Drug-loaded nano-microcapsules delivery system mediated by ultrasound-targeted microbubble destruction: A promising therapy method (Review)

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Abstract. The nano-microcapsules drug delivery system is currently a promising method for the treatment of many types of diseases, particularly tumors. However, the drug delivery efficiency does not reach a satisfactory level to meet treatment demands. Therefore, the effectiveness of delivery needs to be improved. Based on the alterations in the structure and modification of nano-microcapsules, ultrasound-targeted microbubble destruction (UTMD), a safe physical targeted method, may increase tissue penetration and cell membrane permeability, aiding the drug-loaded nano-microcapsules ingress the interior of targeted tissues and cells. The effectiveness and exact mechanism of action of the drug-loaded nano-microcapsules delivery system mediated by UTMD have yet to be fully elucidated. In this study, the latest advancement in UTMD-mediated drug loaded nano-microcapsules system technology was reviewed and the hindrances of UTMD-mediated drug delivery were assessed, in combination with a prospective study. The findings suggested that the drug delivery efficiency of nano-microcapsules mediated by UTMD was distinctly improved. Thus, the UTMD-mediated drug-loaded nano-microcapsules delivery system may significantly improve the efficiency of drug delivery, which may be a promising new therapeutic method.

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Contents

1. Introduction

An optimal drug delivery method is required to ensure safety and high efficiency of delivery. The nanoparticle has recently become one of the most popular and promising non-viral vectors (1) and has several advantages compared to viral vectors, such as lack of pathogenicity, lack of immunogenicity, biodegradability, wide range of host cells or tissues and diversification of loadings. The diameter of nanoparticles is 1/60-1/60,000 that of a cell. Therefore, drug-loaded nano-microcapsules are able to pass through several insurmountable obstacles and ingress the interior of cells and tissues for targeted therapy (1,2). However, drug delivery efficiency does not appear to reach satisfactory therapeutic levels, particularly under specific physiological or pathological conditions.

The oscillation and destruction of microbubbles, as well as microstreaming and radiation forces generated by ultrasound-targeted microbubble destruction (UTMD) may result in the rupture of stalwart barriers, such as the blood-brain barrier, dense connective tissue and the cell membrane structure, allowing more nano-microcapsules into cells and tissues. Recent studies demonstrated that UTMD has considerably

improved the efficiency of the nano-microcapsules drug delivery system (3-7).

2. Material selection and production of nano-microcapsules

Material selection. The materials used for manufacturing nano-microcapsules are classified as non-biodegradable and biodegradable. Non-biodegradable materials are able to protect DNA and RNA from digestion by enzymes, however, they may result in severe cytotoxicity and tissue necrosis (8-10). Biodegradable materials are highly biocompatible and are able to be decomposed by hydrolytic enzymes in the body and absorbed, ultimately metabolize to carbon dioxide and water through the tricarboxylic acid cycle and are excreted by the lungs, kidneys and skin. Therefore, biodegradable materials are considered the optimal choice and are widely used (11). Poly(lactic-co-glycolic acid) (PLGA) copolymer (12,13), one of the most commonly used biodegradable polyester materials, may be used in all types of drug-loaded nano-microcapsules embedding proteins (3), amino acids (3), genes (3), vaccines (9), antigens and growth factors (4). PLGA has been approved by the FDA for human medical use, and is non-toxic and harmless (10,14,15). Its crystallinity, solubility and water absorption capacity are regulated by modifying the proportion of polylactic and polyglycolic acid to control the rate of degradation, in order to meet the needs of the release of different embedded drugs (16-19).

Production of nano-microcapsules. Nanoparticle-producing technologies are currently classified into three categories, the mechanical pulverization, physical dispersion and chemical synthesis methods. Different types of nanoparticles are manufactured by different techniques and processes. The mechanical smashing method is a technique during which the mass is broken into nanoparticles by a high-speed rotary mill, jet mill, ultrasound, ball mill or colloid mill. The solvent evaporation and emulsification/solvent diffusion methods (physical methods) are suitable for producing nanosuspensions. The chemical synthesis method uses the hydrophobic segments of polymers to synthesize surface-active block copolymers. Several studies on nanoparticles successfully loading DNA (7), siRNA (7), anticancer drugs such as cisplatin (20) and mitoxantrone (21), and antiparasitic drugs such as pentamidine (22) and albendazole (22) were recently published. The encapsulation efficiency of drugs is affected by factors such as material and emulsifier concentration and intensity of the ultrasonic irradiation and the release rate is regulated by the proportion of various components of the nanomaterial and the pH (20,23).

3. Factors affecting the targeted delivery efficiency of nano-microcapsules

Size of the nano-microcapsule. Nano-microcapsules may be used for the treatment of a variety of diseases, particularly tumors. Different sizes of nanoparticles are selective for different tumor tissues. In general, nano-microcapsules ~150-300 nm readily accumulate in the liver and spleen and nano-microcapsules ~30-150 nm are prone to accumulate in the bone marrow, heart and kidneys. Particularly small nano-microcapsules, with a diameter of ~20-30 nm are usually cleared by the kidneys prior to ingressing the target tissues (24).

Electric charges borne on the surface of nano-microcapsules. The negative electric charges on the surface of nano-microcapsules limit their combination with certain gene drugs as well as with several target tissues and cells, particularly tumor cells (25,23).

Monitoring of the immune system. Nano-microcapsules that enter the human body may be cleared away as foreign bodies by the mononuclear phagocytes of the reticuloendothelial system in the liver and spleen (24).

High expression of specific antigens or receptors on the surface of tumor cells. High expression of specific antigens or receptors on the surface of tumor cells or tumor vascular endothelial cells is moderately or not expressed on the suface of normal cells or normal vascular endothelial cells (26,27).

4. Research on overcoming the hindrances of nanoparticle delivery

Prolonging the circulation time of nano-microcapsules. Modifying PLGA with monomethyl ether polyethylene glycol (mPEG) may shield some of the surface charges of the complex and evade clearance by the body's immune system, consequently prolonging the nano-microcapsules residence time in the systemic circulation (28,29).

Increasing the rate of gene drug encapsulation by increasing the amount of surface positive charges to promote delivery efficiency. The positive charges on the surface of PLGA are distinctly increased following its combination with poly-L-lysine (PLL), which is able to generate electrostatic interactions with the negative charges carried by DNA/siRNA to improve the loading effect (27).

Active targeted delivery by targeting molecules modifying nano-microcapsules. Recently, several investigators reported that drug-loaded nano-microcapsules modified with specific target antibodies may actively recognize target tissues or target cells, increasing the efficiency of drug delivery (26,27,30). The integrin αvβ3 is a receptor that is highly expressed on the surface of a variety of tumor cells or malignant tumor vascular endothelial cells and not expressed or detected in normal tissue cells or mature vascular endothelial cells. mPEG-PLGA-PLL polymers modified with ligand analogs that contain the Arg-Gly-AsP sequence combine with αvβ3 as antagonists to modify targeted delivery (29). Yoo *et al* (31) successfully constructed PEG-PLGA polymers modified with folic acid that encapsulated adriacin. Human oral squamous cell carcinoma cells exhibited increased uptake of nano-microcapsules modified with folic acid compared to unmodified ones in an *in vitro* study (32).

5. Research progress on the delivery efficiency of drug-loaded nano-microcapsules

Nano-microcapsule-targeted delivery technology has achieved some success; however, the gene transfection efficiency and

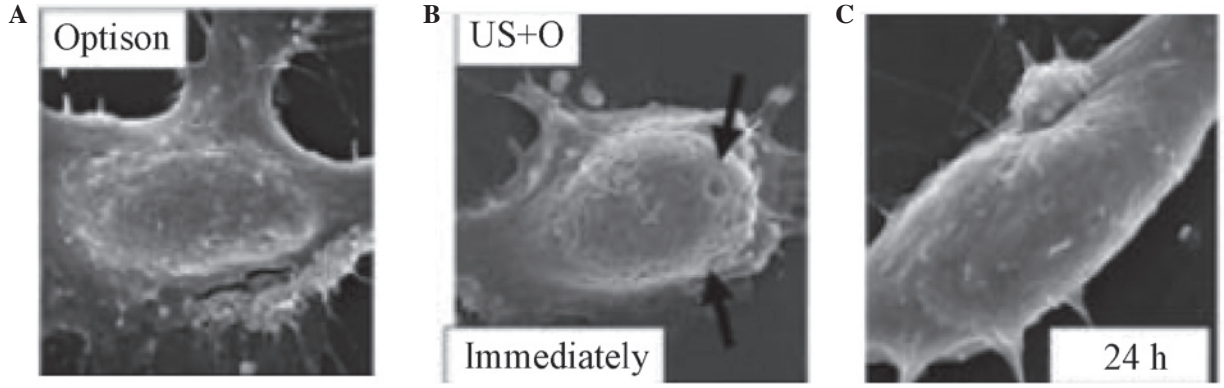

Figure 1. Formation of transient pores on the surfaces of cell membranes by ultrasonic irradiation under the electron microscope. (A) Pores in the cell membranes are not observed prior to ultrasonic irradiation. (B) Pores in the cell membranes are observed immediatedly following ultrasonic irradiation (arrows). (C) Pores in the cell membranes disappear 24 h after ultrasonic irradiation, van Wamel *et al* (37). US+O, ultrasound + Optison.

drug delivery efficiency remain low and do not satisfy the treatment demands.

A previous study conducted by de la Fuente *et al* (33) reported that plasmid DNA was delivered to the cornea and conjunctiva cells by a new type of nanocarrier synthesized by the bioadhesive polysaccharides hyaluronic acid and calcium silicate, the transfection efficiency of which was 15%. Chen *et al* (20) suggested that the targeted therapy effect of nano-microcapsules containing mitoxantrone was slightly superior to intravenous chemotherapy in mouse breast cancer only to a certain extent. A previous study demonstrated that drug-loaded nano-microcapsules were extremely difficult to pass through the vitreous cavity, a grid-like barrier consisting of collagen fibers bridged by proteoglycans (34). However, the treatment of retinal diseases requires drugs to cross this barrier, which remains an intractable problem. Similarly, in pancreatic cancer (referred to as 'the king of cancer'), which exhibits a special pathological anatomy structure, drug-loaded nano-microcapsules faced significant resistance. A previous study (31) demonstrated that the peripancreatic tissue of normal pancreas as well as the leaf gap tissues that act as ingress and egress pathways to the blood, nerves and lymphatics of the normal pancreas are loose connective tissues. Furthermore, little leaf gap tissues of normal pancreas are connected to the retroperitoneal and peripancreatic loose connective tissues. By contrast, the tissues surrounding pancreatic cancer are dense connective tissues and the little leaf gaps of pancreatic cancer tissues are immersed in a substantial amount of fibrous tissue and lymphocytes. Therefore, it is difficult for nano-microcapsules to ingress pancreatic cancer tissues and identifying a way to promote nano-microcapsule delivery efficiency is of utmost importance. UTMD was recently verified to be a helpful tool to enhance nano-microcapsule delivery, for which possible mechanisms have been described.

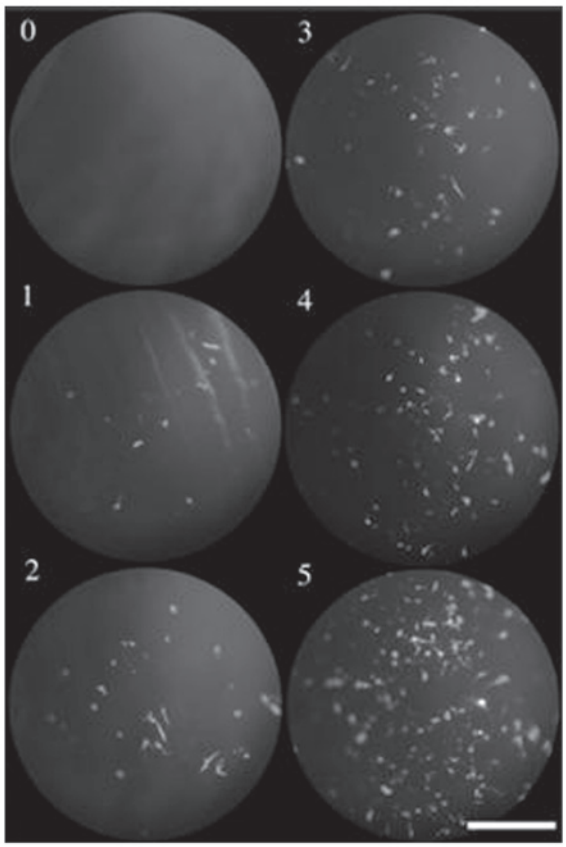

Figure 2. Representative photographs of scoring criteria. Gene transfer efficiency was expressed as a score of 0-5. Scoring was performed by three masked observers according to the following criteria: 0, no positive cells; 1, 1-25 positive cells per field; 2, 26-50 positive cells per field; 3, 51-75 positive cells per field; 4, 75-150 positive cells per field; and 5, ≥151 positive cells per field. Bar, 400 μm, Sonoda *et al* (41).

6. Possible mechanisms of UTMD for the promotion of nano-microcapsule delivery

First, UTMD leads to the formation of transient openings on the surfaces of cell membranes through which nano-microcapsules are able to enter cells and deliver drugs and genes (35-38) (Fig. 1). Second, the greatly increased oxyradical generation in cells under the effect of ultrasound (US) improves the permeability of cell membranes and promotes cellular uptake of nano-microcapsules (39). Third, US may increase endocytosis and activate cell membrane transport, thus enhancing the uptake of nano-microcapsules (40). US enables the local temperature of cell membrane to rise, which alters the liquidity of the membrane phospholipid bilayer and maximizes the cell membrane permeability.

Although the mechanism underlying its action has not been fully elucidated, UTMD has played a significant role in mediating drug and/or gene delivery to several targets, such as eyes, tumors, skeletal muscle, heart and bone marrow stem cells (3,4,34,40,41-46).

7. Research progress on nano-microcapsules delivery system mediated by UTMD

Eye. Sonoda *et al* (41) demonstrated that under the combination of US and Optison albumin-coated microbubbles, the green fluorescent protein (GFP) gene transfer to *in vivo* and *in vitro* rabbit corneal cells was greatly increased without apparent tissue damage, whereas US alone exerted a minimal enhancing effect on gene transfer (Fig. 2). Wu *et al* (42) also reported that using US in conjunction with commercially available SonoVue microbubbles safely enhanced GFP plasmid transfer to the mouse cornea *in vivo*. Another example of a successful gene transfer to the ocular surface mediated by UTMD was a study conducted by de la Fuente *et al* (33). By using a novel hyaluronic acid-Chitosan nanoparticle mediated by UTMD successful transfection of plasmid DNA in retinal pigment epithelium (RPE) cells *in vitro* and *in vivo* was achieved. Du *et al* (43) reported that UTMD is able to safely and effectively enhance siRNA-loaded nano-microcapsule delivery to RPE cells. Moreover, the most notable benefit of UTMD-mediated Cy3-siRNA loaded by nano-microcapsules was using the least amount of nano-microcapsules while maintaining a higher rate of uptake, which was achieved in rats *in vivo* and *in vitro*.

Tumor. Chumakova *et al* (44) reported that DNA-loaded nano-microcapsules produced from PLGA and PEI triggered by UTMD were successfully delivered to tumor cells *in vivo*. In addition, the gene transfection rate with UTMD was at least 8 times higher compared to that without UTMD. Hosseinkhani *et al* (45) demonstrated that cationic Dextran modified by PEG and US may target transfer plasmid DNA to fibrosarcoma cells efficiently. Rapoport *et al* (46) succeeded in synthesizing doxorubicin-containing polymer microcapsules and nano-microbubbles filled with gas, which were used for the treatment of mice bearing xenograft breast tumors, triggered by US. Doxorubicin was released from the polymer microcapsules to infiltrate target tumor interstitial tissues, leading to significant tumor shrinkage. Hauff *et al* (47) demonstrated that plasmid pU t651-MB packaged in inflatable nanoparticles combined with UTMD was effective in treating hepatocellular carcinoma in rats and gene expression in liver cancer cells was significantly increased. In the same manner, plasmid p16 as an anti-oncogene may effectively inhibit the growth of human pancreatic cancer cells. Yang *et al* (3) reported that gene-loaded Chitosan alginate particles combined with US significantly promoted the transfection efficacy of plasmid GFP in HeLa and 293T cells.

Heart and muscle. Bekeredjian *et al* (48) reported that luciferase reporter gene was target delivered to rat heart cells mediated by UTMD. After measuring the activity of luciferase and mRNA at different time points within 4 weeks, the investigators observed that the heart gene transfection efficiency mediated by UTMD was higher than that mediated by virus. Moreover, the transfection rate peaked after the first 4 days. Chappell *et al* (4) suggested that nano-microcapsules containing fibroblast growth factor 2 were largely deposited on the muscle tissue of rats mediated by UTMD.

8. Conclusion

Nano-microcapsule drug-loaded systems triggered by UTMD prolong the circulation time of the drug in the body and improve the drug concentration in target tissues, thus enhancing their efficacy. In addition, they reduce the frequency of drug administration. Therefore, they are regarded as fairly promising, particularly in cases with intractable malignant neoplasms. A previous study demonstrated that tumor cells were visualized through magnetic resonance concurrently with nano-microcapsule targeted therapy (49). Ke *et al* (50) of the Third People's Hospital affiliated with Peking University and Harbin Industry University, have synthesized a type of novel drug-loaded gold nano-microcapsule which may be useful for diagnosis and treatment. The gold nano-microcapsule combines the function of ultrasound contrast imaging with the function of photothermal therapy triggered by UTMD. Tumor position and size during the course of treatment is visualized and evaluated by enhanced ultrasound imaging of the polymer microcapsules. In addition, gold shells irradiated by laser generate high temperatures and destroy tumor tissues. However, the size of the gold nano-microcapsule is so minute that lesions may be visuaized only by using a great number of nanoparticles.

In conclusion, nano-microcapsules drug-loaded systems triggered by UTMD may play a critical role in therapy as well as imaging, which is a subject requiring further investigation.

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