β-TM-induced structural and functional variations in BM-MSCs important because it may provide basic understanding of hematopoetic stem cell (HSC)-MSC interactions in such a pathological bone marrow microenvironment. In this scope, firstly, BM-MSCs were characterized in terms of their morphological, immunophenotypical and differentiation properties. Then, variation in the macromolecular concentrations in between studied groups was obtained visually. The spectral results reflected that there were significant changes in the concentrations of lipids, proteins, glycogen and nucleic acids in children and adolescent group BM-MSCs when compared with the infants, early and mid adults. In β-TM disease study, the differences in chemical maps belonging to different macromolecules clearly indicated the succesfull differentiation of healthy control, pre- and post-transplant BM-MSCs by FTIRM.

1737-Pos Board B629

Characterization of Sodium Butyrate Induced Differentiation in Colon Cancer Cells by Fourier Transform Infrared Spectroscopy and Microscopy

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Colon cancer is a major cause of morbidity and mortality throughout the world.The pathogenesis of colon cancer with respect to loss of cellular differentiation has not been clearly understood. To understand this loss in colon cancer and to differentiate the cancer cells, sodium butyrate (NaB), one of the differentiation inducer, is commonly used. NaB is produced in the colonic lumen as a consequence of bacterial fermentation of complex carbohydrates and it alters colon cancer cell morphology and reduces the cell growth and motility. Although, the differentiation process of colon cancers, which are stimulated with NaB, has been resolved at genetic level, the underlying mechanisms regarding structural and functional alterations regulating the differentiation of colon cancer cells have not been well characterized. Therefore, in this study, it was aimed to explore the NaB-induced macromolecular structural and functional changes in differentiation of colon cancer cells by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and FTIR microspectroscopy. These changes were determined from the spectral analysis of control and NaB treated colon cancer cell spectra and their chemical maps. Based on the spectral differences, cluster analysis was applied to discriminate the groups. The spectral analysis indicated the differences in saturated and unsaturated lipids, protein and nucleic acid content between the control and NaB treated cancer cells. The variation in membrane fluidity and lipid order was also determined in the NaB treated cells. Moreover, the successful discrimination between control and treated cells was obtained. The results of this study not only shed light on better understanding the loss of differentiation mechanisms in colon cancers but also strongly support the power of ATR-FTIR spectroscopy and FTIR microscopy as novel, simple, reagent-free methods for the identification of differentiated cancer cells.

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Optical Method to Asses Ex-Vivo the Extent of Atherosclerosis in Mouse Aortas

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The main cause of Coronary Artery disease, the first cause of death in most industrialized countries, is Atherosclerosis. Atherosclerosis is a chronic inflammatory disease in the walls of arteries, promoted by low density lipoproteins (LDL) without adequate removal of cholesterol. Over the years, the response of the body to the continuous buildup of cholesterol is the formation of a plaque (atheroma) that can obstruct blood flow.

Many therapeutic strategies focus on reducing the size of the atheroma and the preclinical studies require methods to measure lesion extension in an accurate, reliable manner. One of the most used methods in mice is called *en face.* This technique consists of dissecting the aorta, opening it longitudinally to expose the luminal side, and staining it with dyes to reveal lipid-laden plaques. Photographs of the labeled arteries are taken after the staining and the area occupied by the stained structures (atheroma) is determined by using image-processing software. The pitfall of this method is the lack of tri-dimensionality that may lead to misestimation damage extension.

Here we show a fast and relatively simple solution for the lack of tri-dimensionality of the *en face* method by tri-dimensionally imaging the dissected aortas with a femtosecond pulsed infrared (IR) laser. We used mice prone to develop Atherosclerosis (*Apoe*$^{-/-}$) having different diets and age and compared the extension of the atheromic damage using *en face* and our optical method. Our results show the advantages of using volume for assessing atherosclerotic damage quantification and also the potential of the method in the characterization of single atheroma and atherosclerotic damage in preclinical studies.

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Spatial Organization of RNA Polymerase II Revealed by Super-Resolution Imaging of Mammalian Cell Nucleus

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Super-resolution microscopy based on single-molecule centroid determination has been widely applied to biology in recent years. However, quantitative imaging of mammalian nucleus has been challenging due to the lack of 3D optical sectioning methods for normal-size cells as well as the inability to accurately count the absolute copy numbers of biomolecules in highly dense structures. Here we report a Reflected Light Sheet Super-Resolution Microscopy (RLS-SRM) that allows counting of protein and RNA molecules inside a mammalian nucleus with single-copy accuracy, at 25-nm lateral and 50-nm axial resolutions. Applying RLS-SRM to probe the organization of mammalian transcription by RNA polymerase II (RNAP II), we observed that RNAP II distribution exhibit a punctate pattern with discrete foci, consistent with previous studies. Spatio-temporal clustering analysis showed that the average number of RNAP II molecules in each of the foci is one, and no statistical evidence is found for clustering of RNAP II molecules within 250 nm from each other, arguing against the hypothesis of 'transcription factories'. Two-color imaging revealed that 20% of the bound RNAP II is poised while 80% is actively transcribing. Using single-molecule FISH, we also imaged the distribution of U2 spliceosomal snRNA inside the nucleus, ~25% of which is found to colocalize with transcribing RNAP II, suggestive of co-transcriptional splicing. This study provides a way to quantitatively image and count key biomolecular species inside the mammalian nucleus with unprecedented level of detail.

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Potential and Limitation of Microparticle-Based Immunoassays: A Thermodynamic and Kinetic Study

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The fundamental significance of immunoassays in diagnostics and research drives the ongoing quest for improving assay formats, sensitivity and instrument technology. Early on, the advantage of immobilizing antibodies to a surface to allow for separation of bound and unbound reagent was realized in the immunometric (sandwich) assay format. Today, this approach is established in most immunoassays as performed using ELISA (enzyme linked immunosorbent assay), GCSPR (grating-couples surface plasmon resonance) and microparticle-based chemiluminescence. However, antibody immobilization to a surface hampers reaction kinetics and imposes mass transport limitation and sterical hindrance. Theoretical considerations indicate that these effects can be minimized by using a spherical surface underlining why recently developed ultra-high-sensitivity techniques rely on the utilization of microparticles. In the present study, we investigate the potential and limitation of microparticle-based immunoassays in respect to impact of mass transport and surface immobilization on the intrinsic thermodynamic and kinetic properties of antibody-analyte interaction.

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Vibrational Spectroscopy, Microscopy and Imaging Probes Cutaneous Wound Healing and Artificial Skin Structure

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Wound healing in human skin consists of a complex series of spatially and temporally organized events to prevent infection and restore barrier function. Multiple biological pathways are activated after an injury. We used a human skin model to study re-epithelialization of excisional wounds. Infrared (IR) microscopic imaging at ~ 10 micron spatial resolution revealed a population of disordered lipids in the migrating epithelial tongue (MET) which has not been previously reported. Particular spectral features allowed us to elucidate lipid structure/organization during the healing process (from day 0 to day 6 post-wounding). Although the role of lipids in the MET is currently unclear, this finding may ultimately aid in the development of improved therapeutic agents for wound care.