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Ultrasound-targeted microbubble destruction: toward a new strategy for diabetes treatment

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Ultrasound-targeted microbubble destruction (UTMD) is a promising technique with an immense target-specific gene delivery potential deep inside the human body. The potential of this technique has recently been confirmed for diabetic patients. This technology allows the genes to transfer specifically into the inefficient pancreas using ultrasound energy without viral vector utilization. It has been speculated that this idea and the advent of modern gene therapy techniques could result in significant future advances. Undoubtedly, this strategy needs further investigation and many critical questions have to be answered before it can be successfully advanced. Herein, we introduce the salient features of this approach, the hurdles that must be overcome, the hopes associated with it and practical constraints to develop this method for diabetes treatment.

Introduction

The need for localized therapy in specific tissues and organs has led us to a variety of delivery techniques using viral vectors or a wide range of therapeutic nanoparticles [1]. In the case of gene delivery, although the idea of using viral vectors seems to be efficient and interesting, it is always a high risk process compared with using naked DNA [2]. Some novel physical techniques using ultrasound energy have been under investigation as the next generation of gene or drug delivery systems, such as microbubbles [3]. Previous studies showed that by circulating microbubbles in the bloodstream they can act as cardiovascular delivery agents, through the rupture in the specific areas of interest using ultrasound energy [4]. Such microbubbles have been conventionally used to enhance the reflectivity of perfused tissues in clinical ultrasonography but current studies are focusing on their significant potential in therapeutic applications. This technique was further developed as ultrasound-targeted microbubble destruction (UTMD) and different research groups are working on this strategy for different tissues and organs. For example, Phillips *et al.* [5] have recently used this idea for gene delivery to vascular smooth muscle cells using ultrasound-triggered delivery of plasmid DNA from electrostatically coupled cationic microbubbles. They successfully showed that DNA can be locally delivered to vascular smooth muscle cells using microbubble carriers and focused ultrasound.

During the past few years, UTMD has evolved mainly because of its ability to focus deep inside the human body, and to provide a modality for targeted delivery [6]. Many proof-of-principle studies have confirmed the high potential of UTMD as a noninvasive and targeted delivery tool [7,8]. This technique has the potential to transport and release specific substances into target tissues or organs [9], change the microenvironment [10] and promote stem cell homing [11]. Presently, a growing number of researchers are considering UTMD technology as a successful solution for delivery of specific substances in blood vessels [7], skeletal muscle [12], heart [13], lung [14], liver [15], among others. As can be seen from the growing number of publications in this field (Fig. 1), there has been heightened interest in the use of this strategy recently.

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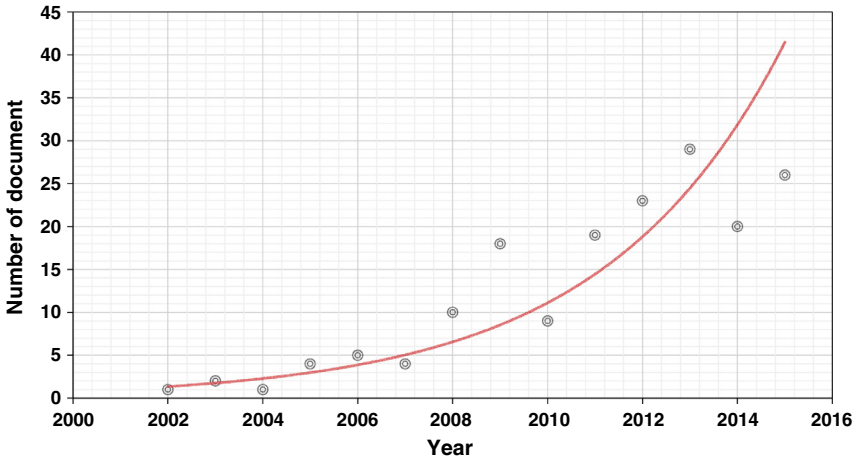

FIGURE 1

Number of scientific papers published per year using ultrasound-targeted microbubble destruction (UTMD) technique, compiled from a literature search in the Scopus database.

UTMD and diabetes

According to the literature review, it is expected that UTMD will also play a significant part in all aspects of diabetes treatment in the near future. Some primary studies have shed light on the potential benefits of this technique for diabetic patients [16]. Diabetes affects ~200 million people, and it is the sixth-most debilitating disease in the world [17]. Diabetes is a serious endocrine disorder that is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, causing defects in insulin secretion, insulin action or both. Diabetes has been classified into three main categories: type 1 insulin-dependent diabetes (IDD); type 2 non-insulin-dependent diabetes (NIDD); and type 3 gestational diabetes mellitus [18]. People who suffer from type 1 diabetes do not produce adequate amounts of insulin to sustain life, so they become dependent on exogenous insulin, and daily injections of insulin become necessary for survival. Although people who suffer from type 2 diabetes are not dependent on exogenous insulin for existence, many of them show reduced insulin production over time, which requires exogenous insulin for suitable blood glucose control [19]. Type 3 diabetes occurs mostly in pregnant women without any previous history of diabetes and can either disappear or change to diabetes type 2 after pregnancy [20]. Both types 1 and 2 diabetes involve either partial or complete destruction of beta cells of the pancreatic islets, which can be restored via one of the medical regeneration of islet beta cell methods [21]. Because the rate of beta cell changes in the human pancreas is slow, even after injury, regenerative medicine aims to propose new techniques for either beta cell replication or neogenesis [22,23]. Despite the few pharmacological treatments available for diabetes (e.g. insulin therapy and adequate blood sugar control), new treatment strategies focus on replenishing the deficiency of beta cell mass in the main types of diabetes by either islet transplantation or beta cell regeneration or improvement of beta cell function. Recent studies demonstrate that gene therapy can assist the pancreatic islets in normal rats using UTMD [16]. In this context, the gene delivery to beta cells using UTMD turned out to be successful and promising. This technology allows the genes to transfer specifically into the inefficient pancreas using ultrasound energy without viral vector utilization [24]. Moreover, this novel technology can be used for delivering various bioactive molecules, including therapeutic genes, to tissues available to receive ultrasound energy, such as the heart [to which glucagon-like

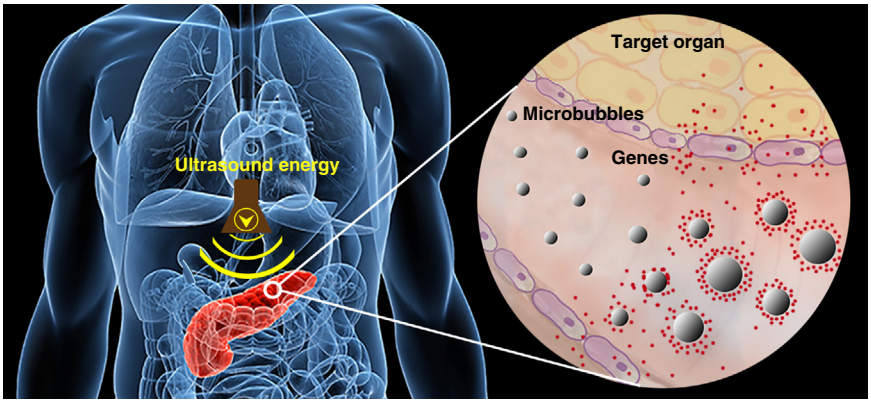

FIGURE 2

Schematic diagram of gene therapy mediated by ultrasound-targeted microbubble destruction (UTMD) for the treatment of diabetes.

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peptide (*GLP*)-1 was delivered] [25,26] and liver [27].

In our previous study, human islets were transplanted into liver via the portal vein, and human vascular endothelial growth factor (VEGF) was overexpressed in the liver by UTMD, resulting in the increase of neovascularization and improvement of the graft islet function [27]. This provided the community with a hint that islet cell transplantation could be an effective cure for treating diabetes in which UTMD allows almost noninvasive delivery of genes to pancreatic islets with an efficacy comparable to moderate beta cell function in adult animals.

In the concept of improvement of beta cell function, Chen *et al.* [16] showed that the delivery of genes to islet beta cells can be successfully achieved by utilizing a plasmid containing rat insulin promoter (RIP) via UTMD technology. The above described system could effectively control gene expression by glucose in animals that received a RIP–luciferase plasmid. In addition, they delivered a RIP–human-insulin plasmid to islets of adult rats by the system resulting in the increase in circulating human C-peptide and decrease in blood glucose levels. They also delivered a RIP–hexokinase-I plasmid resulting in the increase in hexokinase I protein expression in islets and insulin levels in blood. The study also revealed a novel approach in local gene expression targeted to beta cells by a modified rat insulin promoter (RIP3.1), in which intravenous microbubbles carrying plasmid DNA were destroyed within the pancreatic microcirculation by ultrasound energy.

Generally, UTMD offers a unique mechanism for gene delivery owing to the use of lipid-shelled microbubbles. The objective genes could be efficiently delivered from the shells to the target organs. In fact, under the ultrasound exposure, the microbubbles burst and the created energy makes the cell membranes permeable for the genes, which has a relatively low toxicity, low immunogenicity, organ specificity and broad applicability to acoustically accessible organs [26]. The regeneration of beta cells is a promising concept. Surprisingly, this technique enabled not only *in vivo* islet regeneration and restoration of beta cell mass but also normalization of blood sugar, insulin and C-peptide in rats without viruses [28]. In this study, human ANGPTL8 gene, which promotes pancreatic beta cell proliferation, was delivered to rat pancreas by UTMD, resulting in the expansion of the beta cell mass, improvement of glucose tolerance and increase of the fasting blood insulin level. In fact, UTMD allows various transcription factor genes related to beta cell development and function – betacellulin and pancreatic duodenal homeobox-1 [24], Nkx2.2 [29], NeuroD1 [30] – to transfer specifically into the inefficient pancreas using ultrasound energy without viral vector utilization resulting in beta cell regeneration. In a recent study, Chen *et al.* [31] proposed a novel approach in which a nonviral gene was targeted to pancreatic islets using UTMD technology *in vivo*. Treated animal models received a gene cocktail comprising the genes that control cell cycle and proliferation [cyclinD2, cyclin-dependent kinase (CDK)4 and GLP-1], which in baboons results in robust and durable islet regeneration with normalization of blood glucose, insulin and C-peptide levels. The overexpression of the gene cocktail led to beta cell regeneration which might be achieved by proliferation of the existing beta cells or differentiation of progenitor cells, although one might be concerned about these genes causing uncontrollable cell proliferation and even tumors. The preliminary results indicate that gene therapy via UTMD can be achieved *in vivo* by normalization of the intravenous glucose tolerance test (IVGTT) curves in streptozotocin (STZ) hyperglycemic-induced conscious tethered animals. These studies demonstrate direct evidence of successful islet regeneration and restoration of beta cell mass with the application of UTMD technology [30,31].

Fig. 2 shows a schematic diagram of gene therapy mediated by UTMD for the treatment of diabetes.

Concluding remarks

Like any other novel medical therapy, a number of crucial challenges and opportunities have yet to be considered for UTMD technology. Although this technology seems to be a promising approach for many aspects of diabetes treatment, more-detailed analyses are needed to confirm its efficacy before testing in the human body. The optimal genes or their combination, promotor, vector, size and composition of microbubbles, condition of ultrasound and target organ or cells should be further investigated. In addition, it remains to be elucidated whether the repeated treatment by UTMD can be safely carried out. Therefore, there are important obstacles that need to be addressed first. The efficiency of this technology needs to be tested in large animal models, because the ability of beta cell regeneration in rodents has been much higher than humans or large animals [32,33]. Another issue is the long-term efficacy of beta cell regeneration and the reproducibility of UTMD technology along with safety. A recent study indicated that a PiggyBac™ transposon plasmid might be useful for prolongation of the effects [25,30,31]. Moreover, the application of this technology for people who suffer from type 1 diabetes needs special attention for the prevention of destruction of the newly regenerated beta cells by autoimmunity. Hence, applying immunosuppressive drugs or immunotolerance methods would be required.

For that reason, extended research in all aspects of this technology needs to be conducted to understand the processes involved in the treatment. In addition, future *in vitro* and *in vivo* studies should systematically assess the various effects of UTMD on the human body. Although there are still major issues to overcome, the proponents of this strategy are optimistic that UTMD will be an effective approach in the treatment of diabetes, and will, one day, have an increasing impact on clinical applications. Interdisciplinary research areas and effective collaborations can potentially overcome the major issues and make this technique a viable option in the near future.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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