

SINGLE FACTOR STRESS RESPONSE STUDIES OF MCF-7 BREAST CANCER CELLS BY FTIR SPECTROSCOPY

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Introduction The response of cells to various growth factors via the biochemical composition is well known and has been studied by FT-IR spectroscopy. Lately, there has been an increase of studies of nanoparticles. Unique molecule-like behaviour and excellent biocompatibility make gold nanoparticles very promising photoluminescent probes for tumour imaging. Bovine serum albumin stabilized gold nanoparticles (Au-BSA NPs) have been widely studied due to their possible applications in biomarkers as sensors. Biomarkers or multi-modality markers and nanoparticles agents. It also has been shown that cell cultivation under hypoxic conditions enhances the uptake of nanoparticles. Despite previous proposals, the stress response of cells cultivated in presence of nanoparticles or under hypoxic conditions remains poorly understood.

Materials and Methods MCF-7 cancer cells were cultured (37°C, 5% v/v CO₂) in Dulbecco's Modified Eagle's medium (DMEM) (Biocrom, Germany) supplemented with 10% (v/v) fetal bovine serum albumin (BSA) (Gibco, US), 100 U/ml penicillin, 100 mg/ml streptomycin (Biocrom, Germany). The cells were grown in 25 cm² cell culture flasks with up to 90% confluence. The cells were incubated with BSA and Au-BSA NPs (200 nm) for 48 hours. For oxygen stress cells were put in no serum media and placed in hypoxic (5% CO₂+11% O₂+94% N₂) conditions where grown for another 48 hours. After 48 hours the cells were trypsinized using 0.25% trypsin/EDTA (Biocrom, Germany) and centrifuged at 400 g for 5 min. The cells were washed with phosphate buffered saline (PBS) (Biocrom, Germany) couple of times. After washing BSA was fully discarded and the cells were frozen in liquid nitrogen.

For FT-IR measurements cells were re-suspended in distilled water. Spectra were registered on a microplate reader HTS-KT (Bruker, Germany) and the data processed with OPUS 6.5 software. Quantitative analysis of carbohydrates, nucleic acids, proteins and lipids was carried out on the basis of spectral data (Grubiņa, et al., 2014, Vib. Spectr. 28, 277-285).

Results MCF-7 cells showed little response to the presence of BSA or Au-BSA NPs. As can be seen from table 1 compared with control, the content of carbohydrates in cells incubated with BSA or Au-BSA NPs was slightly lower - 10.12% and 9.92% of dry weight (dw) compared to 10.7% in control. The content of nucleic acids and proteins was slightly increased - from 2.36% and 64.42% to 2.52% and 64.91% dw, respectively. Lipid content increased from 8.96% to 9.21% dw for BSA and 9.83% dw for Au-BSA NPs incubated cells. Hypoxic conditions evoked stronger stress response. The content of carbohydrates, nucleic acids and proteins all slightly decreased to 12.14%, 7.58% and 61.07% dw, respectively. Whereas the lipid content increased 1.84 times from 5.96 to 9.21% dw.

Growth condition	CH, % dw	NA, % dw	P, % dw	L, % dw
Control	10.70	2.36	64.42	8.96
Hypoxia	12.14	7.58	61.07	9.21
BSA	10.12	2.52	64.91	9.21
BSA + Au NPs	9.92	2.52	64.91	9.83

Table 1. Quantitative data of MCF-7 cells grown under different conditions, which total amount of carbohydrates from dry weight (dw) - nucleic acids, P - proteins, L - lipids.

Figure 1 shows the FT-IR spectra of MCF-7 cells cultivated under different conditions. While it is clear that there are differences in total lipid amount, cells cultivated under hypoxic conditions and the rest. There are changes to the profiles of carbohydrates spectra region are cultured under hypoxic conditions. That could suggest significant changes within these components.

Fig. 2. Normalized FT-IR spectra of MCF-7 breast cancer cells cultivated under different conditions: control - black, BSA - red, Au-BSA - green and hypoxia - blue.

Conclusions Results showed minor differences in the macromolecular composition of cells incubated with BSA or Au-BSA NPs thus suggesting Au-BSA NPs do not evoked stress effect on cells and are relatively safe to use. However enhancing of nanoparticles uptake by hypoxic conditions due to the noticeable effect on the total lipid content in cells.

Acknowledgement This study was supported by the IGA and project "Cancer-derived exosomes - a source of novel biomarkers and therapeutic targets for cancer".



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