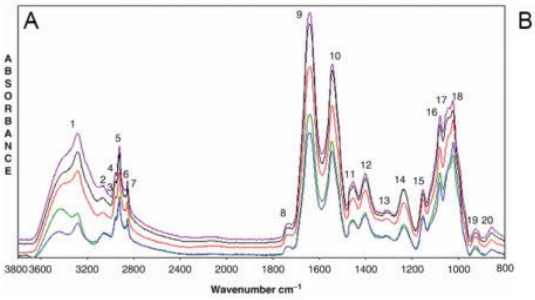
FTIR spectroscopy and ımaging to understand donor age effect on bone marrow mesenchymal stem cells

Stem cells, like Bone Marrow Mesenchymal Stem Cells (MSCs), have enormous potential to develop new therapies for tissue regeneration and repair with their ability to differentiate into many different types of cells. The aging process and the impact of donor age are important factors to achieve favorable clinical outcome with cellular therapy. Identification of molecular differences in stem cells depending on the donor age may contribute to better selection of donors in regenerative medicine and development of in vitro conditions for stem cell-based clinical applications. Therefore, understanding of the cellular, biochemical, and molecular interactions between MSCs and their microenvironment(niche) will contribute to development of better in vitro modeling strategies for stem cell studies.



B

Peak no.	Wavenumber (cm^{-1})	Definition of the spectral assignments
1	3339	*Amide A*: N–H and O–H stretching vibrations of polysaccharides, proteins
2	3065	*Amide B*: N–H vibrations of proteins
3	3015	*Olefinic – CH stretching*: unsaturated lipids, cholesterol esters
4	2957	CH_3 *antisymmetric stretching*: lipids, protein side chains, with some contribution from carbohydrates and nucleic acids
5	2924	CH_2 *antisymmetric stretching*: mainly lipids, with the little contribution from proteins, carbohydrates, and nucleic acids
6	2873	CH_3 *symmetric stretching*: protein side chains, lipids, with some contribution from carbohydrates and nucleic acids
7	2852	CH_2 *symmetric stretching*: mainly lipids, with the little contribution from proteins, carbohydrates, and nucleic acids
8	1740	*C=O stretching vibrations* of triglycerides, cholesterol esters
9	1638	*Amide I*: C=O stretching vibrations of proteins
10	1543	*Amide II*: N–H bending and C–N stretching vibrations of proteins

Peak no.	Wavenumber (cm^{-1})	Definition of the spectral assignments
11	1453	CH_2 *bending vibrations* of lipids
12	1402	COO^- *symmetric stretching*: fatty acid side chains
13	1310	*Peptide side chain vibrations*
14	1234	$PO^-{}_2$ *antisymmetric stretching*: fully hydrogen bonded, mainly nucleic acids with the little contribution from phospholipids
15	1152	*CO–O–C antisymmetric stretching vibrations* of glycogen and nucleic acid ribose
16	1080	$PO^-{}_2$ *symmetric stretching*: nucleic acids and phospholipids; *C–O stretching*: glycogen, polysaccharides, and glycolipids
17	1045	*CO stretching vibrations* of carbohydrates, glycogen; deoxyribose/ribose of nucleic acids
18	1025	*Mainly from glycogen*
19	975	*Sugar vibrations* in backbone of DNA-Z form
20	835	Vibrations in N-type sugars in nucleic acid backbone

Fig. 1. A. The representative infrared spectra of healthy BM-MSCs from different age donors. Red line represents infants' BM-MSCs, black line represents children's BM-MSCs, purple line represents adolescents' BM-MSCs, the green line represents early adults' BM-MSCs and the blue line represents mid-adults BM-MSCs in the 3800-800 cm-1 region. (The spectra were normalized with respect to the amide A band).
B. General band assignment of bone marrow mesenchymal stem cells (BM-MSCs).

There is a strong evidence that the aging process has an adverse effect on stem cells and their niche functions. Current studies have showed that stem cells of elderly donors have increased senescence and decreased proliferation capacity compared with stem cells obtained from younger donors. Reduction in the function of stem cell with age may be due to intrinsic molecular alterations or extrinsic changes in the stem cell niche. In this scope, an investigation of cellular markers is important to deeply understand the underlying mechanisms of aging and interconnected roles of intrinsic and extrinsic changes which cause aging process. In the bone marrow microenvironment, there is a mutual interaction between hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), non-stem cells, extra cellular matrix components and molecular signals to control stimuli for differentiation and self-renewal. The factors contributing the maintenance of HSCs in their niches have been recently investigated whose results indicated that MSCs facilitate HSCs maintenance through the secretion of soluble factors and cell-cell contact in bone marrow. Therefore, understanding of interactions between HSCs with MSCs will provide valuable information through the design of stem cell based cellular therapies. In this context, this study was designed to investigate the effect of aged bone marrow microenvironment on MSCs by using MSCs

of different aged healthy donors via infrared (IR) spectroscopy and imaging which may be useful through donor selection both for cellular transplantation and other stem cell therapies. Fourier transform infrared (FTIR) spectroscopy and microspectroscopy can be used as a novel, nondestructive, operator independent research methods to identify novel molecular marker(s) by enabling real-time chemical monitoring and high-quality data collection with less experimental complexity. Attenuated total reflection (ATR) mode of FTIR spectroscopy is a effective and easy tool to study biomedical samples because small amount of samples (~one drop) can directly be placed on ATR crystal. FTIR microspectroscopy (FTIRM) is an imaging technique in which a microscope is coupled with an infrared spectrometer. It provides spatially resolved information on unstained thin tissue samples or cell monolayers by allowing the generation of infrared (IR) images with high image contrast. Unlike staining techniques, IR microscopy with its label-free, noninvasive, and nondestructive properties generates information about relative concentrations and structure of macromolecules by considering alterations in the infrared spectra and the specific heterogeneities.

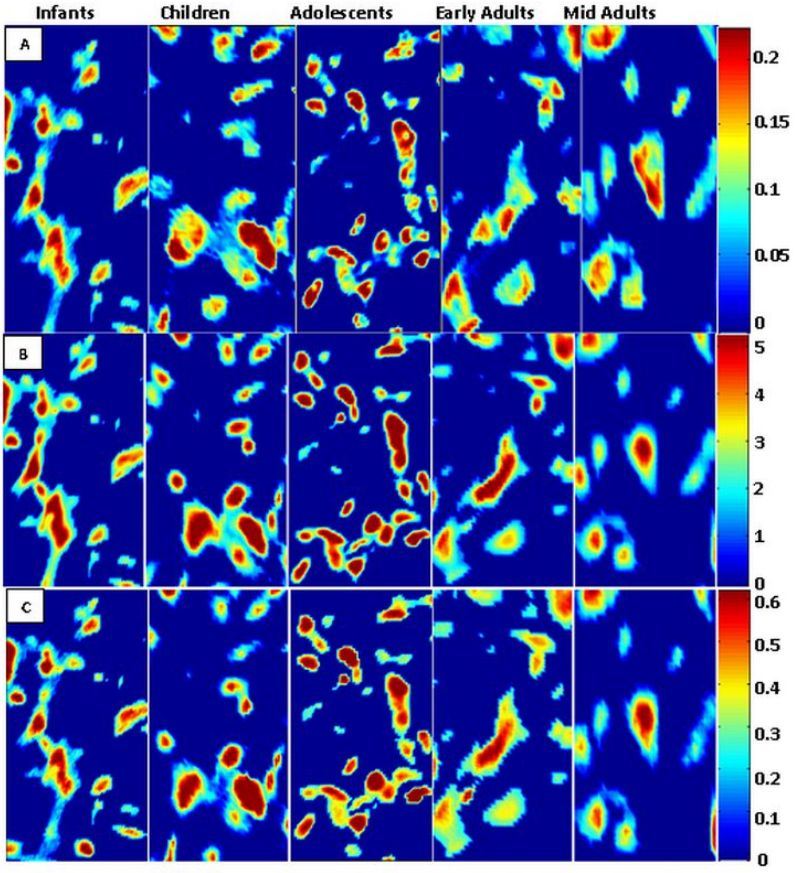


Fig. 2. Spectral image maps that reflect the distribution of lipids, proteins, and nucleic acids in the BM-MSCs from five different age groups. These maps were derived respectively by taking the peak integrated areas of (A) CH2 symmetric stretching bands of lipids, (B) amide II band of proteins, and (C) PO_2^- symmetric stretching bands of nucleic acids.

FTIRM of biological systems is used to investigate cells in different stages such as their growth cycles, cancerous states and contaminated states with pathogens. Hence, FTIR spectroscopy and microspectroscopy can successfully be used to characterize the biochemical makeup of intact live stem cells. Optical signals are generated intrinsically from the sample that are used to obtain information about relative concentrations and structure of biomolecules such as protein, lipids, carbohydrates, and nucleic acids (Fig. 1 and Fig. 2). This information can be used to understand similarities and differences between stem cell populations, lineages, the level of maturation, and differentiation of stem cells under healthy and disease states.

Ceren Aksoy [1], ***Feride Severcan*** [2,3]
[1]*Reasearch Coordination Department, Middle East Technical University, Ankara, Turkey*
[2]*Department of Biophysics, Altınbaş University School of Medicine, İstanbul, Turkey*
[3]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey*

Publication

Infrared Spectroscopy and Imaging in Stem Cells and Aging Research.
Aksoy C, Severcan F
Methods Mol Biol. 2018 Feb 21