Introduction and Objectives
Our recent study demonstrates that micronized liposaprate acts as a natural scaffold for stem cells and give rise to spontaneous cell outgrowth, together with a paracrine effect on resident cells [1]. Lipogems® is a commercial device that washes and micronizes adipose tissue preserving the structural properties, as an alternative to the extracted stem cells, avoiding the regulatory limits, manipulation processes and costs deriving from isolation, culture and differentiation [2].

On the basis of the results obtained on cartilage regeneration, in this work we tried to demonstrate in vitro if micropiposaprate can promote and accelerate around wounds the extensive time and manipulation necessary for the use of MSCs therapy in tissue regeneration. We evaluated:

1) the ability of resident cells in liposaprate to grow out from adipose tissue and their capability of repopulating an organ culture of human skin;
2) the effect of liposaprate on the proliferation rate and migration of fibroblasts and keratinocytes and on fibroblast contraction;
3) the release of trophic/reparative cytokine from liposaprate;
4) antibacterial effect of liposaprate.

Materials and Methods
Liposaprate were obtained from five healthy female patients (age range 30–45) undergoing a liposuction (abdominal subcutaneous fat tissue) at the IMAGE Institute using Lipogems®® dispositive**.

Micronized fat, was cultured 24h in RPMI with 5% FBS, supplemented with 2 mL 1 glutamine (0.5L Lipogems/mL). Human adipose stem cells (hADSCs) were isolated as stromal cell fraction from Lipogems® with enzymatic digestion and after growth used for the test at a concentration 2.5×10^6 cells/mL.

The outgrowth study was done in a 3D collagen matrix culture using clusters of liposaprate transduced by Lenti-GFP® cocultured with an organ culture of healthy or mechanically damaged skin.

Proliferation migration and contraction tests on fibroblasts and keratinocytes have been done using MRC5 and HaCat cell lines using respectively ATP quantification kit, scratch Wound healing, and collagen contraction assay.

Antibacterial effect of Lipogems® was tested using agar test and CFU count of E. coli and P. aeruginosa. Assessment of inhibition of bacterial growth was done comparing the activity of the clusters to that of hADSCs known to have an antibacterial activity [3].

To understand the paracrine effect of micronized fat, the cytokine released in culture media were assessed using the Human Cytokine Antibody Array Membrane kit (Panomics). Moreover in order to test if Liposaprate modify the cytokine pattern when infected, Liposaprate clusters (0,5mL/RPMI) were co-incubated with 300 CFU E. coli for 24h and the cytokines released were compared to the untreated one.

LL-37 peptide concentration in culture media of Lipogems® (0,5 mL) or hADMSCs (2,5×10^6 cells/mL) incubated with or w/o 300 CFU E. coli for 24h was quantified using the LL-37, Human, HyCult Biotechnology ELISA kit.

The data were analyzed using the statistical software R (n=15; p<0.05).

Conclusions
The ability of resident cells in microfragmented fat to grow out without enzymatic digestion and Lipogems paracrine effect on the proliferation rate, migration and contraction of fibroblasts and keratinocytes ………….

These results together with the release of trophic/reparative cytokine and high antibacterial role of Lipogems® could constitute a promising adjuvant to antibiotic therapy, determining an effective therapeutic advantage for the healing of wounds in the field of regenerative medicine.

References

** Design of the study approved by the ethical committee of the University of Milan. Exclusion criteria were BMI >30, diabetes, hypertension, and nicotine or alcohol abuse. Written informed consent, specifying that residual material destined to be disposed of could be used for research, was signed by each participant before the biological materials were removed, in agreement with the 2004/24/CE of the Committee of Ministers Council of Europe on research on biological materials of human origin.

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