ChemComm

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c7cc09613e

Received 15th December 2017,
Accepted 16th January 2018

DOI: 10.1039/c7cc09613e

rsc.li/chemcomm

Rock the nucleus: significantly enhanced nuclear membrane permeability and gene transfection by plasmonic nanobubble induced nanomechanical transduction†

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Efficient delivery to the cell nucleus remains a significant challenge for many biomolecules, including anticancer drugs, proteins and DNAs. Despite numerous attempts to improve nuclear import including the use of nuclear localization signal (NLS) peptides and nanoparticle carriers, they are limited by the nanoparticle size, conjugation method, dependence on the functional nuclear import and intracellular trafficking mechanisms. To overcome these limitations, here we report that the nanomechanical force from plasmonic nanobubbles increases nuclear membrane permeability and promotes universal uptake of macromolecules into the nucleus, including macromolecules that are larger than the nuclear pore complex and would otherwise not enter the nucleus. Importantly, we show that plasmonic nanobubble-induced nanomechanical transduction significantly improves gene transfection and protein expression, compared to standard electroporation treatment alone. This novel nanomechanical transduction increases the size range and is broadly applicable for macromolecule delivery to the cell nucleus, leading to new opportunities and applications including for gene therapy and anticancer drug delivery.

The cell nucleus is a subcellular compartment where genetic information and the transcription machinery reside, and is also the target of numerous therapeutic agents.[1] For example, gene therapy aims at restoring dysfunctional or missing genes by delivering therapeutic genes into the cell nucleus.[2] Some anticancer drugs, such as doxorubicin, induce tumor cell apoptosis by oxidative DNA damage and DNA enzyme inhibition, *i.e.*, topoisomerase II in the nucleus.[1,3] Upon entering the cell through endocytosis, many biomolecules, including anticancer drugs, proteins, and DNAs, need to escape the endosomal barrier and reach the nucleus to elicit their therapeutic effects.[4,5] Due to size limitations of the nuclear pore complex, it is extremely difficult for nanoparticles or macromolecules to enter the cell nucleus.[6,7] This challenge has been approached *via* numerous strategies in an effort to enhance nuclear delivery, for example, using ligand modified nanocarriers or biomolecules.[8–12] For instance, a nuclear localization signal (NLS) peptide-functionalized nanoparticle was reported to target and deliver doxorubicin into the cell nucleus.[13] Despite their nuclear-homing capability, there are still several limitations. Firstly, nanoparticles must be small enough to enter the nucleus.[14,15] The cationic surface charge due to NLS modification and small size makes the nanoparticles undergo rapid body clearance, and thus these nanoparticles are unsuitable for *in vivo* applications.[16] Secondly, the efficacy of NLS modifications are dependent on the conjugation method. For instance, it has been reported that the direct conjugation of NLS peptide to linearized DNA did not enhance the nuclear entry, while conjugation of NLS peptide to DNA *via* a cross-linker was able to enter the nucleus.[17,18] Thirdly, functional nuclear import and intracellular trafficking mechanisms are crucial for NLS modified nanoparticles to enhance nuclear targeting. For example, in glioma cells, NLS surface coating on nanoparticles even reduced the nuclear delivery due to the aberrant nanoparticle intracellular trafficking and nuclear import.[19] With these limitations in the nanoparticle size, biomolecule structure, dependence on the functional nuclear import and intracellular trafficking, new methods to enhance nuclear delivery are highly desirable to improve therapeutic gene transfection.

Here we report a novel mechanism based on nanomechanical transduction to increase the nuclear membrane permeability and biomolecule delivery (Fig. 1). Plasmonic nanoparticles efficiently absorb laser energy at plasmonic resonant wavelengths.[19,20] Under ultrashort laser pulse irradiation (ps, fs), plasmonic nanoparticles locally heat up to high temperatures, leading to transient

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† Electronic supplementary information (ESI) available: All experimental details, Fig. S1, S2 and Tables S1, S2. See DOI: 10.1039/c7cc09613e

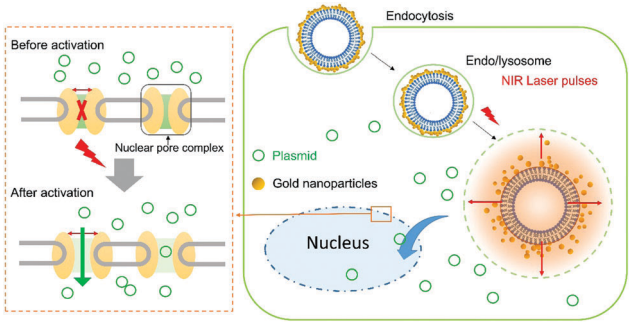

Fig. 1 Schematic illustration of nanomechanical transduction enhanced nuclear entry of biomolecules. A near-infrared laser pulse activates plasmonic nanoparticles to create plasmonic nanobubbles that grow and collapse within nanoseconds. The nanomechanical force from plasmonic nanobubbles increases the nuclear membrane permeability, thus leading to enhanced nuclear entry for biomolecules.

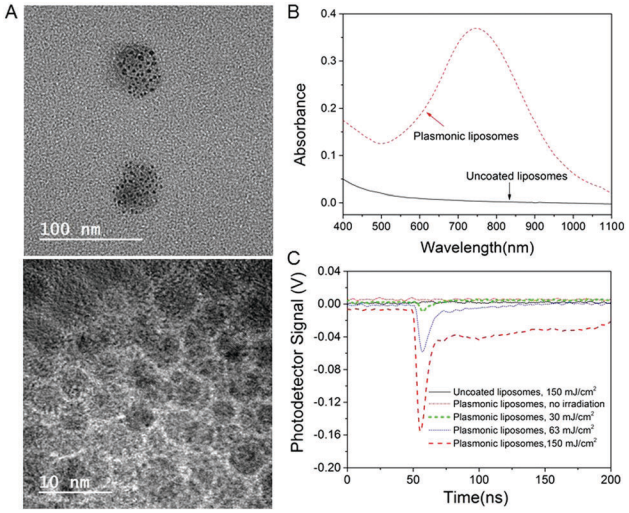

Fig. 2 Characterization of plasmonic liposomes and detection of plasmonic nanobubbles. (A) TEM images of plasmonic liposomes; (B) UV-Vis spectra of plasmonic liposomes and uncoated liposomes; (C) a plasmonic nanobubble signal of plasmonic liposomes irradiated with a single 750 nm laser pulse under different laser fluence.

nanoscale vaporization bubbles that grow and collapse on the order of nanoseconds.[21] The growth and collapse of nanobubbles lead to nanoscale mechanical effects and thus act as nanomechanical transducers, which convert optical energy into nanomechanical energy. Using this novel nanomechanical transduction mechanism, we demonstrated that single near-infrared laser pulse (750 nm, 28 ps) significantly increases the nuclear membrane permeability and allows nuclear uptake of macromolecules (dextran, 40 kDa), which would otherwise not have entered the nucleus. Importantly, nanomechanical transduction in combination with electroporation significantly increased the gene transfection efficiency by 2.7-fold compared to electroporation alone. Finally, this nanomechanical transduction technique is highly localized, does not rely on specific functional ligands, and is promising for nuclear delivery of many biomolecules—especially DNAs and anti-cancer drugs.

We first characterized the nanomechanical transducers that are capable of converting near-infrared laser pulse energy into nanomechanical forces. While many near-infrared absorbing plasmonic nanoparticles have been reported, we followed previously reported methods to synthesize gold-coated plasmonic liposomes by a one-step deposition of gold nanoparticles directly onto the liposome surface (Fig. S1, ESI†).[22] Gold coating increased the liposome size by 10–15 nm (ESI,† Table S1). Fig. 2A shows that small gold nanoparticles form discrete gold clusters surrounding the liposomal core. The plasmonic coupling between gold nanoparticles leads to a near-infrared absorption at around 750 nm for plasmonic liposomes, while non-coated liposomes did not show any resonant peak in the near-infrared wavelengths (Fig. 2B). We measured the plasmonic nanobubble generation upon near-infrared pulsed laser activation using an optical pump–probe technique. The refractive index mismatch of vapor nanobubbles strongly scatters the probe laser beam, leading to a transient decrease in the transmitted laser intensity. The results show that increasing the pump laser energy above the nanobubble generation threshold leads to a greater drop in the transmitted intensity, while uncoated liposomes do not generate nanobubbles even at a high laser pulse fluence (150 mJ cm^{-2}, Fig. 2C). The lifetime of the plasmonic nanobubbles ranges from 20–50 ns as a function of the laser pulse energy and is a transient event.

Next, we assessed how nanomechanical transduction affected the intracellular transport of macromolecules. The nuclear pore complex has an estimated cutoff size of 5 nm[23] and larger macromolecules do not readily enter the nucleus. To demonstrate this point, cells (Raw 264.7) were first subjected to electroporation to allow trans-membrane uptake of fluorescein isothiocyanate-dextran (FITC-dextran, 40 kDa, or FD-40) in the cytosol. Due to the large size (6 nm), FD-40 does not diffuse through the nuclear pores and thus cannot enter the nucleus without external forces.[24] This is confirmed by the confocal imaging that shows the distribution of FD-40 in the cytosol after electroporation (Fig. 3 and Fig. S2, ESI†), with a very weak fluorescence signal in the nucleus region. To test the effect of nanomechanical transduction, the cells were incubated with plasmonic liposomes to allow the endosomal uptake of nanomechanical transducers. After a single near-infrared laser pulse (60 mJ cm^{-2}), nanomechanical transduction leads to significant nuclear uptake of FD-40, up to 70% fluorescence intensity in the nucleus compared to the cytosol. In contrast, when using standard liposomes without gold coating or plasmonic liposomes without laser activation, no mechanical transduction occurred and no change in the nucleus uptake of FD-40 was observed. These results suggest that nanomechanical transduction induced by plasmonic nanobubbles enhanced the permeability of the nuclear membrane, thus enabling nuclear entry of macromolecules with a size larger than the nuclear pore. We anticipate that the molecular transport induced by nanomechanical transduction is a "two-way street" and it will also facilitate the leakage of nuclear substances into the cytosol. This feature is also common for other physical approaches including electroporation and ultrasound (*i.e.* sonoporation).

Finally, we tested whether the enhanced nuclear membrane permeability by nanomechanical transduction leads to higher

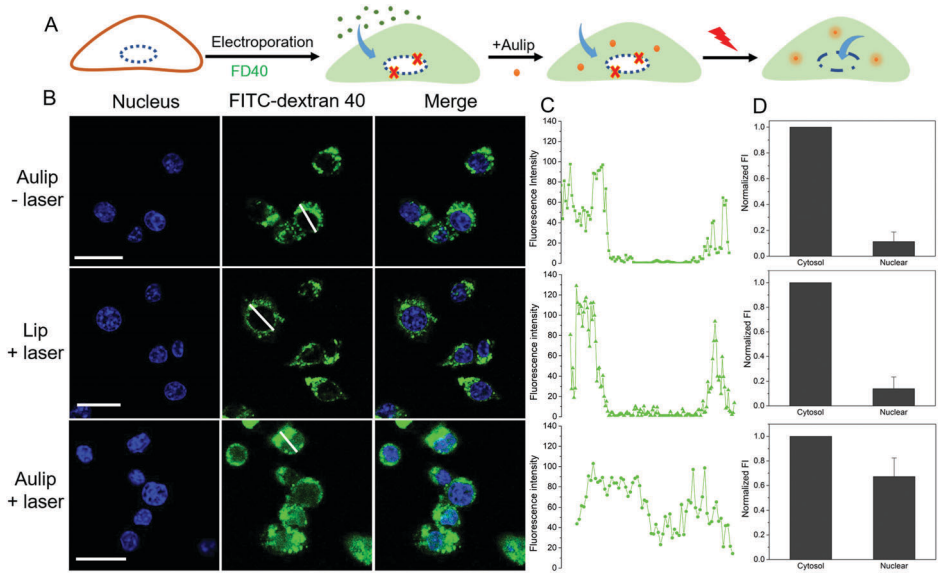

Fig. 3 Nanomechanical transduction by plasmonic nanobubbles leads to enhanced nucleus membrane permeability. (A) Schematic illustration of the experimental procedure for macromolecule nuclear membrane permeability. Fluorescein isothiocyanate-dextran (FITC-dextran, 40 kDa, or FD-40) was introduced into the cells by electroporation. Gold-coated plasmonic liposomes (Aulip) were loaded into the cells 3 h after electroporation. The endocytosed intracellular plasmonic liposomes were activated by a single laser pulse (750 nm, 60 mJ cm^{-2}) to generate nanomechanical forces. (B) Confocal images of cells for experimental groups including: plasmonic liposomes alone (top), with uncoated liposomes (Lip) and laser pulse (middle), and with plasmonic liposomes and laser pulse (bottom). (C) Fluorescence intensity plots of FD-40 across the cell (white lines in the confocal image). (D) Average fluorescence intensity in the cytosol and nucleus. Scale bar, 20 μm.

gene transfection efficiency. As a testbed, we performed gene transfection in the Raw 264.7 cells using stomatitis virus-encoded glycoprotein tagged with enhanced green fluorescence protein plasmid (pEGFP-VSVG) as a reporter gene. Gene transfer into macrophage cell lines is frequently impeded by extremely low transfection efficiencies[25] even with the use of electroporation. We first introduced pEGFP-VSVG into the Raw 264.7 cells by electroporation and then allowed the cells to uptake the nanomechanical transducers (*i.e.* plasmonic liposomes), followed by single near-infrared laser pulse treatment (Fig. 4).

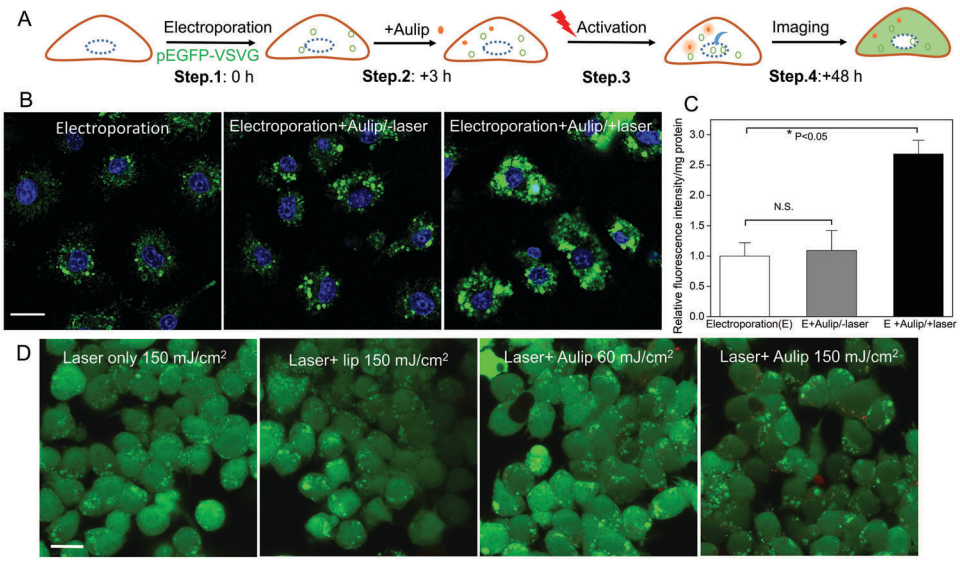

Fig. 4 Nanomechanical transduction by plasmonic nanobubbles leads to significantly improved gene transfection. (A) Schematic illustration of the experimental procedure for gene transfection; (B) confocal images of Raw 164.7 cells 48 h after gene transfection. Blue: nucleus; green: EGFP-VSVG, (C) relative transfection efficiency of EGFP-VSVG determined using a fluorescence microplate reader. Values indicate the mean ± SD of the experiments, n = 3. (D) Live-dead staining of Raw 264.7 cells. Green staining represents live cells and red staining represents dead cells. No dead cells were observed under current experimental conditions. This suggests that nanomechanical transduction under current experimental conditions does not induce cell apoptosis. Scale bar: 20 μm.

Qualitative confocal imaging suggests a higher level of EGFP expression with nanomechanical transduction treatment compared with electroporation alone (Fig. 4B). Further quantitative measurement of EGFP showed a 2.7-fold increase in the EGFP expression as a result of the nanomechanical transduction (Fig. 4C). As a comparison, the cells with plasmonic liposomes but without near-infrared pulse activation did not show significant improvement in EGFP expression compared with electroporation alone. Thus, the enhanced nuclear membrane permeability by nanomechanical transduction leads to improved plasmid accumulation in the nucleus and, as a result, significantly higher gene transfection efficiency.

This study builds upon previous investigations on the mechanical stimulation of nuclei and reports a novel nanomechanical transduction mechanism to enhance nuclear membrane permeability.[26,27] There is some evidence suggesting that mechanical stimulation regulates the nuclear entry of endogenous biomolecules.[28] However, the exact mechanism is still unknown, and there are several proposed mechanisms. One hypothesis is that mechanical stimulus transiently deforms the nuclear envelope to activate nuclear pore complexes. This in turn increases the nuclear membrane permeability and leads to enhanced nuclear entry of biomolecules (Fig. 1). Further fundamental studies on mechanical transduction are required to elucidate the exact mechanism. Nanomechanical transduction is well tolerated by the cells as evidenced by cell viability (Fig. 4D). As a critical cellular compartment, the nucleus plays numerous important roles in physiology and therapy. Regulation of nuclear delivery of biomolecules has many applications in understanding the signaling pathway in cell nucleus, enhancing gene transfection[29] and drug therapeutic efficiency.

In conclusion, plasmonic nanoparticles can be used as intracellular nanomechanical transducers to enhance permeability of the nuclear membrane. Near-infrared laser pulses activate these nanoparticle transducers to generate short-lived plasmonic nanobubbles, which exert a nanomechanical effect inside the cell close to the nucleus. The nanomechanical transduction increases the nuclear membrane permeability to allow accumulation of macromolecules that are larger than the nuclear pore complex. We further demonstrated that nanomechanical transduction significantly improves gene transfection efficiency in Raw 264.7 cells, which are notoriously difficult to transfect.[30] This technique is nanomechanical force based and highly localized, does not rely on specific nuclear receptors, and can be applied to a broad range of biomolecules and cells. We anticipated that this technique will be useful for nuclear delivery of anti-cancer drugs and DNAs. Future studies include further understanding the mechanisms and integrating with common liposome vectors for gene delivery.

XL wants to thank Prof. Yihong Wang from UT southwestern for kindly providing us with Raw 264.7 cells, and Dr Peter van Hoogevest from The Phospholipid Research Center, Heidelberg, Germany, for helpful discussions. This work was supported by a postdoc research grant from The Phospholipid Research Center, Heidelberg, Germany (1603574), CPRIT (Z. Q.: RP160770, J. J. G.: RP170752 and L. Z.: RP160617) and NSF (Z. Q.: 1631910 and J. J. G.: DMR-1654405) grants.

Conflicts of interest

There are no conflicts to declare.

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