Broadband high-frequency laser ultrasound generation and applications towards biological membranes

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Background – Photoacoustic light-to-pressure conversion offers the ability to generate ultrasound pulses with high peak pressures, high central frequencies and large bandwidths, using laser pulses with fluences below 100 mJ/cm2. Generation of those intense ultrasound pulses is achieved by the pulsed irradiation of high absorption coefficient thin films. Dyes with fast and high quantum yield of non-radiative decay imbedded in polystyrene, nanostructured carbon particles or nanostructured TiO2 with adsorbed dye permeated with high thermal expansion polydimethylsiloxane, are among the materials being used.[1-3] Laser ultrasound pulses have shown remarkable application versatility, namely towards transient breaching of biological membranes, e.g. transient permeabilization of the outer layers of cell membranes or skin.[1] The non-destructive laser ultrasound pulses interaction with biological membranes is exemplified by the unloading of Green Fluorescence Protein from giant unilamellar vesicles,[4] and by enabling the transfection of plasmid DNA towards COS-7 monkey fibroblast cell line.[5] The ability to transiently and without pain permeabilize the skin is exemplified by the efficient permeabilization of the stratum corneum induced by photoacoustic waves of targets of therapeutic and aesthetic relevance: a 1.1 kDa bacteriochlorin, minoxidil, a precursor of vitamin C and hyaluronic acid.

Methods – Ultrasound pulses were generated by the photoacoustic effect. Pulsed Nd:YAD lasers coupled to an optical parameter oscillator when needed were used: EKSPLA OPO Model PG-122 pumped by a EKSPLA NL301G laser with pulse width 6 ns; a Quantel Big Sky Ultra 50 with pulse width 8 ns; and a EKSPLA PL2143A laser with pulse width 30 ps. Carbon nanotubes permeated with polydimethylsiloxane films were produced with thicknesses between 30 μm and 200 μm. Large superficial area and a thickness <5 μm TiO2 thin films with a Mn porphyrin dye adsorbed were produced by screen-printing. Mn porphyrin was imbedded in polystyrene making ≤80 μm films by casting. Photoacoustic waves were detected by a high frequency contact transducer with maximum sensitivity at 225 MHz (Panametrics/Olympus, model V2113) and by a needle hydrophone (Precision Acoustics, model NH0200).

Results – The characteristics of laser ultrasound pulses generated by fast and efficient conversion of energy from a laser pulse into a pressure transient were measured. CNT functionalized with siloxane groups produce thin films that generate exceptionally wide bandwidths (170 MHz at −6 dB) and peak pressures >1 MPa when excited by pulsed ps lasers.[3] Vertically-aligned CNT grown to distinct thicknesses and infused with PDMS were tested using 8 ns and 30 ps laser pulses. High pressure transient with a peak of 14 MPa and a bandwidth of 180 MHz at -10 dB using a 1064 nm 100 mJ/cm2 laser pulse for excitation was achieved.[4] Efficient release of FITC-dextran and GFP from a giant unilamellar vesicles (GUVs) core was observed, without damaging the phospholipid bilayer. Laser

ultrasound promotion of the permeabilization of GUVs was imaged using real-time interferometric imaging. At a repetition rate of 10 Hz, ultrasound pulses enable to release 25% of the FITC-dextran content of GUVs in 15 min.[4] High stress gradients, produced when picosecond laser pulses with a fluence of 100 mJ/cm ^{2} absorbed by piezophotonic materials formed by a dye incorporated in a thin polystyrene polymer film, enable transfection of a plasmid DNA encoding Green Fluorescent Protein (gWizGFP, 3.74 MDa) in COS-7 monkey fibroblast cells with an efficiency of 5% at 20 °C, in 10 minutes, and without any significant cytotoxicity observed.[5] By exposing skin to photoacoustic waves for two minutes using a laser pulse frequency of 20 Hz, the increase of transepidermal water loss of healthy human skin by a factor of 2.5 was observed, and the skin relaxes to normal two minutes later.[1] Such ultrasound pulses also increased significantly the initial fluxes of a 1.1 kDa bacteriochlorin and a 28 kDa protein through the stratum corneum of minipigs. Such an effect was also observed with other therapeutically relevant molecules as minoxidil or vitamin C precursors in ex vivo skin experiments. The intraepidermal delivery observed in minipigs does not lead to observable adverse effects.

Conclusions – The broad bandwidths and high central frequencies of the photoacoustic waves generated with nanostructured materials constitute a valuable stimulus for the promotion of cell transfection, through skin permeation and controlled drug delivery. Laser ultrasound pulses appear to be a very generic method to promote the temporary destabilization of the outer layers of the skin and promote the effective passage of molecules and macromolecules of therapeutic and aesthetic interest. Tailor made laser ultrasound may potentially enable non-invasive targeted release of GUVs and cell transfection over large volumes of tissues in a few minutes.

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