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Review

Sonoporation: Mechanistic insights and ongoing challenges for gene transfer

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ABSTRACT

Microbubbles first developed as ultrasound contrast agents have been used to assist ultrasound for cellular drug and gene delivery. Their oscillation behavior during ultrasound exposure leads to transient membrane permeability of surrounding cells, facilitating targeted local delivery. The increased cell uptake of extracellular compounds by ultrasound in the presence of microbubbles is attributed to a phenomenon called sonoporation. In this review, we summarize current state of the art concerning microbubble–cell interactions and cellular effects leading to sonoporation and its application for gene delivery. Optimization of sonoporation protocol and composition of microbubbles for gene delivery are discussed.

Contents

1. Introduction

The principle of gene therapy is to introduce gene or nucleic acids into cells to cure genetic deficiencies. The success of gene therapy obtained with the use of viral vectors demonstrates unambiguously the feasibility of this innovative therapy (Bennett et al., 2012; Fischer et al., 2000; Nathwani et al., 2011). To date, viral vectors remain the best vehicles to introduce genes into cells. Nevertheless, there are still drawbacks inherent to the use of a viral molecule observed in gene therapy clinical trials raising serious safety concerns (Hacein-Bey-Abina et al., 2003; Raper et al., 2003). In addition, size limitation capacity, cell targeting and manufacturing issues are still difficult to handle despite tremendous progress made on viral vector bioengineering. Therefore, there is still some room for the development of alternative approaches of high safety, low immunogenicity and easy manufacture. This last decade, many efforts have been done to search for non-viral options. The goal is to design synthetic gene delivery systems that incorporate viral-like features to transfect efficiently cells (Mahato, 1999; Midoux et al., 2009; Wagner et al., 1998). Among non-viral systems, chemical vectors are the most widely used. These vectors have to face several extracellular and cellular barriers to efficiently reach the target cells. One of the main challenges is the lack of selectivity towards target tissues explaining their narrow therapeutic index. Lack of specificity causes high toxicity which hampers the efficacy at the target site. For that, designing an efficient targeted delivery strategy is of importance to further improve the delivery systems while reducing side effects. To target chemical vectors, it is possible to couple them with ligands specific to receptors present at the cellular surface of target cells. A second option is to use an externally applied trigger to control the gene delivery in the targeted area. These

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; eGFP, enhanced Green Fluorescent Protein; HIFU, High Intensity Focused Ultrasound; VEGF, Vascular Endothelium Growth Factor.

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two strategies are not mutually exclusive and could be combined. A physical trigger can be used either alone or combined with chemical or viral vectors to improve the targeting and/or gene expression efficiency. There are several physical methods starting from hydrodynamic injection to more sophisticated systems such as electroporation based on electric fields or ultrasound-mediated delivery.

2. Ultrasound as a physical method for delivery

Ultrasound can be used for imaging (ultrasonography) and for physical therapy (pulsed ultrasound mode) (Lindner, 2004; Mitragotri, 2005). These last years, therapeutic applications of ultrasound have gained new interests as a result of its exploitation for drug or gene delivery. Depending on the energy delivered by ultrasound, two types of effects can be produced either thermal or non-thermal each of them having their own application. High ultrasound intensities produce heating due to the absorption of acoustic energy by tissues; this property is employed in high-intensity focused ultrasound (HIFU) surgery or ultrasound-based physiotherapy. The "World Federation for Ultrasound in Medicine and Biology Temperature" has stated that an elevation of 1.5 °C is considered safe while an elevation of 4–5 °C during 5 min could be dangerous (Barnett et al., 2000). At low ultrasound intensities, cavitation, mechanical streaming and radiation forces are the main non-thermal effects obtained. These effects can induce some benefits such as tissue healing or ultrasound-mediated delivery. Mechanical streaming and radiation forces could be used to enhance the diffusion of drugs or nanometer sized bubbles across the vessel wall (Stieger et al., 2007; Wang et al., 2010). Inertial cavitation is the process of formation, oscillation and collapse of gaseous bubbles driven by an acoustic field. The presence of preformed microbubbles in the environment allows reducing the threshold of energy needed for cavitation. Used as an external trigger, ultrasound permits to spatiotemporally control the release of a drug encapsulated in microbubbles or in their surrounding in a non-invasive manner (Frenkel, 2008; Kinoshita et al., 2006; Kost et al., 1989; O'Neill and Li, 2008; Rapoport et al., 2007; Schroeder et al., 2007, 2009).

3. Microbubbles and sonoporation

Microbubbles are gas-filled particles consisting of a gas core encapsulated by a stabilizing shell. They have been first developed as ultrasound contrast agents to differentiate blood and their surroundings under ultrasound due to their low acoustic impedance difference. When microbubbles are driven by ultrasound at a frequency close to their resonance frequency, they oscillate and produce sound (Dayton et al., 2002; Morgan et al., 2000). These oscillations lead to an increased permeability of surrounding cells allowing a targeted local drug delivery. The increased cellular uptake has been attributed to the formation

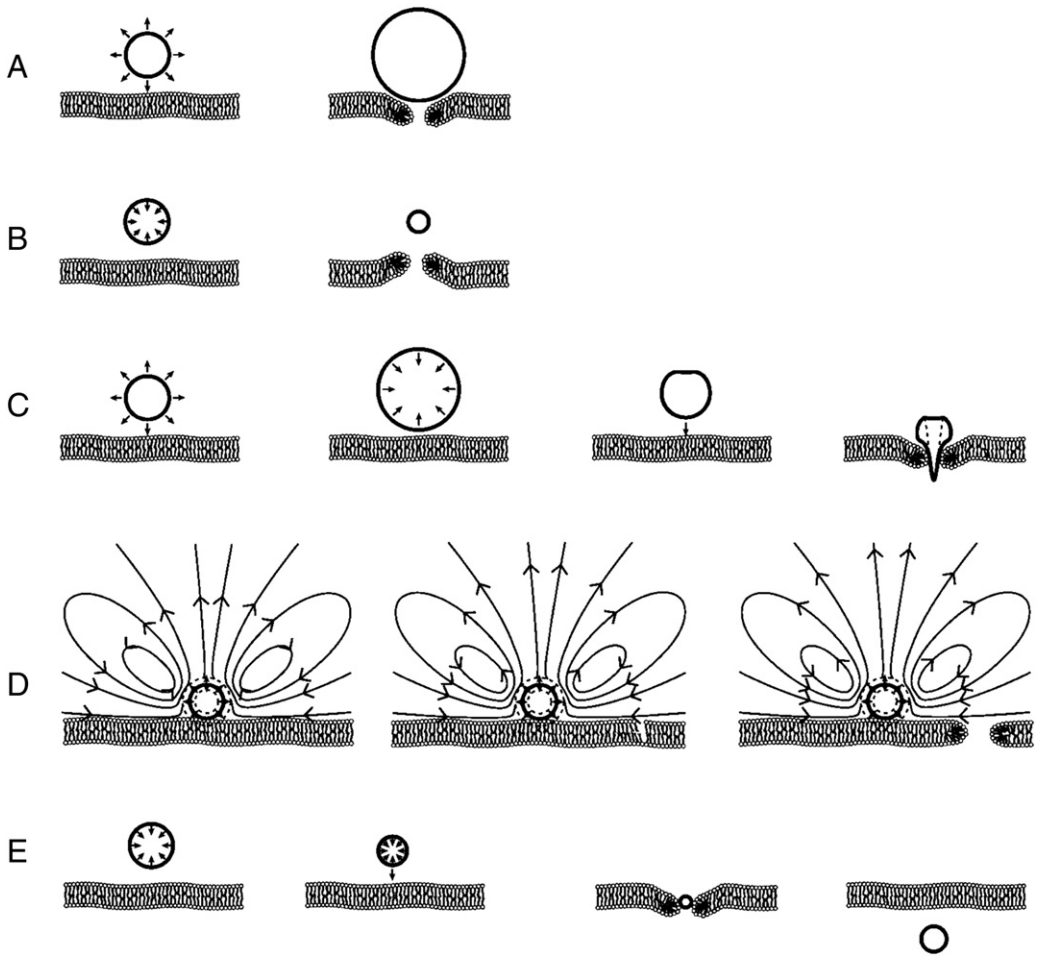

Fig. 1. Five mechanisms of pore formation provoked by microbubbles oscillating under ultrasound. (A) Push: During its expansion phase, a microbubble might touch a cell membrane surface, possibly pushing it apart. (B) Pull: During the contraction phase of an oscillating microbubble, the plasma filling the void left by the contracting bubble might pull the cell membrane towards the microbubble, possibly disrupting the plasma membrane. (C) Jetting: Jetting is the asymmetric collapse of a bubble, creating a funnel-shaped protrusion through the bubble which is directed towards a boundary. (D) Streaming: If a microbubble is attached to a cell membrane, the fluid streaming around the oscillating bubbles creates enough shears that could induce cell membrane rupture. (E) Translation: Owing to radiation forces, lipid-encapsulated microbubbles could translate through cell membrane. The microbubble may lose part of its shell whilst passing through the cell membrane. Figure adapted from (Delalande et al., 2011a).