OP268
Characterization of fibrin microspheres obtained from total blood plasma as cell culture scaffolds for human adipose mesenchymal stem cells

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Introduction: Tissue engineering traditionally requires suitable scaffolds that allow cell maintenance and adhesion. Therefore, it's crucial to evaluate biomaterials as potential cell carriers. In this work, the microbeads initially developed by Gorodetsky et al. 1999 (1) using pure fibrinogen and thrombin, were obtained using complete blood plasma making possible the production of autologous biocarriers. The resulting particles were characterized and used as culture support of human adipose mesenchymal stem cells (hAdMSC).
Materials and methods: The general aspect of produced microbeads was scrutinized by scanning electron microscopy and their sizes determined by sieving and laser diffraction. Topography was evaluated with Atomic force microscopy. The protein composition was analyzed by SDS-PAGE. Human mesenchymal stem cells expressing GFP were used to verify cell adhesion and proliferation.
Results: Size and surface features found were heterogeneous (Fig. 1, Left). Our electrophoretic analysis showed similar pattern of bands as those of pure fibrin. Cell cultures confirmed adhesion (Fig. 1, Right), growth (Fig. 2) and optimal viability.

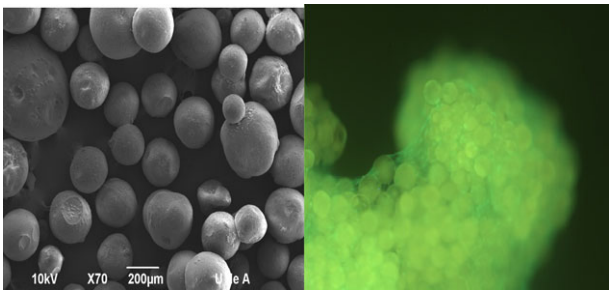

Figure 1 Left, Aspect of microspheres by SEM. Right, AdMSC bound to microspheres.

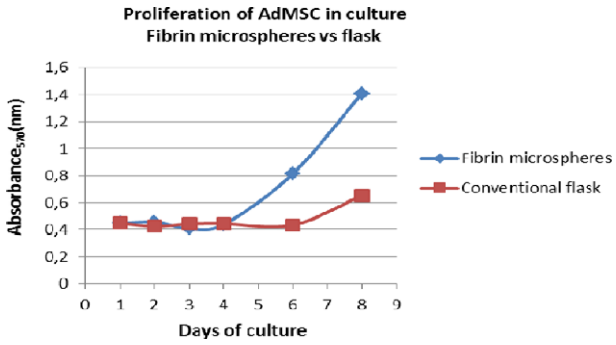

Figure 2 Proliferation of hAdMSC in fibrin microspheres vs conventional flask.*Absorbance is shown instead cell number because of the high growth observed, that was out of the range predicted by the calibration curve.
Discussion and conclusions: Our microspheres are favourable cell culture scaffolds that support cell adhesion, promote cell proliferation and can be obtained in a wide range of sizes. They are also cheap to produce and can be used with advantage for developing autologous implants.
Acknowledgments: To the health care facility of the U de A, for financial aid. To different research groups in U de A, those without their collaboration, our work would be not possible.

Disclosures: The authors state that some of the results of this work are being object of a patent request. Those results could not be exposed in detail.
References
1. Gorodetsky R, et al. Fibrin Microbeads (FMB) as Biodegradable Carriers for Culturing Cells and for Accelerating Wound Healing. 1999.

OP269
Xeno-free cGMP isolation and cryopreservation of clinical grade stromal vascular fraction

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Introduction: In the last decades, adipose tissue (AT) has attracted attention of the scientific and industrial communities for its high therapeutic potential. When focusing on the clinical translation of such type of cells, aspects such as Good Manufacturing Practices (GMP) compliance, scalability, reproducibility, and protocol validation must be ensured. Manufacturing of cells must warrant high quality concerning cell viability, cell yield immunophenotype, and absence of any microbial or pathological contamination. This project focused on the development of a complete manufacturing and cryopreservation process of therapeutic cells for human clinical delivery, ensuring all parameters and characteristics listed above.
Materials and methods: The new protocol developed is particularly compact and ensures economies of scale when increasing the volume of processed AT, which reduces manufacturing cost. In this project 100–200 g AT/human donor, obtained from liposuction or lipectomy procedures (n = 15) were processed under GMP conditions. Stromal vascular fraction (SVF) isolation and cryopreservation was performed using xeno-free and GMP grade reagents. Cell number and viability, before and after cryopreservation, was determined by trypan blue exclusion dye by three operators. Immunophenotype of SVF subpopulations was evaluated by flow cytometry (FACSCanto and FACSDiva software) for the expression of mesenchymal, endothelial, hematopoietic and pericytic markers: CD31, CD34, CD45, CD73, CD90, CD105, CD146 and HLA-DR. Mesenchymal trilineage differentiation was performed to demonstrate potential of the isolated cells.
Results: The developed xeno-free cGMP procedure for isolation of clinical grade SVF cells resulted in a cell yield of $2.4 \times 10^5 \pm 0.9$ cells/g AT, and cell viability of 85.4%±7.1, which demonstrated differentiation into mesodermal lineages. After xeno-free cryopreservation cell viability was 80.0%±10.1. Regarding immunophenotype of SVF subpopulations (Fig. 1), when considering mesenchymal markers co-expressed with CD34[1], 12%±5 of the cells presented the $CD90^+CD34^+CD73^+CD105^+$ phenotype, while 1.3%±0.8 of cells expressed concomitant mesenchymal markers in the absence of CD34. Regarding endothelial markers, 15%±6 of the cells are $CD45^-CD34^+CD31^+$. Preliminary analysis of samples demonstrated no significant difference of phenotypic expression before and after xeno-free cryopreservation.

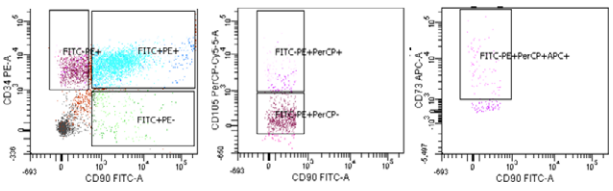

Figure 1 Representative strategy of gate analysis used for hSVF characterization.

Discussion and conclusions: To the best of our knowledge this project is pioneer in providing clinical grade cryopreserved hSVF, ensuring extremely high viability post-thaw, and maintenance of immunophenotypic characteristics of its sub-populations. This achievement is of great relevance for therapeutic use of adipose tissue regenerative cells.

References

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OP270
Role of the stromal vascular fraction from adipose tissue associated to a biomaterial to regenerate bone in irradiated area

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Introduction: We have previously demonstrated that the association of Total Bone Marrow (TBM) and Biphasic Calcium Phosphate (BCP) is the best combination to regenerate bone in irradiated area.[1] Recently, Stromal Vascular Fraction from adipose tissue (SVF) has been described as an alternative to TBM as a source of mesenchymal stem cells.[2] Studies showed that SVF could promote osteoformation in healthy bone.[3] The subject of this study was to identify the capacity of the SVF to induce new bone formation in irradiated area.

Materials and methods: Four weeks after an external 20 Gy of irradiation on the hind limbs of 20 rats, 4 bone defects were created per animal and filled with SVF or TBM, associated or not with BCP. Three weeks after implantations, the bones were removed. Histological and scanning electron microscopy analyses were performed to obtain qualitative and quantitative analyses. The different conditions were compared using Mann & Whitney tests for unpaired comparisons after validation by an analysis of Kurskal & Wallis.

Results: The BCP-TBM mixture significantly improved bone ingrowth ($p < 0.05$). The BCP-SVF mixture did not provide new-bone formation over and above that induced by BCP alone (Fig. 1). However, the histological staining of blood vessels showed that the BCP-SVF association induced more angiogenesis than BCP (Fig. 2).

Discussion and conclusions: Instead of the lack of vascularisation, the lack of cytokines and growth factors in the SVF could limit bone formation when compared to TBM.

BCP associated to TBM appears to be the most efficient combination for bone reconstruction after radiotherapy today.

Disclosures: Authors have nothing to disclose.

References

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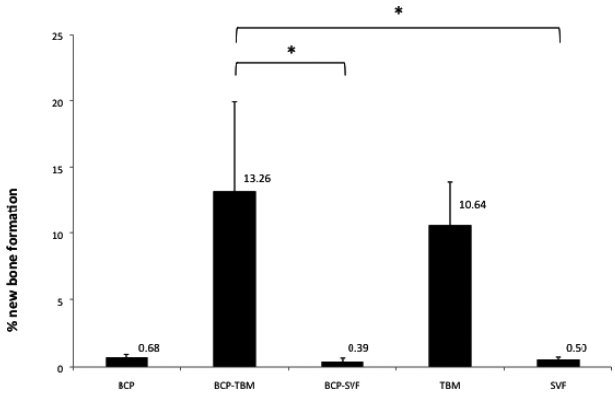

Figure 1 Bone ingrowth in osseous defects (*p = 0.021).

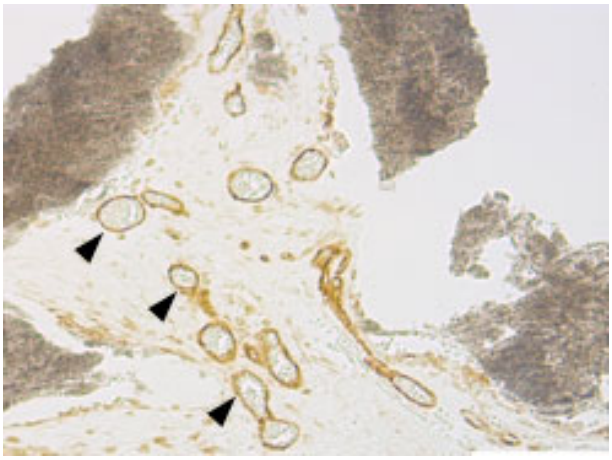

Figure 2 Sample of BCP-SVF filling with specific CD31 labeling of blood vessels (arrow heads).

OP271
Adipose stromal cells-conditioned medium is less efficient in modulating osteoarthritic chondrocytes and synovyocytes behavior

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Introduction: Adipose stromal cells (ASC) have been shown to exert anti-fibrotic, anti-inflammatory and anti-apoptotic properties, through secreted growth factors(1,2). Basing on these characteristics have been proposed their use for stem cell-based therapy of degenerative disease for the treatment of osteoarthritis (OA). Therefore the aim of the study was the analysis of trophic potential effects of ASC-conditioned medium (CM) on chondrocyte and synoviocyte from OA patients.

Materials and methods: Good manufacturing practice (GMP)-clinical grade ASC were isolated from subcutaneous adipose tissue. Chondrocytes and synoviocytes were isolated from cartilage and synovial of OA patients undergoing total joint replacement. Chondrocytes or synoviocytes were treated with different ratio of ASC-CM or co-cultured with ASC in transwell. Specific markers of fibrosis (collagen type 1 and 3), and matrix degrading factors and inhibitors (ADAMTS4, ADAMTS5, TIMP1, TIMP3) were tested by RT-qPCR analysis. Secreted inflammatory (IL6, CXCL8/IL8, CXCL1/GROα, CCL2/MCP-1, CCL3/ MIP1α, CCL5/RANTES) and anabolic (HGF, PGE2) factors by multiplex bead--based sandwich immunoassay or ELISA test.

Results: Chondrocyte treated with ASC-CM significantly inhibited collagen type 1 and 3 and did not affect matrix degrading factors and inhibitors. Synoviocytes treated with ASC-CM decreased ADAMTS5 and increased ADAMTS4 expression while no effects were observed on TIMP1 and TIMP3. By contrast, synoviocytes in co-culture experiments confirmed a decrease of ADAMTS5 associate to an increase of TIMP1expression. The release of all the inflammatory factors analyzed