Ultrasound-Induced Cell Permeability in Endothelial Cells for Targeted Delivery

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ABSTRACT

Targeted ultrasound contrast agents (UCAs), provide a novel means for the detection and evaluation of intravascular pathology, cellular imaging and for ultrasound-mediated drug and gene delivery [1, 2]. Targeted UCAs in conjunction with ultrasound have the potential to directly interact with endothelial cells resulting in an increase of their permeability. Endothelial cells in blood vessels form the main barrier for the passage of macromolecules and circulating cells from the bloodstream into the tissues. The ultrasound-induced mechanisms which influence these respective changes are not well understood [3]. In this regard, ultrasound- and integrin-mediated changes in endothelial cell permeability are investigated in real-time using the electric cell substrate impedance sensing (ECIS) methodology [4-6].

INTRODUCTION

UCAs are micron-sized gas-filled bubbles. With the addition of ligands on their lipid shell, UCAs have the potential to target specific sites of interest for molecular imaging and to be used as carriers for drug and gene delivery in a variety of conditions. The molecular mechanisms of the interaction of ultrasound, UCAs and endothelial cells are not well understood. More rigorous studies are needed for the optimization of targeting across blood vessel walls. Few if any studies have attempted to quantify ultrasound-induced transport across the endothelial barrier specifically in terms of either membrane pore formation or gap junction spreading. In this study, we seek to quantify the variations in endothelial cell membrane permeability in real-time with ECIS in terms of both pores and gap junctions [7-9].

EXPERIMENTAL METHODS

Human coronary artery endothelial cells (HCAECs) were cultured according to standard cell culture protocols. HCAECs were seeded at a concentration of $4 \times 10^4$ cells per cm$^2$ and allowed to grow to confluency. The cells were cultured and maintained as monolayers in a humidified atmosphere at 37ºC in 5% (v/v) CO$_2$.

UCAs with a mean diameter of 2.5 µm were conjugated with streptavidin prior to the attachment of the biotinylated antibodies to integrins $\beta_3$ and $\alpha_2\beta_1$. The endothelial cell-to-cell and cell-to-substrate resistivity was measured in real-time with ECIS under a variety of different conditions including the use of integrin-conjugated UCAs and ultrasound at clinically relevant frequencies.

A single-element unfocused transducer with a center frequency of 1 MHz was used for insonification. The peak negative acoustic pressure generated at the cell surface region was measured to be 0.1 MPa.

RESULTS AND DISCUSSION

The antibodies to integrins $\beta_3$ and $\alpha_2\beta_1$ change endothelial cell membrane permeability from the baseline values observed at $3.8 \pm 0.5$ Ω·cm$^2$ to $1.8 \pm 0.3$ Ω·cm$^2$. Moreover, the anti-integrin $\beta_3$ induces permeability changes in HCAECs.

At an acoustic pressure of 0.1 MPa the mean pore size was 0.019 µm while an increase in acoustic pressure resulted in an increase in the respective average cell membrane pore size. At the increased acoustic pressure of 0.3 MPa the mean size of the pores was found to be 0.024 µm. Although the ultrasound-induced pore mean sizes at the acoustic pressures of 0.1 MPa and
0.3 MPa respectively lie relatively close to one another, higher acoustic pressures result in the formation of less pores on the cell membranes but are larger in size. The three plots in Figure 1 show the resistance levels for confluent HCAECs at 0.5 kHz, 4 kHz and 64 kHz respectively. Changes in the lower frequencies correspond primarily to gap junction resistivity as opposed to variations in higher frequencies, which reflect pore formation.

Notice that after a decrease in resistance which corresponds to an increase in permeability the cells recover to their base line levels within 24 hours. This indicates that the pore formation is transient as confirmed by complementary SEM measurements.

CONCLUSION
Real-time impedance/resistance studies using ECIS in conjunction with simultaneous ultrasound- and targeted integrin-mediated permeability changes offer new insights into the underlying molecular mechanisms. We differentiate between the roles of pore formation and gap junction spreading using ECIS. Further investigations into these changes as a function of acoustic parameters and targeted UCAs may significantly increase the uptake for improved targeted drug and gene delivery.

REFERENCES

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