Raman spectroscopy applications in biological testing

Assist. prof. Vladka Lešer
Assist. prof. Nevenka Kregar Velikonja

Faculty of Health Sciences Novo mesto

COST MP1401 Annual Conference
Zadar, 12. - 15. April 2016

Novo mesto, Slovenia

Outline

- Mayor benefits of Raman spectroscopy for investigation of biological samples
- Example of stem cells (and some others applications)
- Conclusion

Raman spectroscopy & biological testing

- Raman spectroscopy
  - has proved to be a versatile technique to study biological samples, providing information regarding molecular structure and interactions and intracellular effects (Notingher et al., 2002).
  - provides a convenient non-destructive and location-specific means of probing cellular physiology and tissue physiology at sub-micron length scales (Huser & Chan, 2015).

Mayor benefits of Raman spectroscopy for investigation of biological samples and processes are:

- the distribution and metabolic products of small molecules that cannot be labelled with fluorescent dyes can be analysed (Huser & Chan, 2015);
- quantification of pathogens according to specific DNA sequences with Raman spectroscopy (e.g.: disease specific DNAbased SERS assay that demonstrates the simultaneous detection of three bacterial meningitis pathogens) (Gracie et al., 2014);
- Raman spectral measurements allow repeated investigations of live biological samples (e.g. cell cultures) (Ghita et al., 2015).

Potentials for R&D colaboration

Development of methods for:

- Non-distuctive monitoring of stem cell differentiation and tracking of differentiation specific metabolites (in terms of evaluation of stemness, differentiation potential and therapeutic potential of isolated/cultured cell population).
- Nanoparticle interaction with cells (in terms of monitoring tests in ecology and nanoparticle delivery for cell regulation).
- Microorganism detection/diagnostics in biological fluids.

Example of stem cells

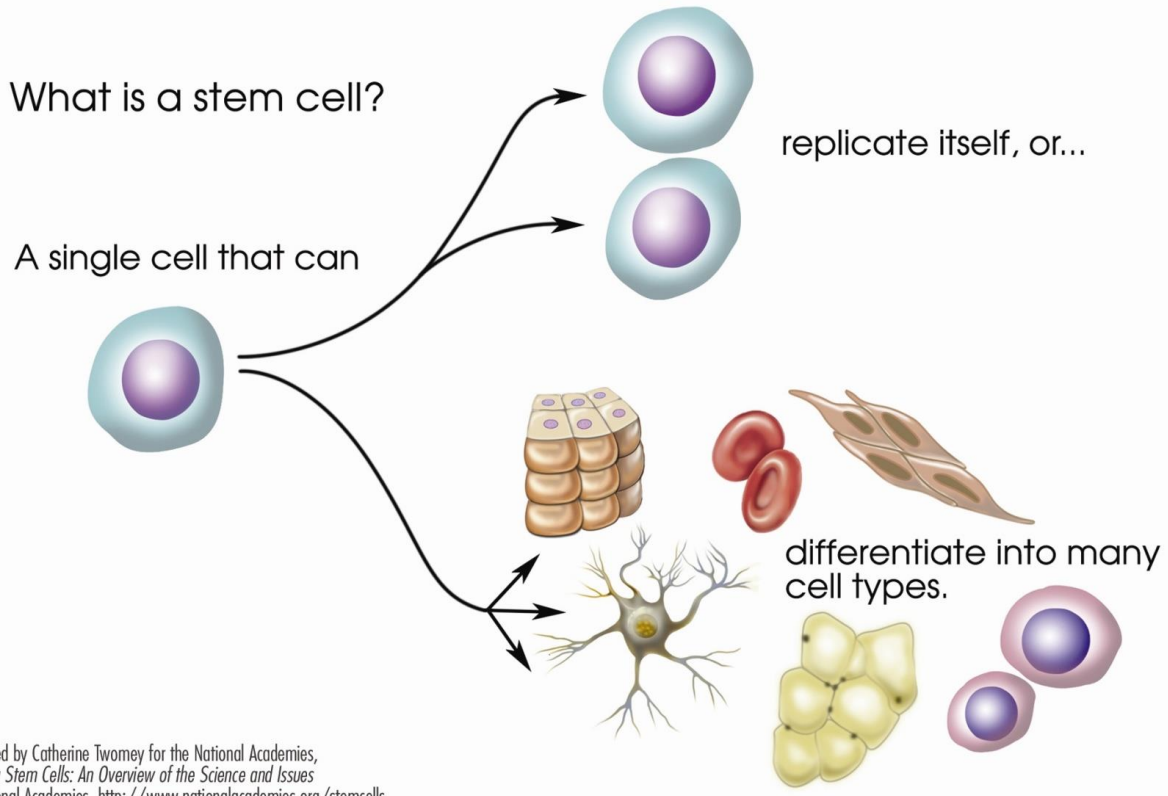
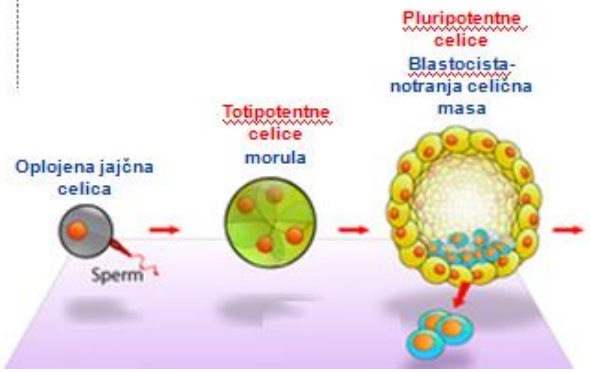
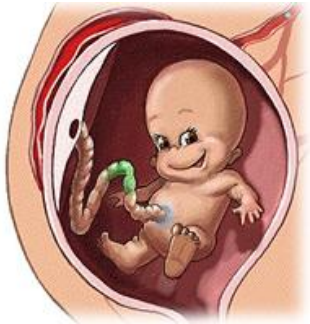


Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.

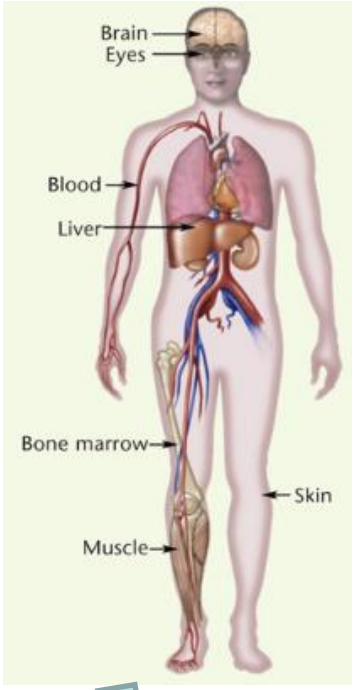
Stem cell types

(pluripotentne celice)
Multipotentne celice
Unipotentne celice





→ **birth** → **adult**



5-7 day

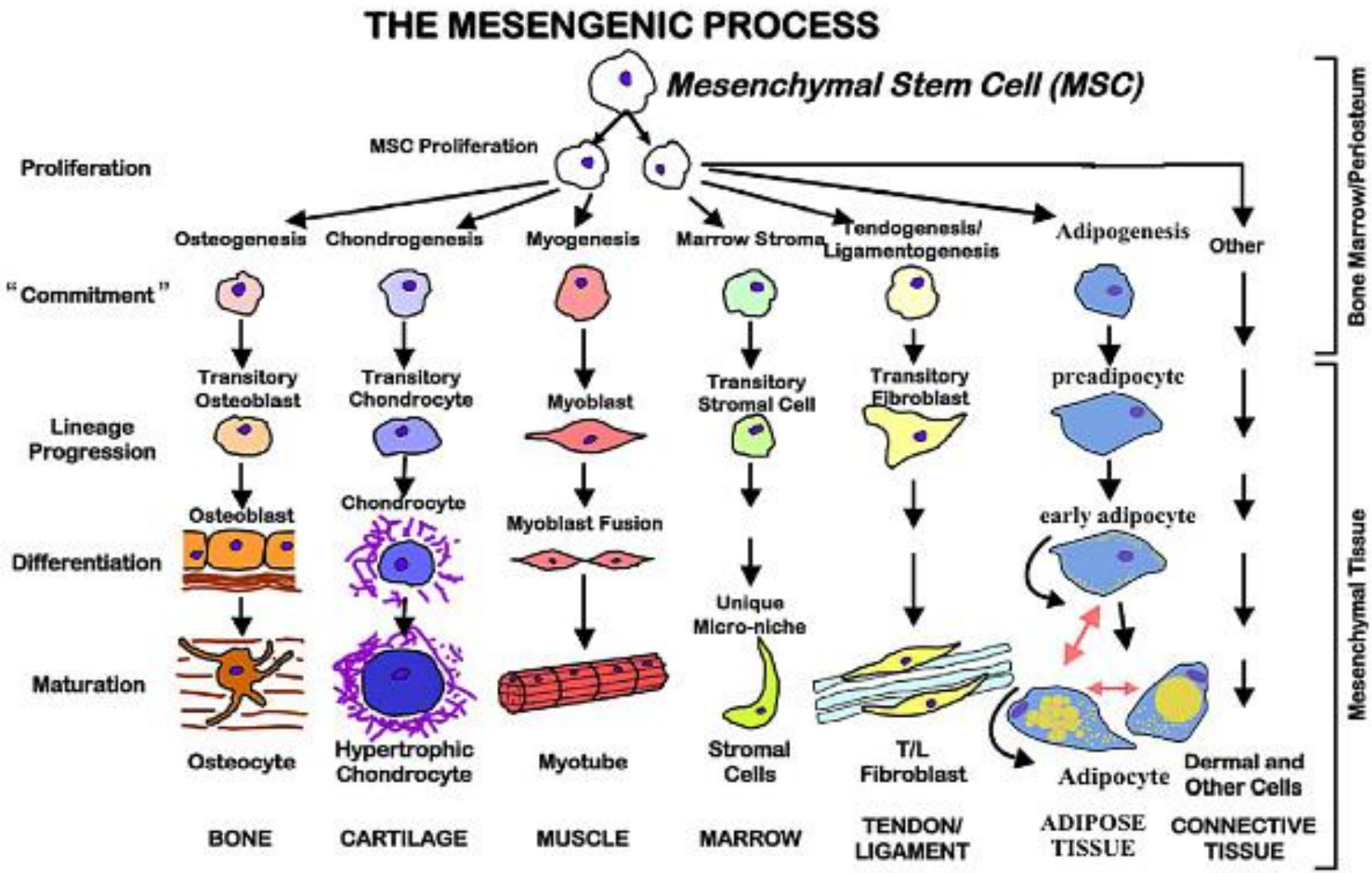
Embrional stem cells

Fetal stem cells

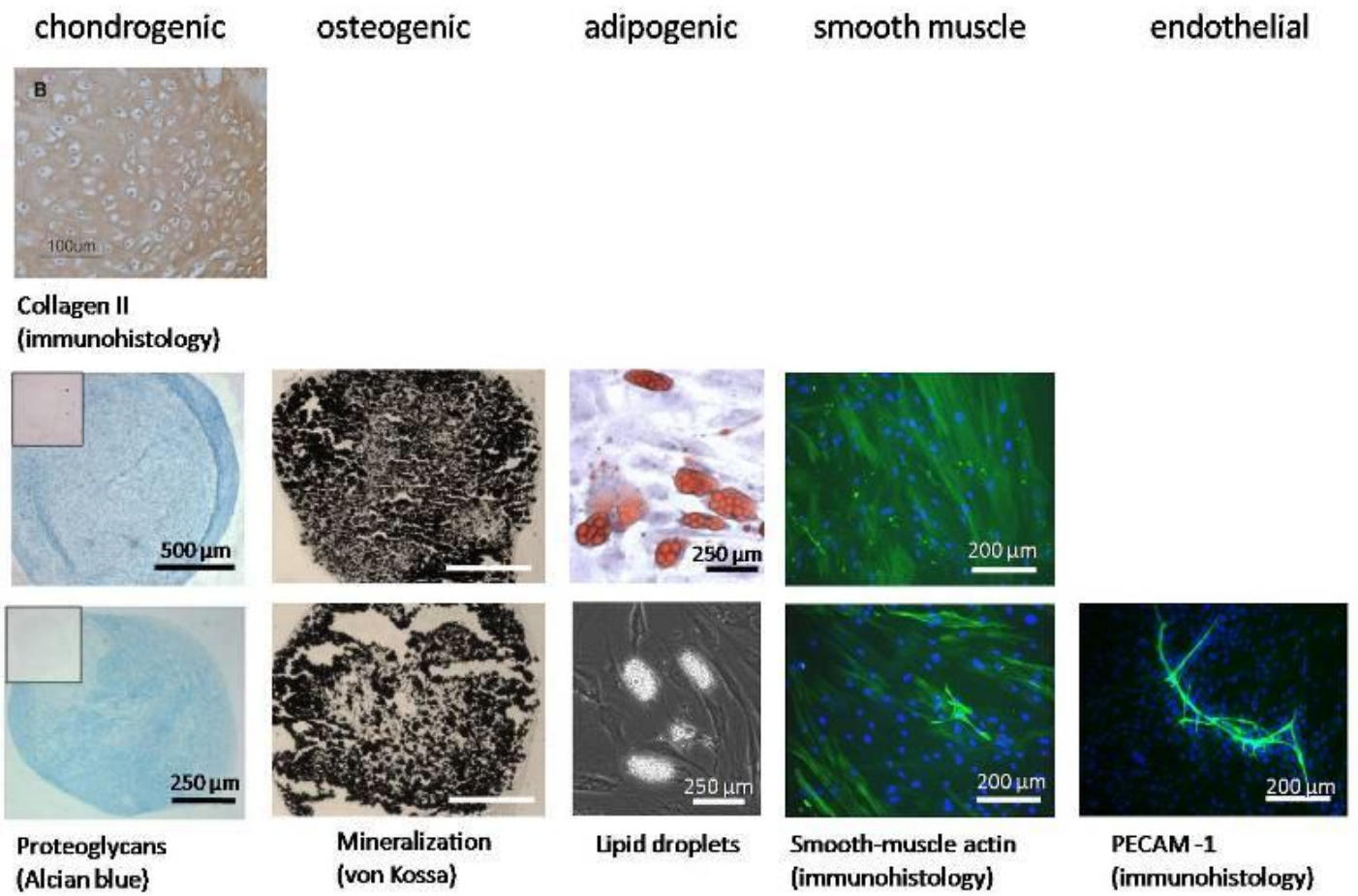
Stem cells isolated from obstetric tissues
-Umblical cord blood
-Umblical cord
-Amnionic membrane

Adult stem cells
-Bone marrow
-Adipose tissue
-Other tissues
Different stem cell types (e.g. **MSC**, HSC, VSEL)

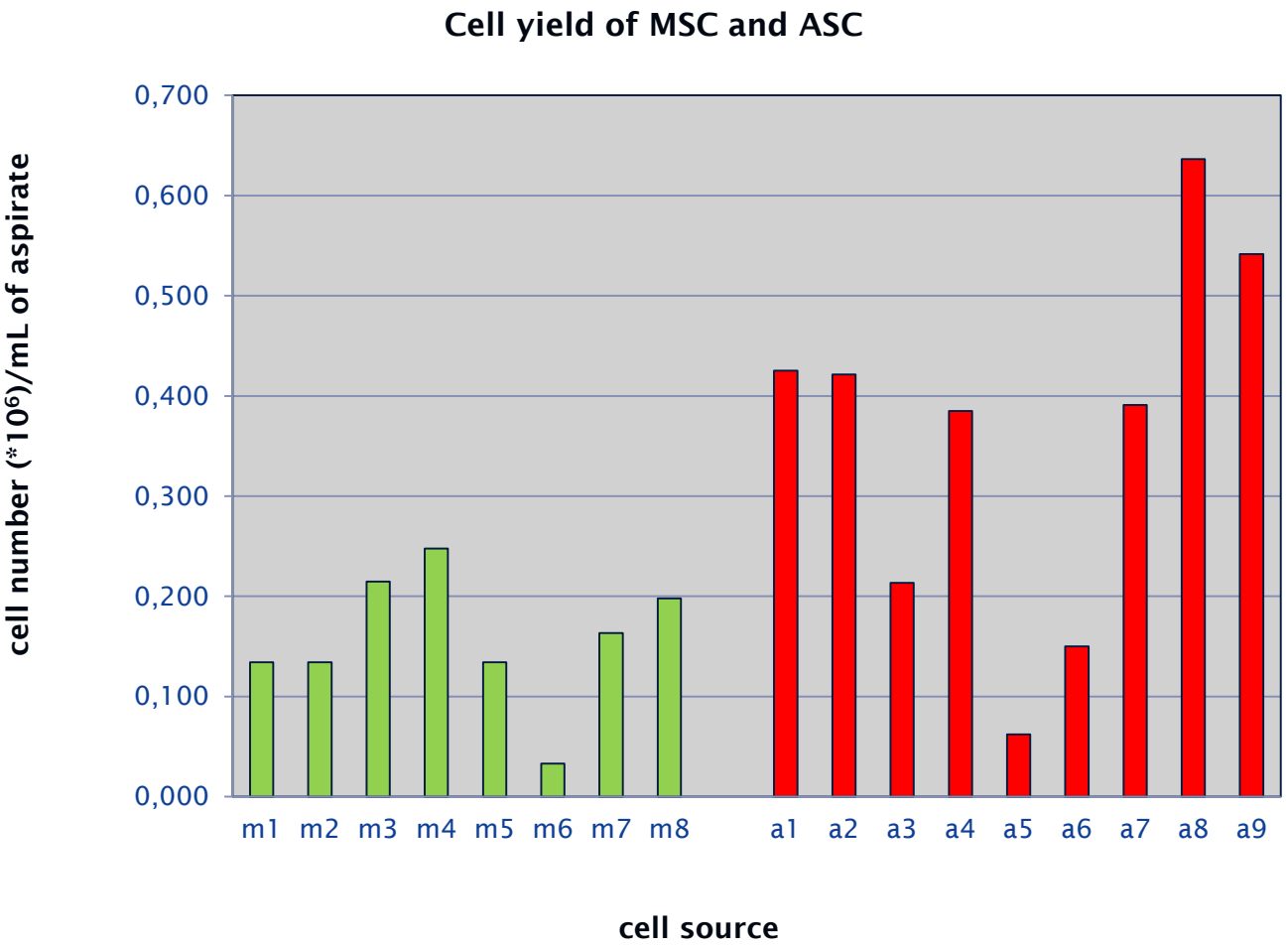
Differentiation & therapeutic potential of MSC



In vitro differentiation of stem cells



Yield of stem cell isolation from primary cultures

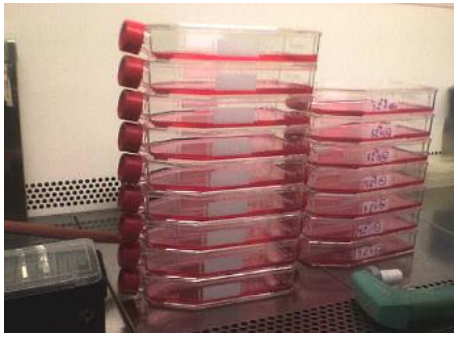


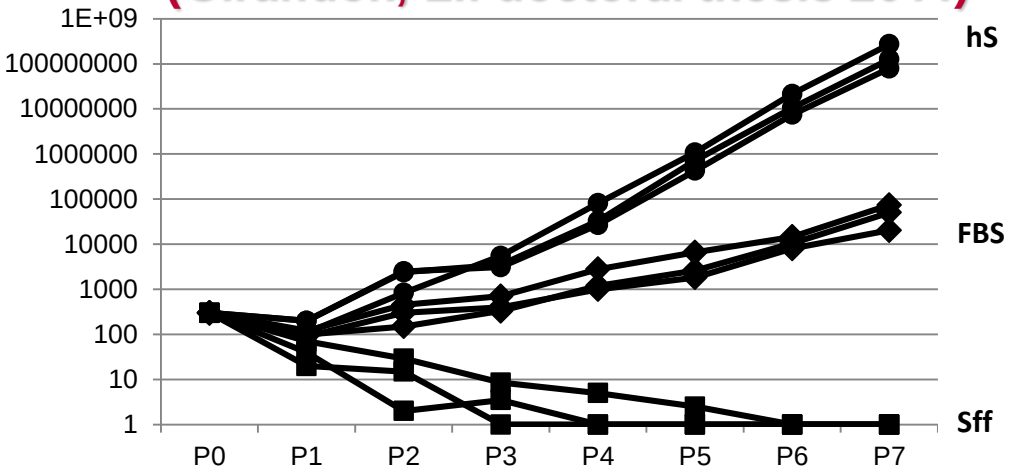
Investigation of stem cell differentiation

- Limited access to stem cell sources
- Time dependant process
- Biological variability
  - among parallel samples,
  - among donors,
  - influence of *in vitro* conditions

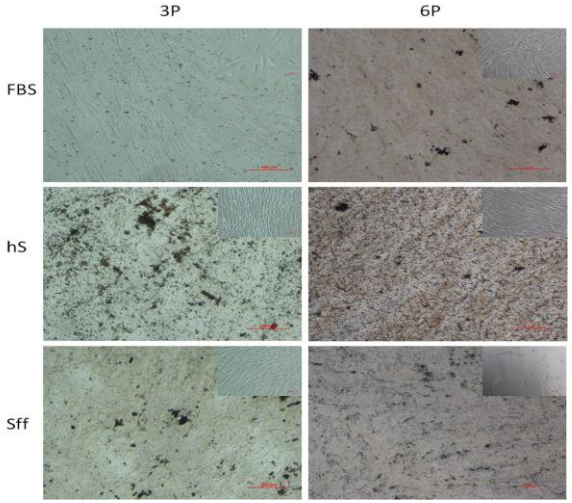
Example of test set-up: Study of adipose derived mesenchymal stem cell differentiation

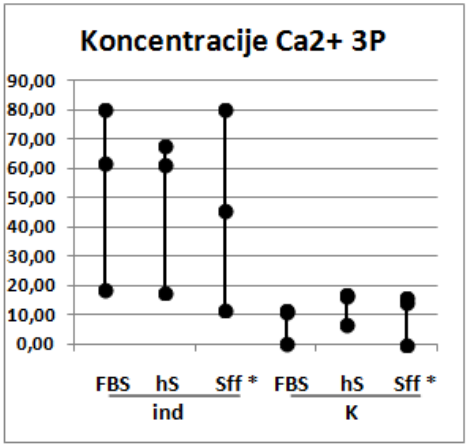
(Girandon, L.: doctoral thesis 2011)





Proliferation of adipose derived stem cells in **different culture conditions**





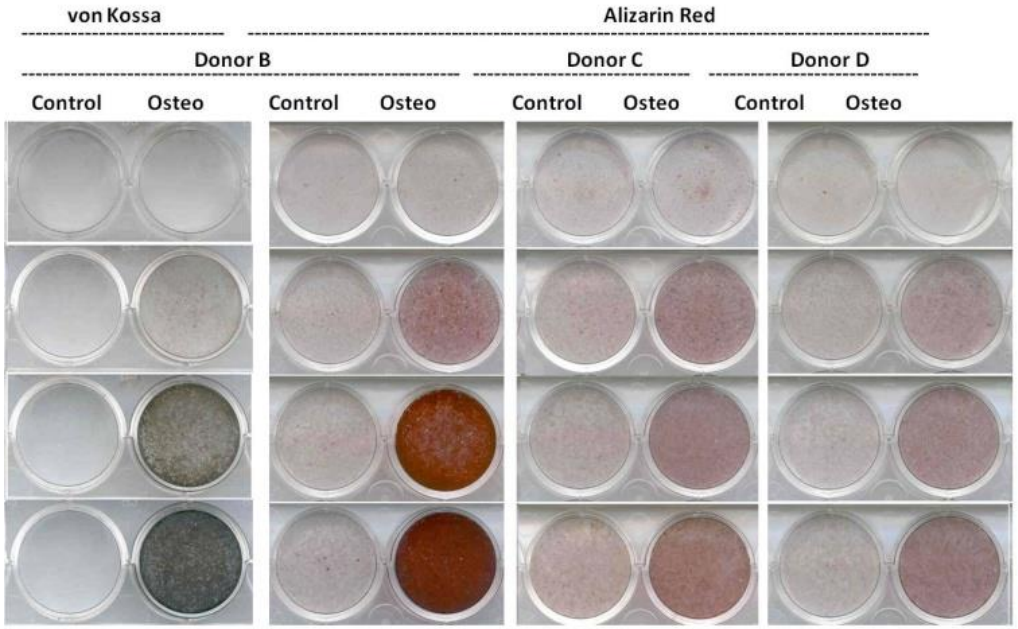
Osteogenic differentiation (Von cossa staining) and Ca+ deposition of adipose derived stem cells in **different culture conditions**

Each dot requires destruction of cell culture

With a relevant non-destructive method we could obtain 8X more data

Example of test set-up:
Study of adipose derived mesenchymal stem cell differentiation

Fröhlich, M.; Grayson, W.L.; Marolt, D.; Gimble, J.M.; Kregar-Velikonja, N. & Vunjak-Novakovic, G. (2010). Bone Grafts Engineered from Human Adipose-Derived Stem Cells in Perfusion Bioreactor Culture. *Tissue Eng* Part A: 16(1):179-89.



Investigation of donor variability

- 3 donors
- 4 time points

Each culture well requires destruction of cell culture
With a relevant non-destructive method we could obtain cultures for 4X more parallel measurements.

Example of test set-up: Study of MSC osteogenic differentiation for tissue engineering of temporomandibular bone

Engineering anatomically shaped human bone grafts

Warren L. Grayson[a], Mirjam Fröhlich[a,b], Keith Yeager[a], Sarindr Bhumiratana[a], M. Ete Chan[c], Christopher Cannizzaro[d], Leo Q. Wan[a], X. Sherry Liu[c], X. Edward Guo[c], and Gordana Vunjak-Novakovic[a,1]

PNAS | February 23, 2010 | vol. 107 | no. 8 | 3299–3304

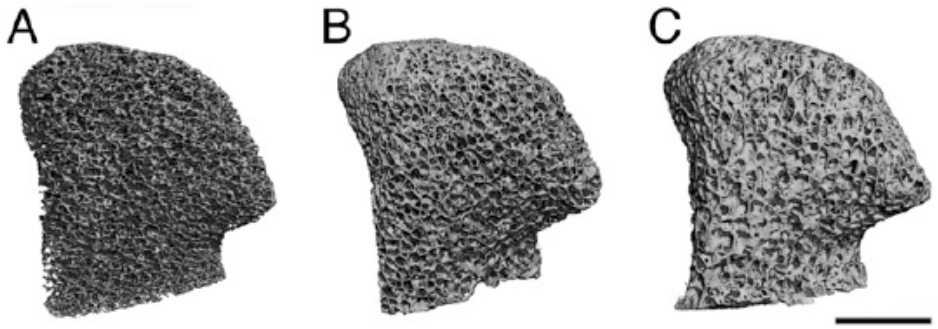


Fig. 4. Architecture of the mineralized bone matrix developed with time and in a manner dependent on culture conditions. The reconstructions of 3D μCT images demonstrate the changes in pore structure (relative to the initial state) that were evident at the end of the 5-week cultivation period. Bioreactor constructs exhibit more rapid deposition of new mineral matrix as compared with static constructs (see Table 1). (Scale bar: 5 mm.)

A: before cell seeding
B: after 5 weeks – static conditions
C: after 5 weeks – bioreactor cultivation

$3x10^7$ cells needed for each of parallel samples:

- DNA content
- Hard tissue histology
- SEM
- Measurements in final time point

Example of monitoring of MSC osteogenic differentiation with Raman spectroscopy

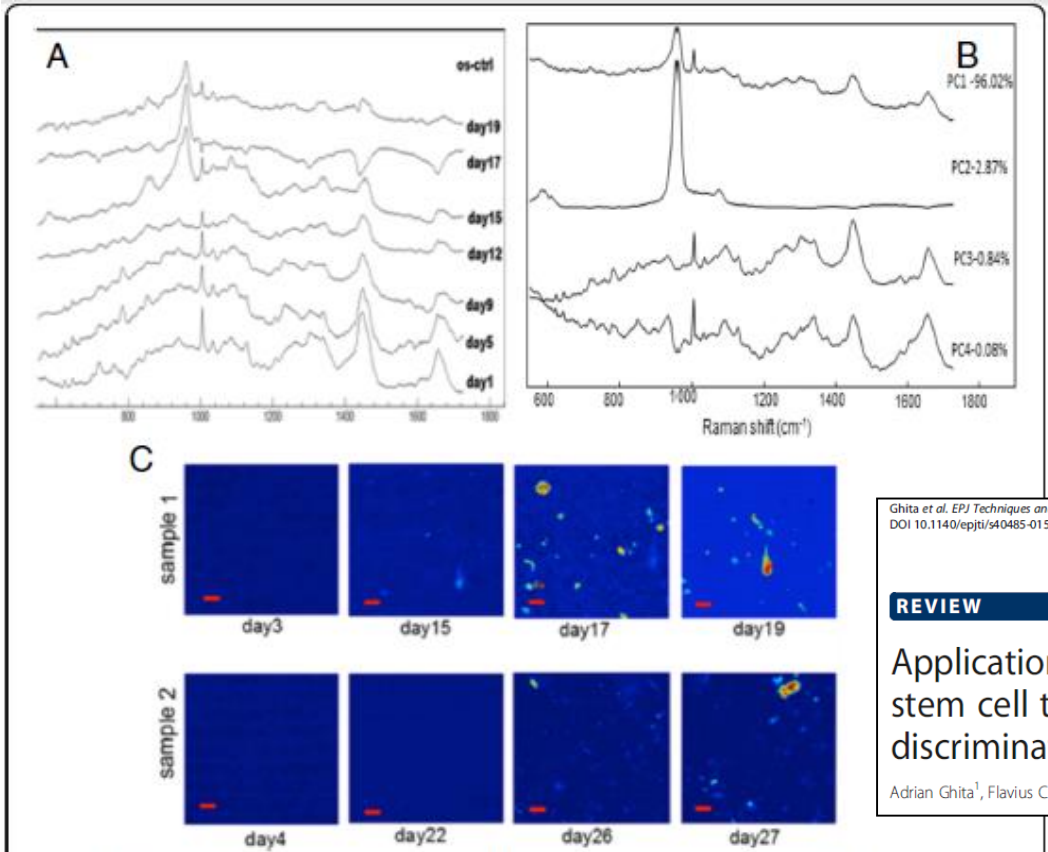


Figure 5 Time-course Raman spectroscopy of MSC grown in osteogenic medium. (A) Mean time-course Raman spectra of typical 210 × 210 mm^2 regions of the cultures shown in C. Raman spectra were acquired at different time points for the same regions of the culture osteoblast in osteogenic medium and non-osteogenic medium. **(B)** Principal components used to extract meaningful chemical information **(C)** Maps corresponding to the PC2 scores recorded in the same culture regions at different measurements days. Scale bar: 10 μm.

Ghita *et al. EPJ Techniques and Instrumentation* (2015) 2:6
DOI 10.1140/epjti/s40485-015-0016-8

EPJ Techniques and Instrumentation
a SpringerOpen Journal

REVIEW Open Access

Applications of Raman micro-spectroscopy to stem cell technology: label-free molecular discrimination and monitoring cell differentiation

Adrian Ghita[1], Flavius C Pascut[1], Virginie Sottile[2], Chris Denning[2] and Ioan Notingher[1*]

Raman spectroscopy in diagnostics of bone disease: distinguishing between osteoarthritic and healthy bone

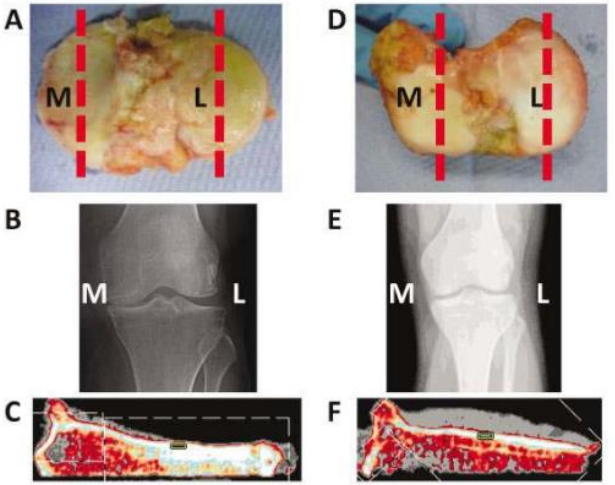


Figure 1. Assessment of the tibial plateau from a patient with osteoarthritis (OA) compared to that from a non-OA control. Postoperative photographs (**A** and **D**) and preoperative radiographs (**B** and **E**) of the medial (**M**) and lateral (**L**) tibial plateaus from a representative patient with OA (**A** and **B**) and a non-OA control (**D** and **E**) are shown. The medial tibial plateau (**C**) and lateral tibial plateau (**F**) from a patient with OA were assessed by peripheral quantitative computed tomography (pQCT). In the pQCT images, the region of interest is demarcated as a green box. The red broken lines in **A** and **D** indicate the plane from which the pQCT measurements were obtained.

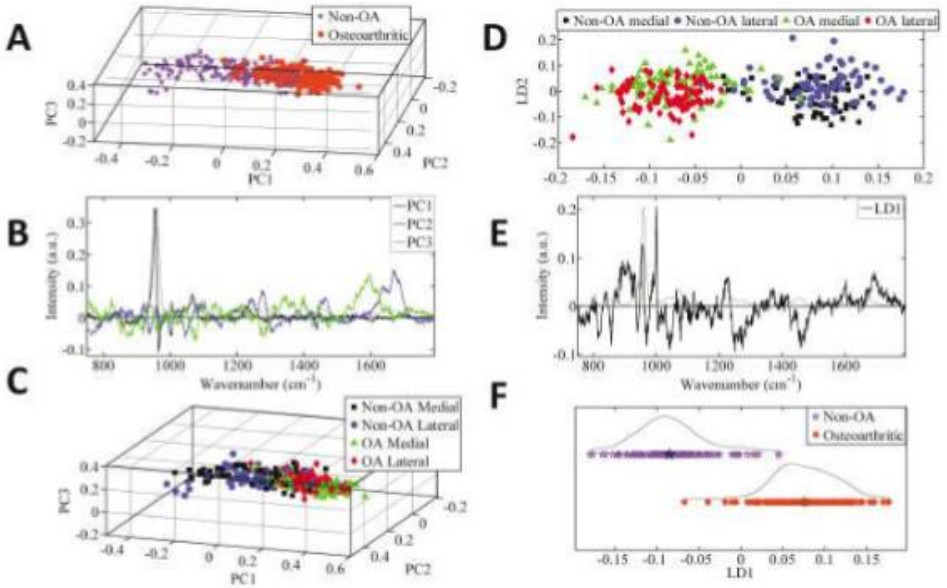


Figure 4. Principal components analysis (PCA) of the Ramen spectra. **A–C,** Plot of PCA scores for the non-osteoarthritis (non-OA) tibial specimens compared to the OA tibial specimens (**A**), the corresponding PCA loadings plot (**B**), and the color-coded plot of PCA scores in the non-OA and OA medial and lateral compartments (color-coded to enable identification of the medial and lateral spectra) (**C**). **D–F,** Plot of PCA–linear discriminant analysis (PCA-LDA) scores for the non-OA and OA medial and lateral compartments (**D**), the corresponding PCA-LDA loadings plot (**E**), and the plot of PCA-LDA scores for the non-OA tibial specimens compared to the OA tibial specimens (**F**). The data in **F** are plotted along the x-axis, and split along the y-axis only for ease of visualization. The loadings plots (**B** and **E**) show the axes (linear combination of the variables) for the PCA-LDA and LDA analyses, respectively. In loadings plots, the larger the peak, the more influence it has on any separation in the scores plotted along a particular axis.

Kerns et al.

ARTHRITIS & RHEUMATOLOGY
Vol. 66, No. 5, May 2014, pp 1237–1246

Quantification of pathogens according to specific DNA sequences with Raman spectroscopy

Simultaneous detection and quantification of three bacterial meningitis pathogens by SERS

Chem. Sci., 2014, **5**, 1030

Kirsten Gracie,[a] Elon Correa,[b] Samuel Mabbott,[a] Jennifer A. Dougan,[a] Duncan Graham,[a] Royston Goodacre*[b] and Karen Faulds*[a]

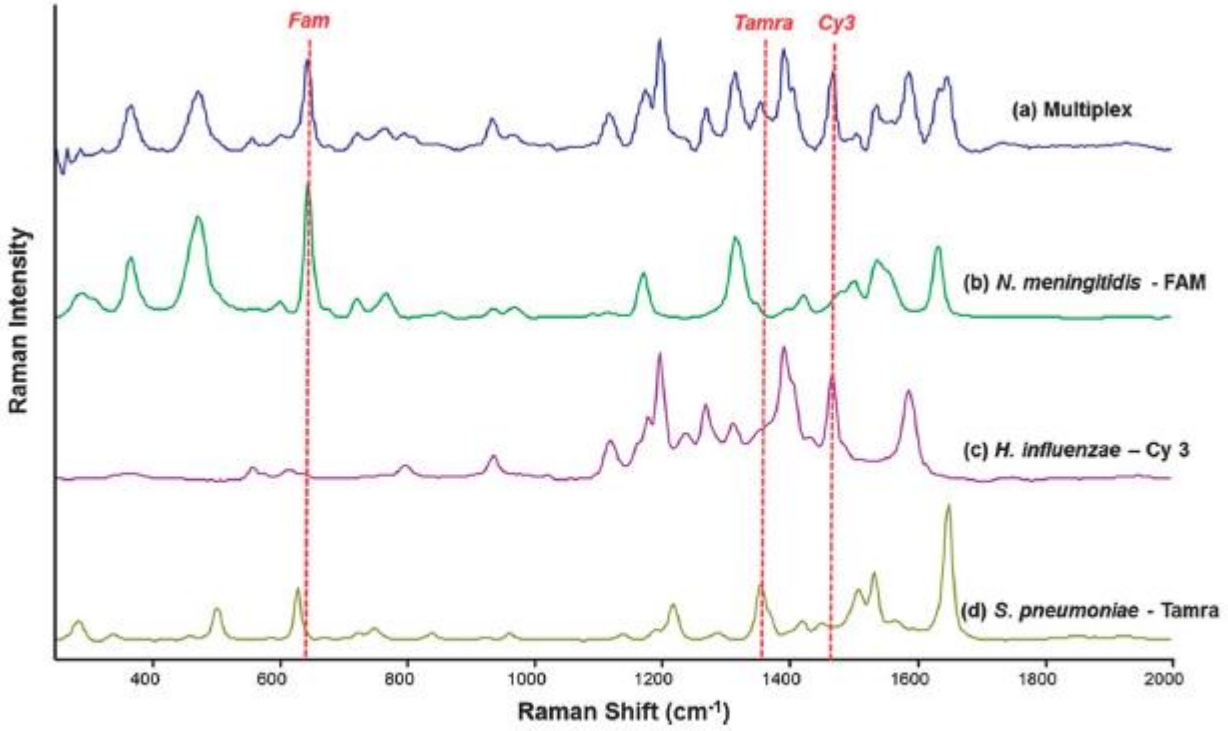


Fig. 4 Stacked SERS spectra showing the spectra obtained from the simultaneous detection of all three bacterial meningitis pathogens using the detection assay (a) and the SERS spectra obtained from the detection of each pathogen separately; *N. meningitidis* using PCR product concentration of 1.13×10^5 copies per μL (b), *H. influenzae* using PCR product concentration of 7.24×10^5 copies per μL (c) and *S. pneumoniae* using PCR product concentration of 2.4×10^4 copies per μL (d). SERS spectra were recorded using an excitation wavelength of 532 nm and a diode laser with a 10 s accumulation time. The red dotted lines show peaks that are unique to each SERS spectrum and hence each pathogen.

Conclusion

- Raman spectroscopy has been proved to be suitable for solving some biomedical problems.
- Future?

Thank you for your attention!