Journal of Plastic, Reconstructive & Aesthetic Surgery (2015) 68, 1491–1497

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

In vitro and *in vivo* biocompatibility, bioavailability and tolerance of an injectable vehicle for adipose-derived stem/stromal cells for plastic surgery indications

Charlotte Lequeux [a,b,d], Jonathan Rodriguez [a,b,*,d], Fabien Boucher [c], Ondine Rouyer [a,b], Odile Damour [a,b], Ali Mojallal [c,e], Céline Auxenfans [a,b,e]

[a] *Cell and Tissue Bank, Edouard Herriot Hospital, 5 Place d'Arsonval, 69437 Lyon, Cedex 03, France*
[b] *Institut de Biologie et Chimie des Protéines, LBTI, UMR 5305, CNRS/Université de Lyon, Lyon, France*
[c] *Department of Plastic Surgery, Croix Rousse Hospital, 103 Grande rue de la Croix Rousse, 69317 Lyon, Cedex 04, France*

Received 28 November 2014; accepted 28 July 2015

KEYWORDS
Adipose-derived stem/stromal cells;
Vehicle;
Adipose tissue engineering;
Plastic surgery

Summary Soft tissue reconstruction is a challenge in plastic surgery, when replacing lost materials and correcting contour defects. Many permanent and temporary fillers have been used to restore the volume of these lesions, but often with poor results and even complications. Adipose-derived stem/stromal cells (ASCs) and adipose tissue engineering have been suggested as valuable alternatives. In order to inject these cultured cells, it was essential to find a suitable vehicle. The purpose of this study was to evaluate Cytocare®, an injectable medical device, composed of hyaluronic acid plus amino acids, vitamins and mineral salts. First, ASC viability and bioavailability in the 3 different available Cytocare® formulations using the MTT test were assessed; then an animal experiment, testing the tolerance after intradermal injections of both Cytocare® alone and with ASCs was carried out.

Our *in vitro* results demonstrate a high biocompatibility of Cytocare® resulting in a better viability of ASCs when cultured in Cytocare® compared to culture medium ($p < 0.05$, Mann and Whitney). Cytocare® also permits their bioavailability and proliferation, making it a

Abbreviations: ASC, adipose-derived stem/stromal cells; CD, cluster of differentiation; DMEM, Dulbecco's modified eagle medium; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; HA, hyaluronic acid; HPS, hematoxylin phloxine saffron; MTT, 3-(4,5-diMethylThiazol-2-yl)-2,5-diphenylTetrazolium bromide; SDS, sodium dodecyl sulfate; SVF, stromal vascular fraction.

* Corresponding author. Cell and Tissue Bank, Edouard Herriot Hospital, 5 Place d'Arsonval, 69437 Lyon, Cedex 03, France. Tel.: +33 (0)4 72 11 06 27.
E-mail address: jonathan.rodriguez@chu-lyon.fr (J. Rodriguez).
[d] Co first authors.
[e] Co last authors.

http://dx.doi.org/10.1016/j.bjps.2015.07.022

potential transfer vehicle that can retain the cells before their integration around the recipient site. Finally, our animal experiment shows that the ASC + Cytocare® combination is well tolerated.

In conclusion, Cytocare® can be used as a biocompatible scaffold for cultured ASCs in therapeutic treatments, ensuring ASC bioavailability, as well as evidence of excellent tolerance in nude mice.

Introduction

The reconstruction of soft tissue defects and volume restoration is a challenge in plastic surgery. Many permanent (e.g. silicone, polymethyl-methacrylate) and temporary (e.g. collagen, hyaluronic acid) fillers have been used to restore the volume of these lesions but often with poor results and even complications. Autologous fat transfer seems to have regained popularity but resorption rates remain unpredictable.[1] To increase fat tissue survival rates, adipose-derived stem/stromal cells (ASCs) and adipose tissue engineering have been suggested as valuable alternatives.[2]

Since 2001, the self-renewal capacity and multipotency of human adipose-derived stem/stromal cells (ASCs) to generate specialized cells has been clearly shown.[3,4]

In order to clinically use these cultured cells in controlled amounts for long-term padding or in wound healing, it was essential to find a suitable vehicle. This had to be safe for the patient and be easily injectable, while protecting both the cells and the patient during the adaptation period and at the same time ensuring the slow release of cells into the environment. When injected in suspension in a conventional liquid medium, ASCs are quickly dispersed into the subcutaneous space.[5] This is why, in most preclinical studies, ASCs have been administered in porous vehicles (collagen) or gels (collagen, hyaluronic acid or fibrin).[6–10] We selected hyaluronic acid (HA), widely used in plastic and esthetic surgery for example for anti-aging and also for administration of cultured cells like chondrocytes,[11,12] or ASC for intra articular injection in osteoarthritis,[13] to support cultured epidermis[14] or as a dermal filler.[15]

In this paper, we are the first to test the tolerance and the bioavailability of ASC in Cytocare® formulations. Cytocare is an injectable medical device not only composed of hyaluronic acid but also a rejuvenating complex (CT50), which is a balanced protection solution of mineral salts, amino acids and vitamins, granted CE approval for intradermal injections and routinely used with good results in our plastic surgery department. This anti-aging product is marketed at three concentrations: Cytocare® 532 (32 mg of HA/5 ml + CT50), 516 (16 mg of HA/5 ml + CT50) and 502 (2 mg of HA/5 ml + CT50).

ASC in Cytocare is now classified as an ATMP. Regulation for these new drugs requests the proof of biocompatibility, bioavailability and tolerance of the cells in their exact vehicle.

We first tested ASC viability and bioavailability in the 3 different forms of Cytocare®, composed of HA and vitamins + minerals versus HA alone.

Then, as part of our clinical trials, with the selected potential vehicle, we conducted animal experiments, to show the tolerance after intradermal injection of both Cytocare® alone and with ASCs.

Materials and methods

SVF isolation

Lipoaspirates were harvested as surgical residue according to French regulations, including written informed consent and a declaration to the research ministry (DC n°2008162). Human stromal vascular fractions (SVF) were isolated from the lipoaspirate of 2 donors undergoing optimized liposuction, using a 3 mm cannula, whilst following the ethical and safety guidelines approved by the local IRB and based on Björntorp's methods.[16]

Briefly, adipose tissue was digested with collagenase (0.120 U/ml, Roche, Indianapolis, USA) at 37 °C for 30 min under constant shaking. Digestion was stopped by adding Dulbecco's Modified Eagle's Medium (DMEM with glutamax, Gibco (Invitrogen, Carlsbad, USA)) containing 10% fetal calf serum (FCS, HyClone, Logan, USA). Floating adipocytes were discarded and cells from the SVF were pelleted, rinsed with medium, centrifuged (300 g for 5 min at 20 °C) and incubated in an erythrocyte lysis buffer for 10 min at 37 °C. This cell suspension was centrifuged (300 g for 5 min at 20 °C) and viability and cell counts were evaluated using a hemocytometer after staining with Trypan blue.

Adipose-derived stem/stromal cell (ASC) amplification

Freshly extracted cell suspensions containing ASCs were cultured in ASC medium containing DMEM (Gibco), HAM-F12 L-Glutamine (Gibco), 10% FCS (Hyclone), 10 ng/ml basic fibroblast growth factor (Sigma, St Quentin Fallavier, France) and antibiotics (20 μg/ml of streptomycin (Panpharma, Fougeres, France) and 100 UI/ml of penicillin (Panpharma, Fougeres, France)). The medium was changed every 3 days until confluence was reached. At passage 2, the cells were detached using 0.125% trypsin containing 0.025% EDTA (Invitrogen) and centrifuged for 10 min at 300 g. Following viability tests and counting in a hemocytometer after trypan blue staining, cells were either resuspended in DMEM or mixed with Cytocare® formulations (hyaluronic acid (HA) + CT50) for *in vitro* experiments or