Ultrasound and Microbubbles Assisted-gene Transfer: what is next?

The goal of gene therapy is to introduce a therapeutic nucleic acid material to cure genetic deficiencies and a large number of acute diseases. Exciting results from recent clinical trials demonstrate without doubt the promise of gene therapy. Despite the high gene transfer efficiency of viral vectors, there are still some drawbacks in their use due to their immunogenicity and mutagenesis features. Therefore, there is still room for non-viral methods to be developed since they are safer. However, gene delivery by non-viral methods is still a major challenge nowadays. The major limiting factor remains the lack of a suitable and efficient vector for gene delivery.

The challenge is to deliver the nucleic acid to the right intracellular compartment. Many efforts have been undertaken to identify the cellular barriers that have to be passed for this issue (Fig. 1). First, the nucleic acid has to be protected from nucleases in the extracellular compartment. Then the plasma membrane has to be crossed. There are two ways to enter in the cells; either a direct transfer in the cytosol through destabilization of the plasma membrane or via endocytosis process. This latter is the main way when chemicals delivery systems are used as carriers. Once internalized, nucleic acids particles end up inside endosomes where they must escape to reach the cytosol, where mRNA and siRNA or oligonucleotides can be translated and find their targets, respectively. In the case of plasmid DNA (pDNA), it must be imported into the nucleus where the expression machinery takes place. The size of pDNA limits its cytosolic motion and passive diffusion through pores of nuclear envelope (Fig. 1).

In chemical based-methods, several strategies have been developed to bypass these limitations. Protection and stabilization of nucleic acids in extracellular medium have been achieved by nucleic acid condensation or encapsulation by chemical vectors. For the endosomal escape, strategies that exploit the proton-sponge effect enabling endosomal membrane destabilization have been proposed [1]. The nuclear importation of nucleic acid has also been improved by addition of devices bearing nuclear localization signal which can be put either on the chemical vector or the nucleic acid. Despite of all of these tremendous efforts, the efficiency of these chemical based-strategies is still far from that of the viral vectors.

In parallel, physical based methods for gene delivery have been developed. Electric, magnetic, light or ultrasound fields have been exploited as physical trigger. Among them, electrotransfer is one of the most used and efficient method but its invasiveness still hampers its wide application. Twenty-five years ago, an alternative method for drug delivery based on ultrasound stimulation was proposed [2]. This method was proven to be more effective when coupled with gaseous microbubbles [3]. These micron-sized structures...
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Ultrasound enables control of both the drug release by activating the microbubbles and the delivery location by positioning the ultrasound probe in a specific area on the skin. The combination of ultrasound stimulation and microbubble oscillation leads to increased uptake by ultrasound has been attributed to the formation of transient pores on the plasma membrane with a phenomenon called sonoporation. However, it is still ill-known how exactly cells that are subjected to ultrasound and microbubbles internalize extracellular compounds, and which cellular responses ultrasound and microbubble evoke. It was also suggested recently that besides transient pore formation, endocytosis mechanism might also be involved in the uptake during ultrasound-mediated drug/gene delivery. The mechanisms of sonoporation are summarized in Fig. 2. These results open a new research area. Indeed, the type of mechanism(s) involved in the delivery could be both dependent on the microbubble chemical composition, the type of drug to deliver and on the type of insonification.

Cells exposed to ultrasound are known to elicit a variety of biological responses that can be deleterious or with therapeutic potential. Recently, Furusawa and colleagues have shown that ultrasound could induce host DNA modifications up to double breaks. One can ask if it is possible to fine tune the setting to act on the accessibility of the DNA structure, thus allowing more efficient transgene integration.
on. This is not as idealistic as it sounds since ultrasound stimulation has been shown to induce an over-expression of some genes, especially in musculoskeletal tissues which can sense external stimuli. Acquiring more knowledge on the cellular effects in terms of molecular signaling will be worthwhile to take advantage of these phenomena for gene delivery purposes.

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References

Shear Wave Elastography

In the last years, liver elastography became an important target for many research groups, which tried to establish the real value of this new method, especially for the evaluation of diffuse hepatopathies. Liver biopsy was considered as the "gold standard" for liver fibrosis assessment and the first elastographic method, transient elastography, was compared to the morphologic evaluation (liver biopsy). Later studies, evaluating new elastographic methods, tried to demonstrate their non-inferiority as compared to this validated method.

In the last years we observed a dramatic decrease in the number of liver biopsies performed for liver fibrosis assessment in chronic hepatitis, as a result of increasing use of the non-invasive modalities, especially in Europe. Biological tests (direct or indirect) and elastographic methods for liver stiffness evaluation became more popular and are used for prognosis assessment, for decision regarding treatment and for the follow-up of patients with chronic hepatopathies. Tissue elasticity can be described qualitatively by evaluating the relative displacements caused by static or dynamic deformations. Quantitative techniques estimate the deformation rate, which indirectly characterize environmental stiffness. The first elastographic method used in clinical practice was transient elastography (TE). Now, after more than 10 years experience with this method, several meta-analyses showed its real value. The weak points of this method are: It is a quite blind method (no real time ultrasound use); it is not feasible in patients with ascites and in approximately 15–20% of patients, reliable measurements cannot be obtained.

More recently, shear wave elastography (SWE) was developed. Quantitative elastography techniques provide high resolution information about tissue elasticity in a region of interest. To obtain a quantitative elastographic assessment, shear waves are generated into the tissue, then tracked and measured. Complex equipment is used to generate them and to make high-resolution measurements of their propagation velocity. Several techniques can be used to generate the shear waves:

Acoustic Radiation Force Impulse (ARFI) Elastography

The acoustic radiation force causes tissue displacement centered on a region of interest. These displacements propagate through the tissue in the form of shear waves and an ultrasound system is used to follow the shear waves’ propagation. The time scale of tissue’s response is much slower than that of the ultrasonic wave propagation. ARFI elastography is integrated into an Acuson S2000 ultrasound machine, from Siemens.

Principle of ARFI Elastography: A short duration, high-intensity acoustic “push pulse” is transmitted by the transducer, followed by a series of diagnostic intensity pulses, which are used to track the tissue displacement caused by the push pulse. The tissue response to the radiation force is observed using conventional B-mode imaging pulses, and it is possible to measure and display the quantitative shear-wave velocity (meters/second – m/s) from the ARFI generated displacements. This velocity is proportional to the square root of the tissue’s elasticity modulus. Because the shear wave velocity depends on tissue stiffness, it is possible to