Positively charged microbubbles to target nucleic acid delivery with ultrasound

Simona Manta, Anthony Delalande, Gilles Renault, Michel Francis Bureau, Matthias Lebertre, C Marie, Daniel Scherman, Patrick Midoux, Nathalie Mignet, Chantal Pichon

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S. Manta[1], A. Delalande[2], G. Renault[3], M-F Bureau[1], M. Lebertre[4], C. Marie[1], D. Scherman[1], P. Midoux[2], N. Mignet[1]* and C. Pichon[2]*

[1]Unité de Technologies Chimiques et Biologiques pour la Santé, UMR8258/INSERM U1022 CNRS Chimie ParisTech, 4 avenue de l'Observatoire, 75006 Paris, France ; [2]Centre de Biophysique Moléculaire, CNRS UPR 4301, Université d'Orléans, France ; [3]Plateforme d'Imagerie du Petit Animal, Inserm U1016, Institut Cochin, 75006 Paris, France ; [4]Transderma Systems, 10 boulevard Tonnelé, 37000 Tours.
*Corresponding author Nathalie.Mignet@parisdescartes.fr

Abstract – Nucleic acid delivery in vivo via physical means or non-viral vectors still need improvements in particular to reach deep tissues. Sonoporation is highly advantageous for this purpose as ultrasound can be focalised to a particular tissue leading to targeted gene delivery, without alteration of the environing tissues. Few examples of transfection using cationic microbubbles (MBs) and DNA have been reported. We ought to improve the existing systems by developing positively charged MBs able to oscillate in order to follow their fate in vivo and gain in understanding of the pharmacokinetic of the MBs, then target DNA delivery with ultrasound. After optimisation, we obtained MBs in the 1-3 µm range (98% below 10 µm) able to adsorb nucleic acid on their surface. In vitro parameters have been optimised to obtain in vitro transfection both with DNA and siRNA. The systemic injection in mice led to the observation of these MBs in the liver in less than 10 seconds. This investigation allows to address the key parameters to tentatively obtain reproducible gene transfection in vivo.

Index terms – Nano medicine, Therapy monitoring, Ultrasound, Targeted DNA delivery.

I. INTRODUCTION

MBs have been investigated for several years to improve ultrasound contrast imaging. More recently they have been proposed as a mean to deliver small or high molecular weight drugs. Among them, nucleic acid delivery has been proposed. The co-injection of MBs and DNA was shown to improve Raf encoding gene delivery in tumor, leading to reduced tumor growth [1]. We also showed that we could transfect the Achilles tendon [2]. These great findings suggested that increasing the association between nucleic acid and MBs could potentially increase nucleic acid concentration in the ultrasound-targeted tissue, which should in turn increase the biological efficacy. Therefore, we chose to develop positively charged, lipid shell gas MBs. Few groups have also proposed this strategy which seems to be reasonable to achieve gene transfer *in vivo*. Based on our experience on lipoplexes, we chose a lipid which had been selected earlier for its strong capacity to transfect cells and investigated the formation of MBs with this lipid (DMAPAP) [3]. After optimizing MB obtention protocols and characterization, we investigated DNA interaction with the MBs, than MBs with DNA interaction with the cells under ultrasound. Finally, the conditions of transfection *in vitro* and *in vivo* were investigated.

II. MATERIALS AND METHODS

II.1. **MBs**. The DMAPAP lipid was synthesized as previously described [4]. The lipid in suspension in H_2O for injection was placed in a closed vial in which a controlled amount of C_4F_{10} was added. The MBs were formed by mechanical agitation using a Vialmix.

II.2. **Characterisation in size**. MBs stability was observed by optical microscopy and the size and concentration were determined using ImageJ.

II.3. **Acoustic properties**. The acoustic properties of the MBs were investigated by attenuation measurements to determine the resonance frequency via a set-up proposed by the company Transderma.

II.4. **Nucleic acid interaction with MBs**. The interaction of pDNA with MBs was evaluated by confocal microscopy and flow cytometry using a Cy5-labelled DNA.

II.5. ***In vitro* gene transfer**. HeLa cells were transfected by a GFP expressing plasmid using a home made motorised system to perform sonoporation in each well and test various MB to DNA ratio and various acoustic pressures. Ultrasound was set at 1 MHz, 180 kPa, 40%DC, 100 µs pulse during 60 sec. Gene transfer was evaluated after 24h.

II.5. ***In vivo* MB imaging**. MBs with pDNA were administrated intravenously in mice. Their distribution in the liver was observed by ultrasound imaging with a Vevo2100 (Visualsonics).

III. RESULTS

MBs produced by mechanical shaking showed a size distribution centered at 2 μm, a stability up to 1 hour, and a resonance frequency around 1.1 MHz (Figure 1). MBs

are able to bind DNA at their surface as we could show by flow cytometry, gel-shift assay and confocal microscopy. The adsorption of DNA did not change the acoustic properties of the MBs (Figure 2). *In vitro*, sonoporation of HeLa cells using the produced MBs resulted in almost 30% of GFP transfected cells (figure 3). MBs were able to reach the liver in a few seconds after injection (figure 4). The enhancement of the B-mode intensity was observed during 40 sec, with a maximum intensity at 10 sec (over 3 times the baseline).

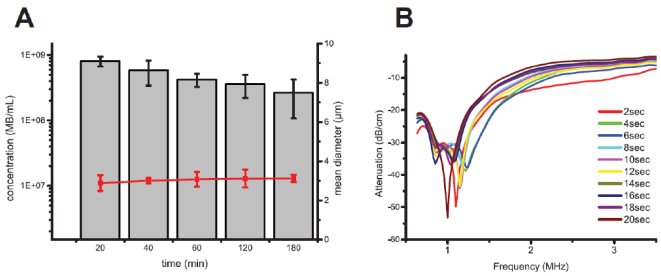

Figure 1. A: Diameter and concentration of the positively charged MBs over the time. B: Acoustic properties determination.

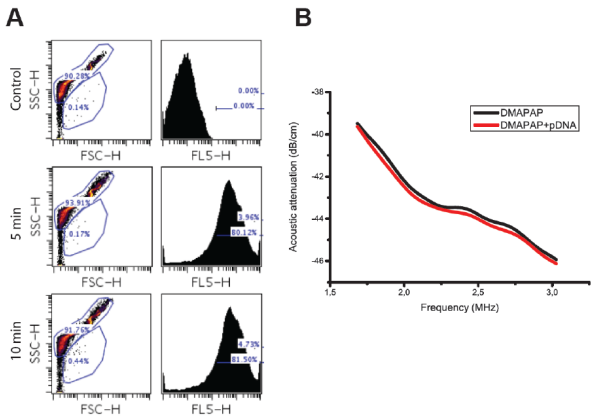

Figure 2. A: Flow cytometry of MBs without and with labelled pDNA. B: Acoustic properties of the MBs with or without pDNA (right).

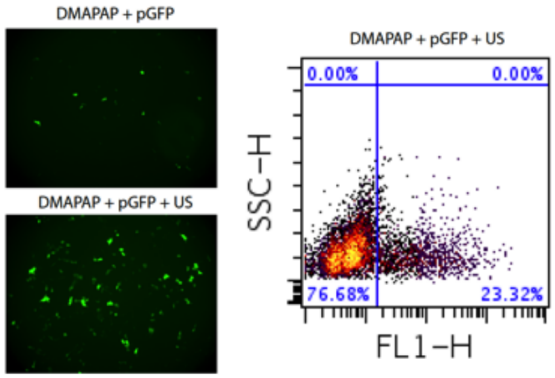

Figure 3. *In vitro* gene transfer efficiency of DMAPAP MBs after ultrasound stimulation on HeLa cells.

IV. DISCUSSION – CONCLUSION

The present study demonstrated that it was possible to form positively charged MBs with a sufficient stability to lead to gene transfer in vitro, to be injected and observed in vivo. The kinetic could be modulated by surface chemistry post formation to get Pegylated MBs. Even though, only data with DNA are shown here, a similar work has been performed with siRNA and showed that the MBs could be used for any type of nucleic acid leading to protein expression or inhibition of protein expression according to the nucleic acid tested. Our interest in this study is to achieve nucleic acid biological effect in deep tissue, where the most efficient physical technique electrotransfer can not be proposed.

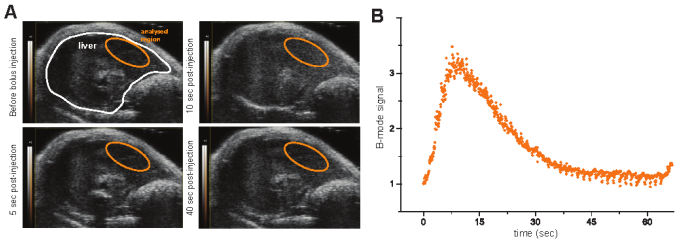

Figure 4. Kinetic of MB observation within the liver using the ultrasound imaging Vevo2100 system.

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