Bone Marrow Derived Mesenchymal Stem Cells from Five Donors Perceived by Raman Spectroscopy at a Single Cell Level

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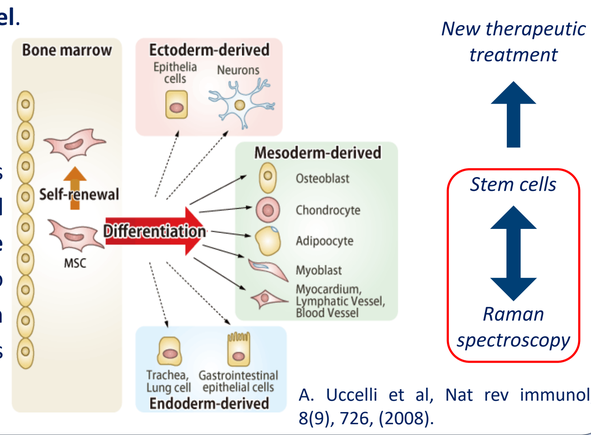
Introduction

- **Mesenchymal stem cells (MSCs)**, as adult stem cells, hold great promise in the field of regenerative medicine. However, significant obstacle for their clinical application is the lack of standardized markers for their isolation and characterization.
- **Raman spectroscopy** is fast, non-invasive optical technique based on an inelastic scattering of the visible light on a probed material, providing a unique (bio)chemical information. Therefore, it is considered that the Raman spectrum is a unique imprint of the analyte, implying that Raman spectroscopy could be the method of choice for the analysis of MSCs properties on a **single cell level**.

OBJECTIVES:

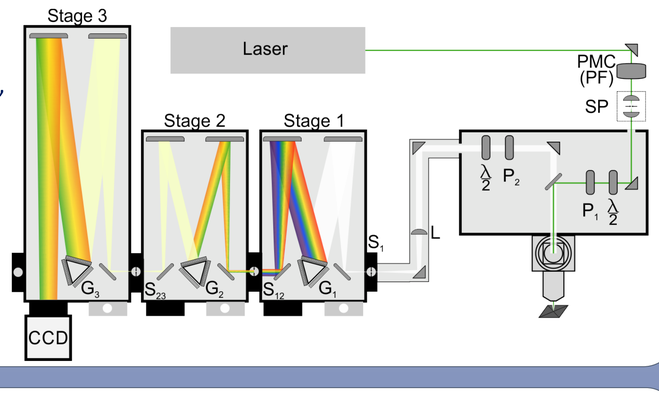
- to **isolate and characterize** bone marrow MSCs (BMMSCs) from **five donors** using **standard biological procedures** and to detect their **biochemical profile using micro-Raman spectroscopy** with the goal to investigate the possibility to employ micro-Raman system **to distinguish individual features** of BMMSCs populations.



A. Uccelli et al, Nat rev immunol, 8(9), 726, (2008).

Method/Design

1. Isolation and Cultivation of Bone Marrow Derived Mesenchymal Stem Cells **(BMMSCs):**
   - Isolated from samples (2ml) aspirated from iliac bone during collection of bone marrow for allogenic transplantation from five healthy donors (age 2-12 years), grown in standard cultivation conditions; cells from 5th passages were used.
   - Confirmed immunophenotype by flow cytometry: expression of positive mesenchymal cell surface markers (CD90, CD44, CD73, CD105) and negative markers (CD34, CD45 and HLA-DR).
   - Multilineage differentiation capacity was investigated based on corresponding histochemical staining: alkaline phosphatase and alizarin red staining were used to detect early and late osteogenesis; chondrogenesis was detected based on Safranin O staining of proteoglycans, while Oil red was used to visualize lipid drops of differentiated adipocytes.
2. Sample preparation
   - BMMSCs were seeded on rounded CaF_2 Raman grade slides in 24-well plate (5 x 10^3 cells per slide) and cultivated in standard conditions during 24 h.
   - Following the adhesion, cells were washed with saline buffer and fixed with methanol for 10 min at the room temperature.
   - Right before Raman spectroscopy was performed, the samples were washed with distilled water.
3. Raman spectroscopy:
   - TriVista 557 Raman system,
   - backscattering configuration,
   - Coherent Ar^+/ Kr^+ ion gas laser, 514.5 nm,
   - x 50 microscope objective, NA=0.50,
   - laser spot diameter ≈ 20 µm,
   - laser power at the sample plain ≈ 5 mW,
   - acquisition time/spectrum 300s,
   - 50-100 cells per each cell population (D1-D5) were analyzed.



Results: Biological Assays

In accordance with the minimal criteria for defining MSCs of International Society for Cellular Therapy (ISCT), we successfully isolated BMMSCs, showing typical adherent, fibroblast-like morphology (**Figure 1A**). All donors (D1-D5) exhibit high expression of positive mesenchymal cell surface markers (CD90, CD44, CD73, CD105) without the expression of negative markers (CD34, CD45 and HLA-DR) (**Figure 1B).**

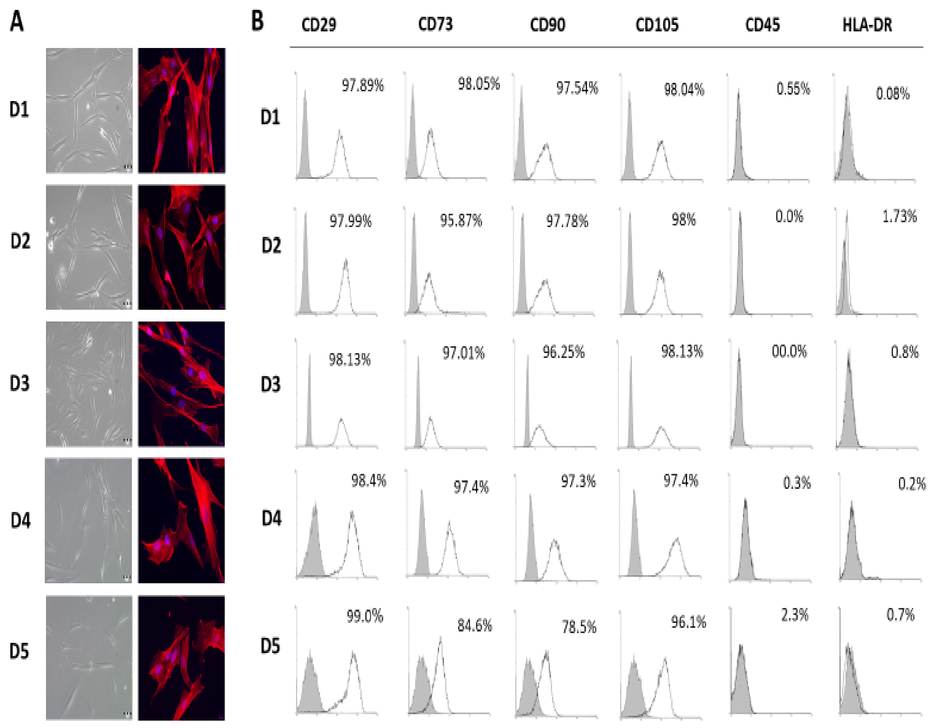


Figure 1. Morphology and immunophenotype of BMMSCs. A) Fibroblaste-like shape of isolated BMMSCs. Cells were grown in GM (Scale bars: 50 µm); TRITC-conjugated phalloidin labeled F-actin (red)/DAPI nuclear staining (blue) merged (Scale bars: 20 µm). **B)** Immunophenotype of cells determined by flow cytometry. Representative flow cytometry histograms are shown. Empty histograms indicate the percentages of positive cells based on the isotype controls (shaded histograms).

Results: Biological Assays

- The multilineage differentiation capacity toward osteogenesis, chondrogenesis, and adipogenesis was confirmed (**Figure 2**) .

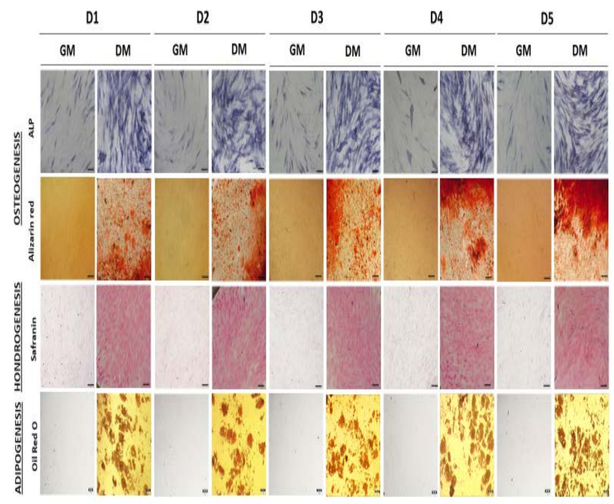


Figure 2. Multilineage differentiation potential of BMMSCs. Cells were cultivated in GM or differentiation medium (DM). Representative photos of osteogenesis determined by ALP and Alizarin staining (Scale bars: 50 µm), chondrogenesis determined by Safranin (Scale bars: 50 µm) and adipogenesis determined by Oil red (Scale bars: 20 µm) are shown.

Results: Raman Spectroscopy

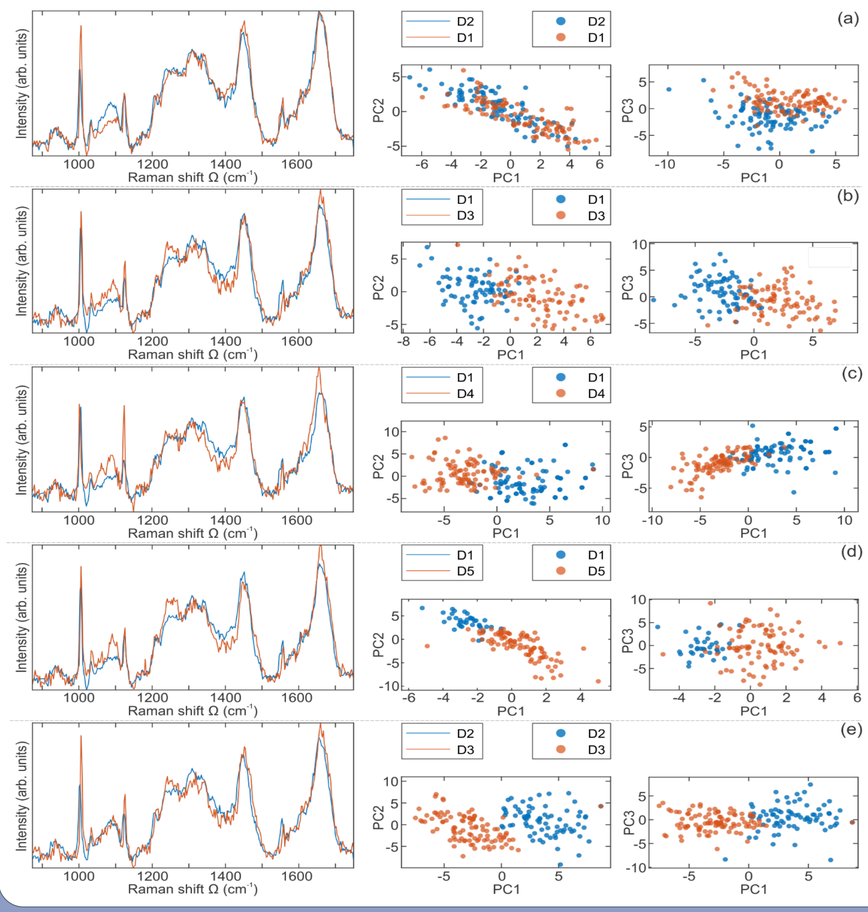


Figure 3. A comparative display of the averaged Raman spectra (red and blue lines), per cell populations: a) D1-D2, b) D1-D3, c) D1-D4, d) D1-D5, e) D2-D3. Principal Component Analysis (PCA) score plots are represented with red and blue dots.

Results: Raman Spectroscopy

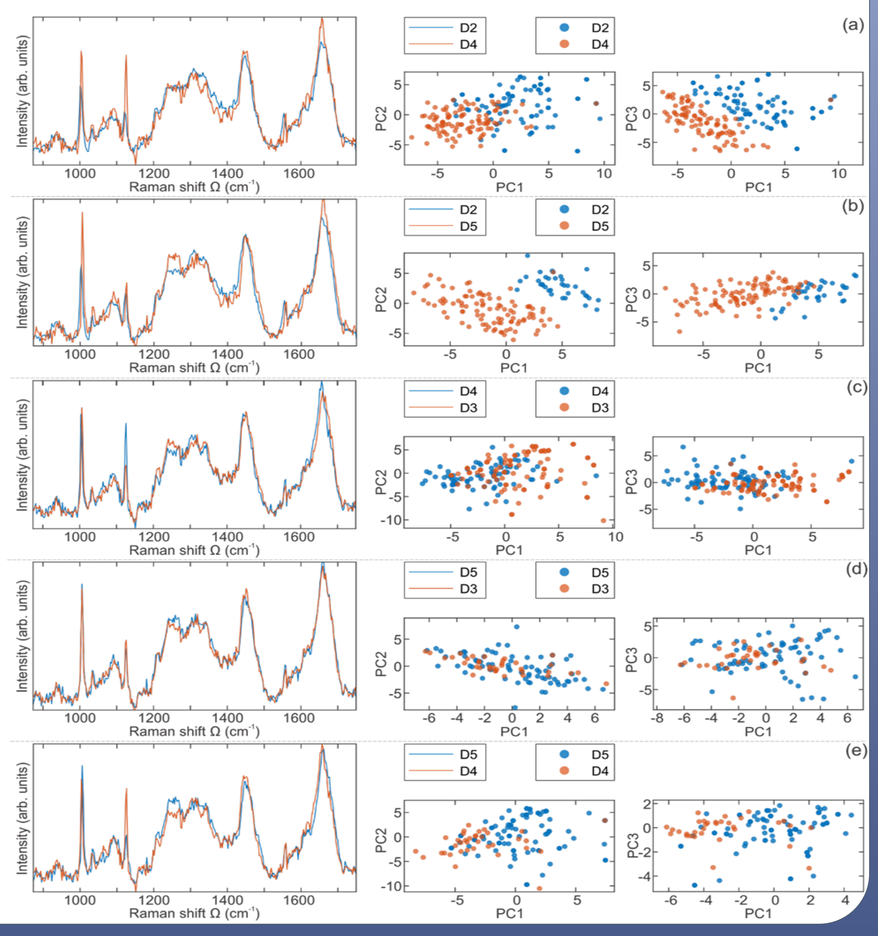


Figure 4. A comparative display of the averaged Raman spectra (red and blue lines), per cell populations: a) D2-D4, b) D2-D5, c) D3-D4, d) D3-D5, e) D4-D5. PCA score plots are represented with red and blue dots.

Conclusions

- Based on *standard biological tests*, **no differences between the donors were detected**.
- *Raman spectroscopy* coupled with multivariate statistical method – **Principal Component Analysis, showed distinct clustering**, based on specific spectral features, **between the donors**, implying that Raman spectroscopy could be a step forward in routine analyzes of BMMSCs (**Figure 3 and 4**).
- Although the significance of standard techniques should not be neglected, a comprehensive analysis can only be achieved by their association with Raman spectroscopy.