P-059	**Adipose-derived stem cells as drug stores in alginate capsules**	

Lucconi G.[1], Chlapanidas T.[1], Mantelli M.[2], Avanzini M.A.[2], Tosca M.C.[1,3], Galuzzi M.[3], Antonioli B.[3], Vigo D.[4], Faustini M.[4#], Marazzi M.[3]* and Torre M.L.[1]
[1]Univ Pavia - Italy [2]IRCCS Policlinico San Matteo - Italy [3]Niguarda Ca' Granda Hospital, Milan - Italy [4]Univ. Milan - Italy *Supervisor [#]massimo.faustini@unimi.it

INTRODUCTION AND OBJECTIVES

The use of adipose-derived stem cells (ADSCs) has recently been proposed in tissue engineering and regenerative medicine to control several inflammatory and autoimmune pathologies, as well as graft-versus-host disease (Yanez 2006). Their therapeutic potential may be explained by the release of growth factors and cytokines mediating paracrine actions (Gimble 2007). ADSCs can be thought of as a "site-regulated drug factory/store" and, for clinical purposes, different approaches may be implemented: cell administration by systemic intravascular perfusion or implantation of a drug delivery system. The aim of this study was to investigate the behavior of ADSCs loaded in implantable microcapsules for advanced therapies.

MATERIALS AND METHODS

Isolation of ADSCs and cell culture

One informed male subject was enrolled (38 years, 90 kg) during abdominoplasty. Adipose tissue was suspended in phosphate buffered saline and delivered to the laboratory at a temperature of 4°C. The sample was digested as reported by Faustini (2010). The adipose stromal vascular fraction was plated on a plastic surface (10.000 cell/cm²) in DMEM/F12, 10% fetal bovine serum, 1% penicillin/streptomycin and 1% Amphotericin B; adherent ADSC were expanded till the 3rd passage and then used for 3D and monolayer culture.
ADSCs were combined with a saturated $CaCl_2$ solution and the resulting suspension was added drop-wise to a sodium alginate solution under stirring. Capsules were collected after filtration, washed, placed in 24-well plate and cultured. Every 2 days, medium was collected and replaced with fresh medium. Adherent cells were also cultured under the same conditions, as a control.

Capsule and ADSC characterization

Capsules were morphologically characterized in terms of capsule diameter, core diameter and gel capsule thickness, using a digital video camera connected to an image analyzer (CV 9000 Ver. 4.0 Image Analyzer, FKV Srl, Sorisole, BG, Italy); the weight of the capsules was also determined. The ADSC morphology, organization and ultrastructure were performed by optical (hematoxylin/eosin staining) and electronic (TEM) microscopy (JEOL JEM 1200 EX). CD73, CD105, CD13, HLA-I, CD33, CD34 and CD45 surface expression was evaluated by flow cytometry for both encapsulated and adherent ADSCs. Cytokine (IL-6, IL-7, IL-8, TNF-α and INF-γ) secretion was quantified in supernatants by ELISA. The encapsulated ADSC shelf life was also evaluated at 4°C.

RESULTS AND DISCUSSION

Capsules exhibited structural integrity until the end of culture. ADSCs maintained their viability, with a spherical shape, and aggregated into clusters migrating to the inner alginate membrane surface from the third day of culture (Figure 1). After twenty-eight days culture, TEM imaging revealed the presence of nuclei, endoplasmic reticulum, mitochondria and a large numbers of vacuoles, typical of active, vital cells (Figure 2).
Lower percentages of encapsulated ADSCs expressed the surface antigens CD73, CD105 and CD13 compared to adherent ADSCs, while they remained negative for HLA-I, CD33, CD34, CD45 expression (Figure 3).
Lower amounts of IL-6, IL-7 and IL-8, were secreted by encapsulated ADSCs compared to adherent cells. Very low levels of TNF-α and INF-γ were detected in supernatants of both encapsulated and adherent ADSC (Figure 4).

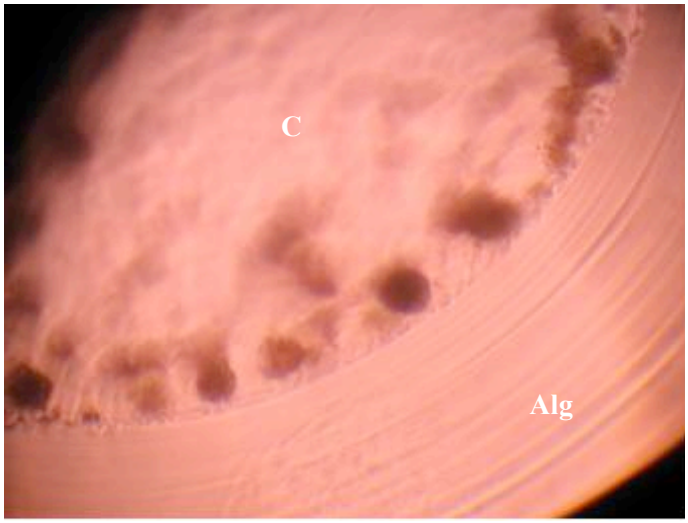

Figure 1: optical microscopy. C: capsule core. Alg: alginate membrane; magnification 10x.

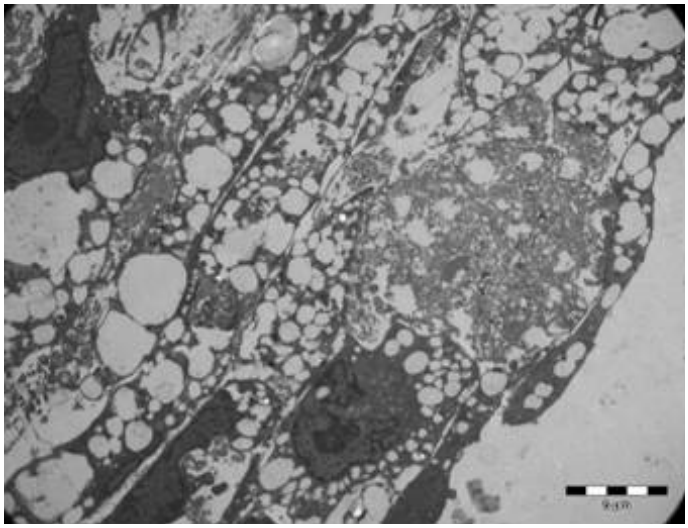

Figure 2: transmission electron microscopy. bars: 5µm.

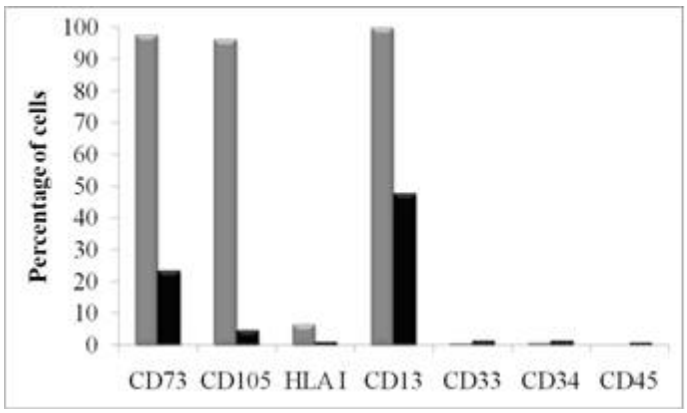

Figure 3: flow cytometric analysis for encapsulated (black) and adherent (grey) ADSCs.

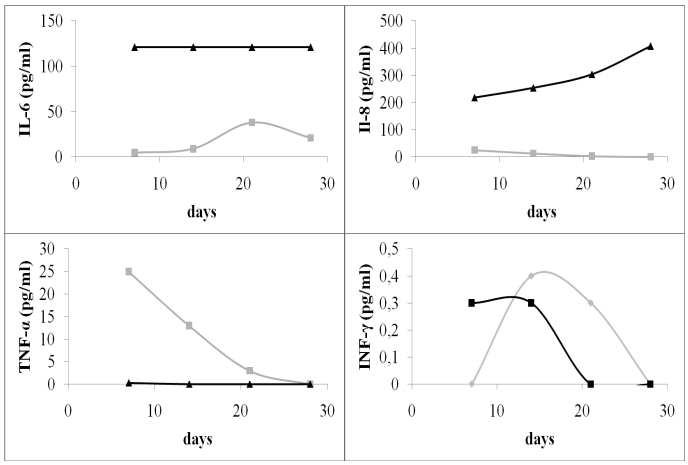

Figure 4: cytokine secretion of encapsulated (grey) and adherent (black) ADSCs.

The capsule diameter, gel capsule thickness, core diameter and weight of the capsules during 56 days of culture are reported in Table 1.

The shelf life was evaluated for encapsulated ADSCs stored at 4°C for one week. The cell viability was greater than 90% at time zero, but it decreased significantly at 24 hours (~60%); over the following hours viability continued to decline (Figure 5).

Table 1: Capsule characteristics: mean values (SD); n: number of samples. Asterisk indicates significant difference with respect to previous time (p<0.001).

Time (days)	n	capsule diameter	Core diameter	thickness	weight
0	20	4.9 (0.2)	4.2 (0.3)	0.3 (0.1)	65.8 (9.6)
7	20	5.6 (0.6) *	3.7 (0.6) *	1.0 (0.1) *	95.7 (11.7) *
14	20	6.3 (0.3) *	4.1 (0.2)	1.2 (0.1)	120.4 (17.0) *
21	12	5.8 (0.4) *	4.2 (0.5)	0.7 (0.2) *	106.4 (18.6)
28	7	5.8 (0.4)	3.6 (0.5) *	1.1 (0.2) *	99.8 (16.2)
35	7	6.0 (0.5)	3.5 (0.5)	1.1 (0.2)	105.8 (17.5)
42	6	6.0 (0.4)	4.3 (0,6) *	0.8 (0,1) *	101.9 (14.7)
49	5	5.8 (0.5)	4.1 (0.3)	0.7 (0.1)	92.4 (21.1)
56	5	5.9 (0.4)	4.3 (0.5)	0.9 (0.1)	96.2 (19.6)

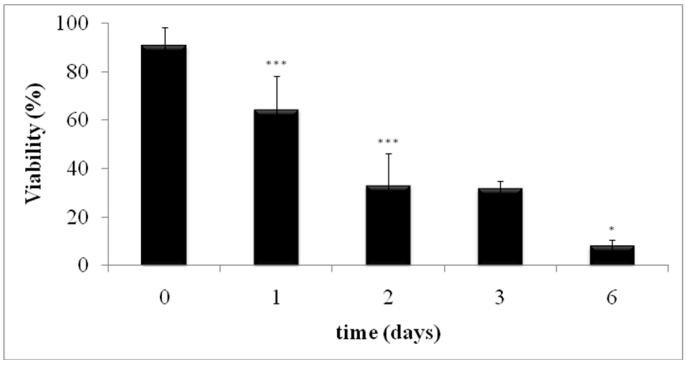

Figure 5: percentage of cell viability for encapsulated ADSCs stored at 4°C for one week. Asterisk indicates significant difference with respect to previous time (*: p<0.001; *: p<0.05).**

CONCLUSIONS

Differences in phenotype and cytokine secretion between encapsulated and adherent ADSCs were observed; whether these differences influence their in vitro and in vivo activity should be further investigated.

REFERENCES

- Yanez et al. (2006) *Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease.* Stem Cells 24 (11) 2582-2591.
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- Faustini et al. (2010) *Non expanded mesenchymal stem cells for regenerative medicine: yield in stromal vascular fraction from adipose tissues.* Tissue Engineering Part C 16 (6) 1515-1521.