Development of Forward-Looking Ultrasound Transducers for Microbubble-Aided Intravascular Ultrasound-Enhanced Thrombolysis

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***Abstract*—In this paper, we report the development of miniaturized forward-looking transducers for microbubble-mediated intravascular ultrasound-enhanced thrombolysis (UET). UET has shown its efficacy for thrombo-occlusive disease by enhancing penetration of thrombolytic drugs into clots, reducing the required dose. In this study, we adopted a previously-developed forward-looking, stacked type transducer for thrombolysis treatment with low dose (approximately 0.4 µg/ml)-recombinant tissue-plasminogen activator (rt-PA). The 650 kHz-prototype transducer with a concave lens enabled a confined ultrasound beam despite the small aperture (diameter <1.5 mm) with respect to the wavelength (2.2 mm), and peak-negative-pressure of 1.35 MPa with a corresponding mechanical index of 1.67 was achieved. *In vitro* thrombolysis tests using the developed transducer with microbubble infusion showed that local administration of low dose-recombinant tissue-plasminogen activator (rt-PA) results in a two-fold increase in average thrombolytic rate versus without rt-PA.**

Keywords*—Intravascular ultrasound; catheter-directed ultrasound; ultrasound-enhanced thrombolysis; microbubbles

I. INTRODUCTION

Ultrasound-enhanced thrombolysis (UET) has been considered as an efficient adjuvant treatment for thrombo-occlusive diseases compared to conventional administration of thrombolytic agent [1]. UET enhances penetration of fibrinolytics, such as recombinant tissue-plasminogen activator (rt-PA), to the target clot, resulting in accelerated treatment time and reducing the required dose of thrombolytic drugs [2]. Ultrasound can be delivered in various approaches, such as transcutaneous-delivered high intensity focused ultrasound (HIFU) and catheter-directed transducer-tipped ultrasound (CTTU) [3]. CTTU has been an attractive approach due its efficient delivery of ultrasound energy. For deep vein thrombus, commercial Ekosonic side-looking array transducers mounted in a catheter (EKOS Corporation, Bothell, WA, USA) has shown its capability of efficient and safe thrombolysis with reduced use of thrombolytic drug [4]. However, it is currently limited by long treatment times (>10 h) [5], and some clinical studies have concluded that such side-fired, low-intensity ultrasound energy (0.5 W/cm^2) at ~2 MHz is not sufficient to produce clinically meaningful improvement in thrombolytic rate [6]. To increase thrombolytic rate, higher intensity, lower frequency ultrasound is required [7], but use of these parameters in a side-looking design could result in damage to the vessel wall because the high intensity ultrasound beam directly towards the endothelium.

We recently developed forward-looking, low frequency (sub-megahertz), intravascular ultrasound transducers for microbubble-mediated sonothrombolysis [5]. Our initial *in vitro* study demonstrated that the custom forward-looking intravascular transducer can generate sufficient ultrasound energy for clot dissolution by cavitation of microbubbles. Although it is promising that the developed transducer enables approximately 90% lysis without use of any fibrinolytics, the ultimate treatment involves low dose of rt-PA administration to avoid the possible distal embolism [5].

Hence, we aim to evaluate the performance of the developed intravascular transducer for rt-PA-combined, microbubble-aided thrombolysis treatment in this study (Fig. 1). This study builds upon our previous *in vitro* study by

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considering the effect of low-dose rt-PA on microbubble-aided sonothrombolysis.

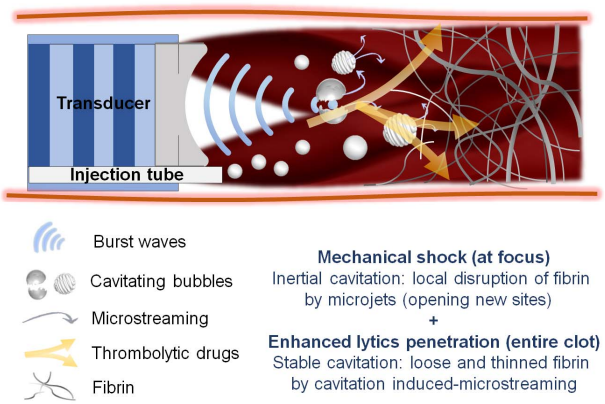

Fig. 1. Schematic of intravascular ultrasound-enhanced thrombolysis by using a custom miniaturized forward-looking focused ultrasound transducer.

II. Materials and Methods

A. Transducer developement

The previous forward-looking design was adopted in this study [5]. The operating frequency was also selected in the range of 600-700 kHz. The development procedure is similar with our previous work described in the reference [5]. Briefly, a 6-layer piezoelectric resonator was prepared utilizing PZT-5A ceramic plates with alternating poling directions. Each layer thickness was 250 µm, and the total thickness was approximately 1.6 mm. Since the lateral dimension of the stack was 1.2 mm, a pure longitudinal vibration mode was considered. The matching layer and concave lens were fabricated using alumina powder/epoxy bond mixture. The approximate wave speed of the matching layer material is 2700 m/s, and a quarter-wavelength-thickness design was considered. A concave lens was prepared for beam focusing. Previous study demonstrated that the confined ultrasound beam can be realized by using a custom plano-concave lens despite its smaller aperture (<1.5 mm) with respect to the wavelength (2.2 mm). In this study, we fabricated new concave lens which has a smaller radius-of-the-curvature (ROC) of 0.75 mm than the previous prototype, which had a 1 mm ROC. The lens fabrication procedure is similar to our previous work but a different size steel ball (1.5 mm in diameter) was used to prepare a polydimethylsiloxane (PDMS) mold.

B. Acoustic characterization

Pressure output of the new prototype transducer was measured for brief acoustic characterization. A needle hydrophone (HNA-0400, Onda Corp., Sunnyvale, CA, USA) was used to measure a pressure output. For high voltage excitation test, a function generator (33250A, Agilent Technologies, Inc., Loveland, CO) and 53 dB RF amplifier (75A250A, AR, Inc. Souderton, PA) were used to apply input voltage of 80 V_{pp}. The -6 dB beam diameter was also determined by controlling the hydrophone position using a 3-axis motion stage.

C. Blood clot preparation

Sample blood clots were prepared by a similar procedure with our previous works [5], [8]. Briefly, fresh bovine blood obtained from Densco Marketing, Inc. (Woodstock, IL, USA) was mixed with 2.75% W/V calcium chloride ($CaCl_2$) solution (Fisher Scientific, Fair Lawn, NJ). The mixture of the blood and $CaCl_2$ solutionwas drained to tygon tubes (6.35 mm ID, 7.94 mm OD). After immersion in a 37°C water bath for 2h, tubes with coagulated blood were refrigerated (3°C) more than 72 h for full retraction [5]. Clot samples for thrombolysis tests were 300±30 mg in mass, and positioned in the vessel-mimicking tube (Tygon ®, ID: 4 mm, OD: 5.6 mm).

D. Thrombolytic agent preparation

A 10 µg-vial of lyophilized recombinant human t-PA was obtained from Aniara Diagnostica LLC (West Chester, OH, USA). 1 ml-distilled water was mixed with 10 µg rt-PA, then divided into 10 test tubes (1.5 ml) according to manufacturer instructions. The test tubes containing the rt-PA solution were stored in a freezer (-20°C), and a thawed rt-PA tube was used for each thrombolysis test. In each test, 2 ml distilled water was stored in a 5 ml syringe to dilute microbubble contrast agent (MCA), and a 0.1 ml rt-PA solution was mixed with this diluted bubble mixture again. Therefore, the concentration of rt-PA at each test was approximately 0.4 µg/ml. Since the infusion rate of MCA+rt-PA was maintained as 100 µl/min, the resulting amount of infused rt-PA for 300 mg±10% clot is very low (approximately 0.4 µg for 10 min treatment).

E. Microbubble prepration

In this study, MCA was used to reduce the cavitation threshold pressure. Lipid-shell microbubbles were prepared by the same preparation procedure described in the previous works [9], [10], similar to Definity® (Lantheus Medical Imaging, North Billerica, Massachusetts) [11]. The diameter of microbubbles was 0.9 ± 0.45 µm, and the microbubble concentration was controlled by mixing with distilled water in this study. With the bubble-water volume ratio of 1:10, the controlled bubble concentration for *in vitro* study was 1×10^9 bubbles/ml.

F. In vitro test procedure

Thrombolytic rate was determined by measuring the mass-reduction after the treatment *in vitro*. Sample clots were positioned inside of a vessel-mimicking tube (Fig. 2). A portion of the vessel-mimicking tube was submerged into water (37°C) to locate the clot sample in the water, and the outlet of the tube was positioned in the air. Blood flow was not considered in this study. A coaxial cable attached to the patterned electrode and the prototype transducer was connected to a function generator (33250A, Agilent Technologies, Inc., Loveland, CO) and 53 dB RF amplifier (75A250A, AR, Inc. Souderton, PA). In this preliminary test, a constant excitation condition was maintained: 80 V_{pp}. 305 cycle-burst, 5 ms-burst duration at 650 kHz. A infusion tube was connected to a micropump (DUAL-NE-1010-US, New

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