Antibacterial activity of lipoaspirate: an *in vitro* study

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Introduction

During wound healing process, bacterial infections may prolong skin regeneration and tissue repair causing a delayed or incomplete healing [1].
Mesenchymal stem cells (MSCs) secrete bioactive molecules with anti-inflammatory, angiogenic, chemostatic, antiapoptotic, and anti-cytotoxic properties [2]. The MSCs have also demonstrated good ability to accelerate skin regeneration with mechanisms still under investigation but which include immune modulation, differentiation into epidermal and dermal cells to replace damaged ones, reduction of fibrosis with reduced hypertrophy in scar formation [3,4]. In addition, it has been detected that soluble products generated by MSCs significantly decrease the growth of Gram-negative and Gram-positive pathogenic bacteria both *in vitro* and *in vivo,* increasing the efficacy of the antibiotics used to treat this type of infection[5]. In particular, literature data have also shown a marked inhibition of bacterial growth following the pre-stimulation of MSCs with viable *E. coli* cultures, due to the release of LL-37 peptide [6].
A promising and alternative source to the bone marrow for obtaining stem cells is represented by the adipose tissue that is available in abundant quantities and is easy and quick to pick up through the liposuction process.
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.

Aims

The study evaluate the in vitro antibacterial activity of lipoaspirate obtained with Lipogems®.

Three aspects were considered:

-direct and indirect antibacterial activity;
-cytokine profile;
-concentration of LL-37 peptide;
-LL-37 peptide antibacterial activity.

Materials and Methods

Human adipose derived MSCs (hADSCs)
Lipogems® was cultured in RPMI with 5% FBS, supplemented with 2 mM l-glutamine.
Human adipose stem cells (hADSC) were isolated as stromal cell fraction from Lipogems® with digestion with type I collagenase 1 mg/ml in PBS at 37°C for 30–60 min, centrifugation for 10 min at 450 × g, resuspension of the pellet in red blood cell lysis buffer (2.06 g/L Tris base, pH 7.2, and 7.49 g/L NH_4Cl) with incubation at room temperature for 10 min. Pellets were collected and filtered sequentially through 100- and 40-mm cell strainers to remove undigested tissue. The pellets were then washed with PBS and the cells grown in DMEM/Ham's F12 medium (v/v 1:1) supplemented with 40% FBS, 100 U/ml penicillin, 100 µg/ ml streptomycin, and 2 mM l-glutamine.

Antimicrobial assays
The strains E. coli ATCC 255922 and P. aeruginosa were used for these experiments. Assessment of inhibition of bacterial growth by Lipogems® and hADSCs, their conditioned medium (CM) and synthetic LL-37 was evaluated by cells counting method. Direct contact: in 24-well plates, Lipogems® (500 µL) or hADMSCs (2 × 10^5 cells/well) in RPMI with 5% FBS, supplemented with 2 mM l-glutamine were coincubated with 300 CFU *E. coli* (in 10 µl of PBS) for 6h at 5% CO_2. Aliquots of culture medium (cm) were taken from each well, serially diluted with sterile PBS, and plated on agar plates (*E. coli*: McConkey, *P. aeruginosa*: Cetrimide) . Colonies (CFUs) were counted after overnight incubation at 37°C. Indirect contact: Lipogems® CM or hADSCs CM was collected from the wells, centrifuged at 15,000 rpm for 10 minutes and stored at −20°C. Prior to the experiments, CM were thawed, and aliquots (90 µl) were transferred to a 96-well plate, inoculated with 100 CFU *E. coli* or *P. aeruginosa* (in 10 µl of PBS) and incubated for 16 hours at 37°C. Then CFU were counted as described earlier. LL-37 peptide: Synthetic LL-37, was dissolved at final concentrations of 1 ng/ml and 10 ng/ml in RPMI with 5% FBS, supplemented with 2 mM l-glutamine. Aliquots (90 µl) were transferred to a 96-well plate, inoculated with 100 CFU *E. coli* or *P. aeruginosa* (in 10 µl of PBS) and incubated for 16 hours at 37°C. Then CFU were counted as described earlier.

Lipogems® Cytokine release
In 6-well plates, Lipogems® (1 ml) in 2 ml RPMI + 5% FBS were coincubated with or w/o 300 CFU *E. coli* (in 10 µl of PBS) for 24h at 5% CO_2. Lipogems® CM was collected from the wells, centrifuged at 15,000 rpm for 10 minutes and stored at −20°C. The cytokines released by the lipoaspirate are evaluated using the Human Cytokine Antibody Array-Membrane kit.

Elisa
In 6-well plates, Lipogems® (1 ml) or hADMSCs (5 × 10^5 cells/well) in 2 ml RPMI + 5% FBS were coincubated with or w/o 300 CFU *E. coli* (in 10 µl of PBS) for 24h at 5% CO_2. Lipogems® CM or hADSCs CM was collected from the wells, centrifuged at 15,000 rpm for 10 minutes and stored at −20°C. LL-37 peptide concentration was quantified using the LL-37, Human, Hycult Biotechnology ELISA kit.

Conclusions

The obtained results regarding the 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.
Furthermore, lipoaspirate has proved to be a source of release of numerous cytokines useful for the tissue repair process.

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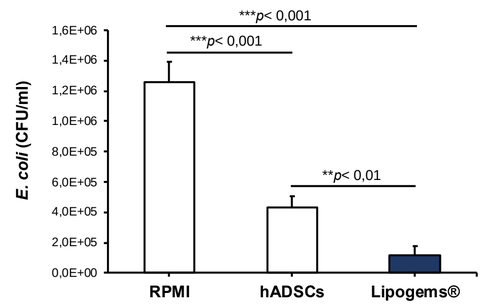
References

[1] Serra et al. 2015 *Expert Rev Anti Infect Ther*. 13:605-13. [2] Caplan A. 2009 *J Pathol*. 217:318-324. [3] Kanji and Das 2017 *Mediators Inflamm.* 2017: 5217967. [4] Larouche et al. 2018 *Adv Wound Care* (New Rochelle) 7: 209–231. [5] Sutton et al. 2016 *Stem Cells Int*. 2016:5303048. [6] Krasnodembskaya et al. 2010 *Stem Cells*. 28: 2229-2238.

Results

Fig. 1 Direct Lipogems® antibacterial activity. After 6h coincubation, Lipogems® directly inhibits *E. coli* growth (91%) and showed a more pronounced antibacterial effect in comparison to extracted hADSCs (66%).



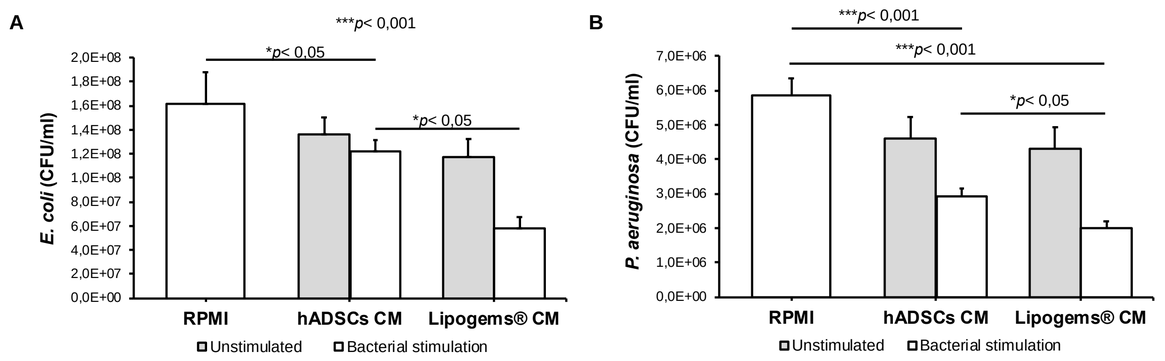


Fig. 2 Indirect Lipogems® antibacterial activity. The CM of Lipogems® or extracted hADSCs w/o prior stimulation was examined for antimicrobial activity against *E. coli* (A) and *P. aeruginosa* (B) after 16h coincubation. The CM of Lipogems ® activated by *E. coli* direct contact determined inhibition percentages in *E. coli* (64%) and *P. aeruginosa* (66%) growth significantly higher than CM of the corresponding hADSCs (36% and 50% respectively) as well as CM of Lipogems ® / hADSCs w/o bacterial pre-stimulation (24% and 26% / 16% and 22% respectively).

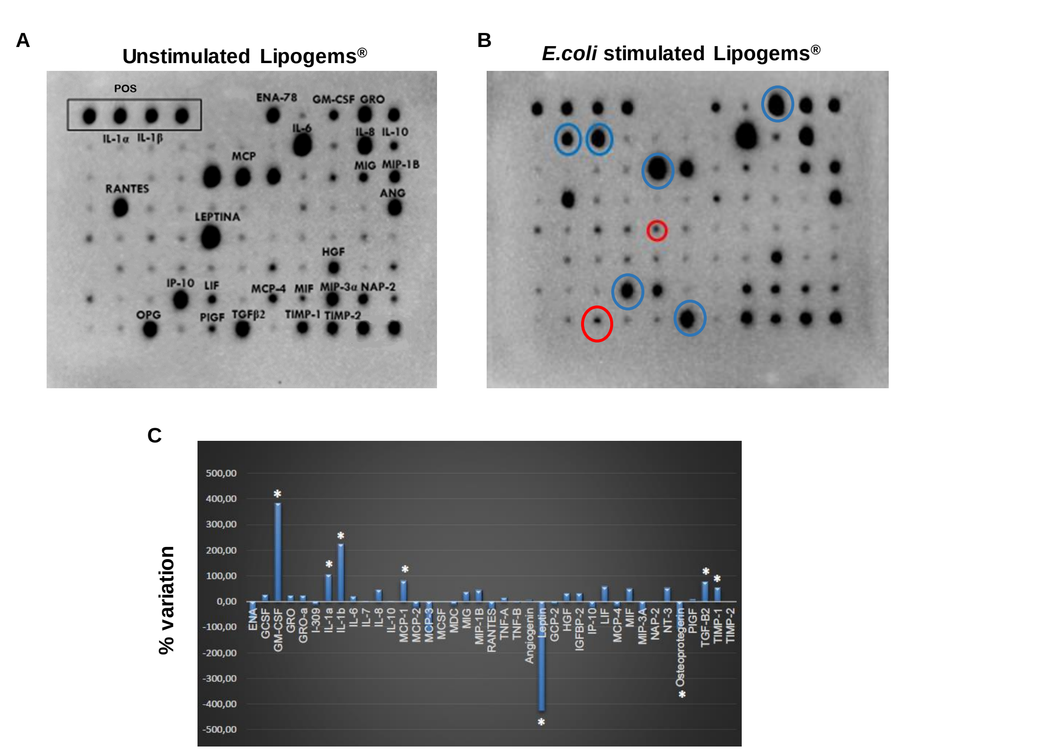


Fig. 3 Cytokines profile. Cytokines release of unstimulated Lipogems® CM (A) and cytokines release of *E. coli* stimulated Lipogems® CM (B) after 24h coincubation. Blue: cytokines with increased level, red: cytokines with lowered levels. Semiquantitative analysis of cytokines profile of *E. coli* stimulated Lipogems ® CM (C). An increase level of release is observed for the GM-CSF (granulocyte monocyte colony stimulating factor), IL-1α, IL-1β, MCP-1, TGFβ 2 and TIMP-1 cytokines whereas a reduction in the release of Osteoprotegerin (OPG) and Leptin is detected.

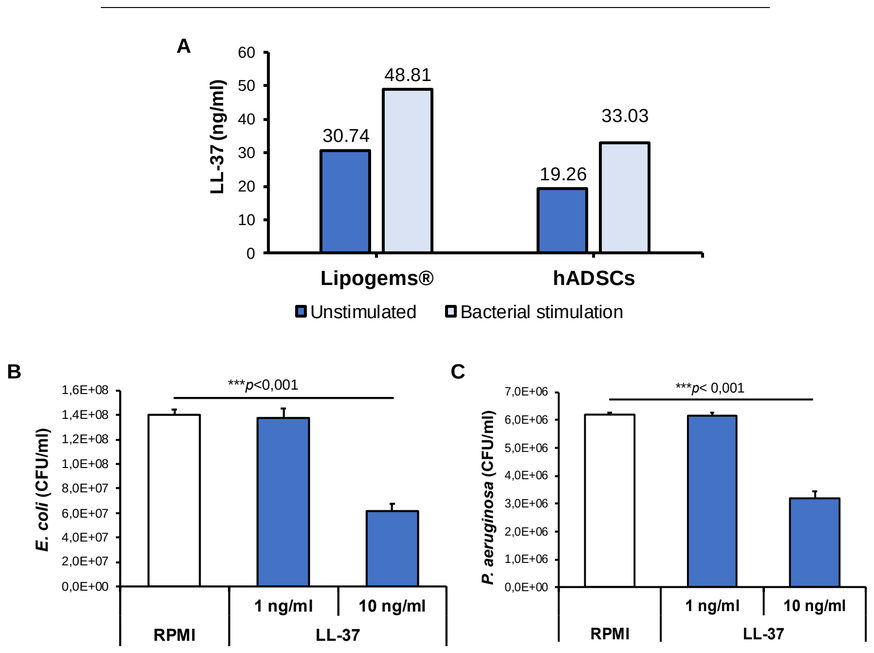


Fig. 4. LL-37 peptide concentration and antibacterial activity. Lipogems® and hADSCs secreted levels of the antimicrobial molecule LL-37 (A). An increase in LL-37 release levels was observed in the CM of Lipogems® and hADSCs due to *E. coli* stimulation. Antibacterial activity of LL-37 against *E. coli* (B) and against *P. aeruginosa* (C). Synthetic LL-37 alone demonstrated a significant antibacterial effect, when tested at 10 ng/ml in the same medium and in the same conditions as CM.