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Translating adipose tissue stromal vascular fraction into regenerative medicine applications: Characterization of an innovative non-enzymatic closed system for minimal tissue manipulation

Claudia Cicione, Barba M, Di Taranto G, Bernardini C, Sciarretta F, Baranzini M, Isgrò MA, Di Stasio E, Michetti F and Lattanzi W
Università Cattolica del Sacro Cuore, Italy

The adipose stromal vascular fraction (SVF) has emerged as a rich and promising source of adipose derived stem cells (ASC). ASCs exhibit high growth kinetics, plasticity and are able to induce efficient tissue regeneration in several biomedical applications without the need of culture expansion. However, the required standards for clinical-grade cell manufacturing (i.e., the current good manufacturing practices, cGMP, guidelines) would be quite hardly to be met by currently experimental protocol for SVF isolation. This problem can be overcome with the development of one-step procedures to treat different disorders by using freshly harvested SVF. Here, it is presented an innovative device (MyStem®), based on GMP-proof non-enzymatic tissue separation and cellular enrichment, enabling a rapid (10-15 minutes) isolation of SVF from human lipoaspirates in a closed sterile system.Adipose tissue (AT) from 5 donors was liposucted using the cannula contained in the kit and alternatively processed using: standard protocol (PLA), lipoaspirate fluid protocol (LAF) and MyStem protocol. All isolated cells were comparatively counted, analyzed morphologically, and characterized by flow cytometry. Isolated SVF was characterized for differentiation capacity towards adipocyte and osteoblast lineages. Furthermore, SVF osteoinductive properties were tested in preosteoblast co-culture experiments. Staining techniques and Real time PCR were used to evaluate the differentiation experiments.The harvested AT comprised small lobules, with intact cell membranes and structurally integer adipocytes. Histology showed the presence of cells without lipid content and placed in clusters along stromal axis of the lobule. The number of cells/gr isolated was respectively: 4x105 PLA, 1.8x105 LAF and 2x105 MyStem. Cells isolated with the three alternative protocols displayed a spindle shaped, bipolar morphology, comparable expression of surface antigens and differentiation capacity. Co-culture assays indicated that the SVF display strong osteogenic potential and osteoinductive properties.These results provided the first proof of principle on the feasibility of using a closed non-enzymatic protocol for collecting intact AT and separate a tissue fraction enriched in ASCs from a lipoaspirate sample. The isolated SVF displays biological properties that can be easily exploited and translated in regenerative medicine applications.

claudia.cicione@libero.it