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Extraction of green fluorescent proteins with sonoporation

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The mechanism of permeabilization with sonoporation is not understood but the recurrent hypothesis assumes the formation of pores in the cell membrane. The aim of our study is to comfort this assumption by investigating whether ultrasound (US) and microbubbles could also facilitate outward transport of molecules across the plasma membrane through these probable pore formations. Stably transfected Hela-GFP cells were used and insonified with a 1 MHz unfocused transducer in presence of BR14 microbubbles (Bracco Research, Geneva) at different acoustic parameters. The percentage of Hela-GFP cells and the mean cell fluorescence intensity (FI) were measured by flow cytometry to evaluate the GFP release. While US alone did not affect the cells, the addition of BR14 microbubbles induced a significant decrease of FI and the percentage of Hela-GFP cells. A reduction of more than 50% of FI and GFP + cells was achieved between 400-600 kPa and 40-75 % of duty cycle during 2 minutes insonation. However a progressive recovery of Hela-GFP cells percentage and FI post-insonation has been observed which indicates a viable situation of Hela-GFP permeabilized cells allowing a restoration of GFP cell content. These results comfort pore formation hypothesis allowing transmembrane transport following molecular concentration gradient.