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Studies on neutral, cationic and biotinylated cationic microbubbles in enhancing ultrasound-mediated gene delivery in vitro and in vivo

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ABSTRACT

Ultrasound-mediated gene transfer is emerging as a practical means of facilitating targeted gene expression and is significantly enhanced in the presence of exogenously added microbubbles. This study explores the influence of microbubble surface modifications on their interaction with plasmid DNA and target cells, and the functional consequences of those interactions in terms of ultrasound-mediated gene transfer. Polyethylene glycol-stabilized, lipid-shelled microbubbles with neutral (SDM201), cationic (SDM202) and biotinylated cationic (SDM302) surfaces were compared in terms of their abilities to interact with a luciferase-encoding reporter plasmid DNA and with target cells in vitro. The results demonstrate that the biotinylated cationic microbubble > cationic microbubble > neutral microbubble, in terms of their abilities to interact with target cells and to enhance ultrasound-mediated gene transfer, particularly at low microbubble concentration. The presence of a net positive charge on both cationic microbubbles promoted the formation of microbubble–nucleic acid complexes, although preformation of the complexes prior to addition to target cells inhibited the interaction between the microbubbles and target cells in vitro. The impact of these findings on potential in vitro or ex vivo therapeutic applications of microbubble-enhanced ultrasound-mediated gene transfer is discussed. All three microbubble preparations could be used to facilitate gene transfer in vivo and the potential advantages associated with the use of the cationic microbubbles for targeted gene delivery are discussed.

1. Introduction

The use of ultrasound as a stimulus for gene transfer offers significant advantage over other gene transfer modalities for application in gene-based therapies exploiting in vitro, ex vivo and in vivo strategies [1–4]. As a physical gene transfer methodology it circumvents many of the problems associated with viral gene transfer and, when compared with other physical gene transfer methods, its non-invasive nature provides significant advantage over alternative approaches. It has been shown that ultrasound-mediated gene transfer is enhanced by exogenously added microbubbles. Currently it is generally accepted that enhanced cell membrane permeabilization is facilitated by a combination of microbubble-induced microstreaming proximal to the target cell membrane resulting from stable cavitation and microbubble-induced microjet formation close to the target cell membrane that results from catastrophic inertial cavitation [4,5]. Microbubbles have been exploited clinically to enhance diagnostic ultrasound imaging and commercially available reagents comprise a significant degree of heterogeneity with respect to gas and the stabilizing shell composition. Shells are chosen to enhance the stability of the microbubble and may be composed of denatured protein (albumin), phospholipid and polymers [4]. Because these microbubbles can respond to an applied ultrasonic field by physically disintegrating and also by inducing site-specific cell membrane permeabilization, it has been suggested that they may be exploited to enhance gene transfer [6,7].

In light of the above-suggested mechanism by which microbubble-based reagents enhance ultrasound-mediated gene transfer, proximity between the microbubble, the nucleic acid and the target cell membrane would appear to be advantageous. Interestingly, many commercially available microbubble-based reagents that are exploited for diagnostic imaging purposes carry either a net neutral or slightly negative surface charge, and this serves to minimize interactions with cellular or molecular components in plasma [4]. However, if microbubbles are to be used in gene delivery, it would certainly be an advantage for the microbubble to carry a positive surface charge as this would enhance interactions with the negatively charged nucleic acid. Indeed, several studies have described the use of cationic microbubbles for the purposes of enhancing ultrasound-mediated gene transfer and have demonstrate electro-

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