Metabolomic studies of mesenchymal steam cells conditioned media and umbilical cord blood plasma

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Mesenchymal stem cells (MSCs) are multipotent progenitor cells that can be isolated from several sources like bone marrow, skeletal muscle, umbilical cord blood (UCB), umbilical cord matrix, etc. These cells have immune-regulatory properties and effects on tissue repair. Human MSCs (hMSCs) are being tested clinically as therapeutic agents for a variety of pathologies and diseases. MSCs can be expanded *in vitro* using culture media containing fetal bovine serum (FBS). Currently, there is no reliable serum-free medium for hMSCs and the animal sera have several disadvantages including economic, ethical and scientific (batch-to-batch variability, unexpected cell growth characteristics, cytotoxicity and risk of possible contaminations with virus, prions, bacteria, etc.). It is important to define FBS replacements for hMSCs *in vitro* culture.

Here we present, NMR and Multiplexing Laser Bead Technology (MLBT) studies, on the composition of human MSCs (hMSCs) conditioned media (CM) and umbilical cord blood plasma (hUCBS) in terms of metabolite content, growth factors and interleukins. The potential of hUCBS as a supplement for hMSCs culture and of CM as an alternative to hMSCs application in regenerative medicine was verified. NMR was used to identify and quantify the metabolites in CM and hUCBS, whereas MLBT - the cytokines and growth factors.

Biological samples:

Human MSCs from Wharton's jelly umbilical cord (hMSCs) were purchased from PromoCell® GmbH. Two MSCs mediums were used for the conditioning: ComMed (PromoCell®) and DMEM (Dulbecco's Modified Eagle Medium, Gibco®). Both, unconditioned (*DMEM, Com. Medium*) and Conditioned media (CM) from hMSCs collected in 24h (*24h DMEM, 24h Com. Medium*) and 48h (*48h Com. Medium*) hours were tested. Umbilical cord blood plasma (hUCBS) from 11 donors.

NMR spectroscopy (Fig.1):

- Bruker Avance III 600 HD, CryoProbe Prodigy;
- H_2O:D_2O (9:1); T= 300K; 0.05mM (TSP)
- NMR spectra: i. 1D NOESY (noesygppr1d, recycle delay-90°-t_1-90°-t_m- acquire): d1 of 5 s, t1 of 4 μs and t_m of 0.01 s. ii. Carr-Purcell-Meiboom-Gill (CPMG, cpmgpr1d, recycle delay-90°-[τ-180°-τ]$_n$- acquire): rel. delay 4 s, echo time (τ) was optimized for each sample and was between 0.3 and 0.8 ms, and a loop for T2 filter (n) 20 ; iii. ^{1}H/^{1}H COSY, TOCSY and ^{1}H/^{13}C HSQC, HMBC.

Multiplexing LASER Bead Analysis (MLBA) (Fig.2):

A simultaneous testing of cytokines, chemokines and growth factors in a single assay; it is based on color-coded polystyrene beads and includes a dual-laser system and a flow-cytometry system.

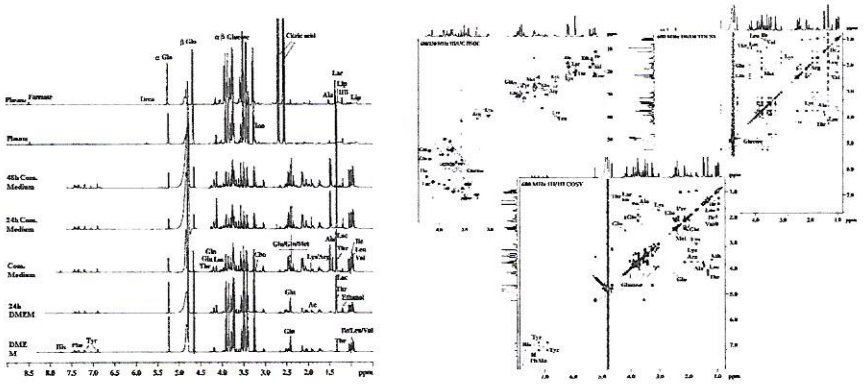
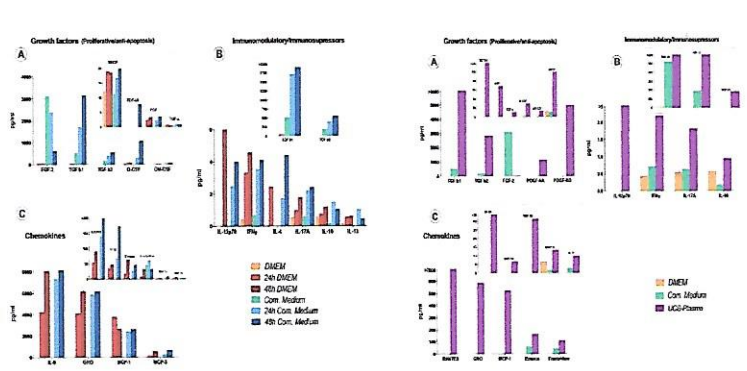


Figure 1: ^{1}H, COSY, TOCSY, HSQC spectra of unconditioned and hMSCs conditioned media, and umbilical cord blood plasma (hUCBS)

Relative quantitative distribution of the main metabolites in the samples



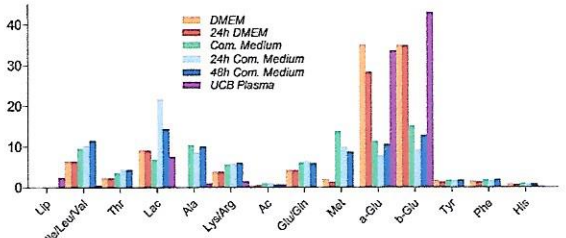


Figure 2: Proliferative and anti-apoptotic growth factors **(A)**, immunomodulatory, immunosupressive cytokines **(B)** and chemokines **(C)** concentrations in the samples studied by MLBA.

The results from NMR and MLBA analysis of hUCBS have allowed the identification of specific components and factors that have *in vitro* promoting effects on hMSCs expansion. It was proved that hUCBS is an alternative for the common FBS culture medium supplement used in hMSCs isolation, expansion and cryopreservation. Also, the CM obtained by hMSCs expansion and the hUCBS are very rich in growth factors with proliferative and anti-apoptotic functions, therefore can be an attractive alternative to the *in vivo* transplantation of hMSCs for tissue regeneration.

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