 10^5$ cells of which not more than 78% attached to the scaffolds [9]. As 5–8 ml of lipoaspirate was digested, the number of cells recovered was in the range of $0.75–1.6 \times 10^6$. After centrifugation (200*g* for 10 min at 17 °C) and removal of the supernatant, cells were resuspended in a preadipocyte culture medium (DMEM/Ham's F-12 supplemented with 10% FCS, 1 nM basic fibroblast growth factor (bFGF), 0.02 IU–0.02 mg/ml penicillin/streptomycin) to obtain a final volume of ~100 to 200 μl. Cells isolated by all samples differentiated into mature adipocytes after 1 week of culture in differentiation medium.

2.3. Characteristics of the scaffolds

HYAFF®11 scaffolds were provided by Fidia Advanced Biopolymers s.r.l. (FAB, Abano, Italy) (Fig. 1B). HYAFF®11 is a linear derivative of hyaluronic acid modified by complete esterification of the carboxylic function of glucuronic acid with benzyl groups [18]. HYAFF®11 biomaterial is spontaneously degraded and resorbed. The structure of the sponges used in this trial showed open, interconnecting pores with pore size of 400 μm. HYAFF®11 sponges with a pore size of 400 μm appeared to be superior to other scaffold types regarding cell attachment rate [9]. HYAFF®11 scaffolds were prepared as semi-cylindrical disks with a diameter of 10 mm and a height of 4 mm and were sterilized by γ-radiation.