Acoustic streaming effects on collagen self-assembly

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ABSTRACT

Anisotropic collagen-based biomaterials have gained significant attention in the fields of tissue engineering and regenerative medicine. They have shown great potential for wound dressing, corneal grafting, and exploring the mechanism of cancer cell invasion. Various external physical field-based methods for the fabrication of anisotropic collagen-based biomaterials have been developed, including electrospinning, microfluidic shearing, mechanical loading, and so on. In this study, we put forward an acoustic streaming-based method that uses acoustic wave-induced fluid streaming to control collagen self-assembly and fiber arrangement. Our acoustic device leverages a piezoelectric transducer to generate traveling acoustic waves in fluids, and the wave-fluid interaction further induces fluid streaming, known as acoustic streaming. If the fluid contains collagen macromolecules, the acoustic streaming is able to affect the collagen self-assembly process to create biomaterials containing directionally arranged collagen fibers along the streaming velocity direction. Therefore, this acoustic streaming-based method allows for manufacturing collagen hydrogel layers that contain acoustically arranged collagen fibers and have controlled anisotropic material properties. We performed a series of proof-of-concept experiments by using a fabricated acoustic device to control the self-assembly process of collagens loaded in a Petri dish. Our results show the effectiveness of arranging collagen fibers that follow the flow direction of acoustic streaming. To better understand the collagen manipulation mechanism, we used particle image velocimetry to characterize the acoustic wave-induced fluid streaming. We expect this study can contribute to the fabrication of collagen-based anisotropic biomaterials for biomedical applications.

Keywords: Acoustic streaming; acoustic waves; collagen fiber arrangement; collagen self-assembly; biofabrication

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1. INTRODUCTION

Collagen-based biomaterials are widely utilized in the biomedical field for wound healing[1], tissue engineering[2], and skin supplementation[3], as collagen, the main protein of the extracellular matrix, has high biocompatibility and biodegradability. Collagen constitutes over 30% of the total protein in the human body to ensure the functionalities of tissues and organs, including skin, vessels, muscle, tendon, and skeleton. Meanwhile, collagen presents in different hierarchical elements, like molecules, fibrils, and collagen fibers. The fibrils are formed by extremely parallel-oriented collagen molecules, and they are repeatedly distributed with a specific fibril D spacing[4]. Collagen fibers can be obtained from the self-assembly of collagen molecules. In collagen-based biomaterials, collagen alignment is considered as a key for controlling cell migration, cell morphology, and so on. To achieve the alignment of collagen fibers, several methods have been developed, including electrospinning[5], microfluidic channels[6], stretching[7], mechanical loading[8], electric fields[9], and magnetic fields[10]. However, there are limited studies on using acoustic waves to control collagen self-assembly and align collagen fibers.

Here, we put forward an acoustic streaming-based method to control collagen self-assembly and align collagen fibers. Acoustic technologies have gained great interest in biological research and biomedicine due to their appealing features, such as non-invasive, label-free, and high biocompatibility. Acoustics-based aligning technologies are normally based on bulk acoustic waves (BAWs) and surface acoustic waves (SAWs). BAWs are commonly generated by the thickness or lateral vibrational mode of a piezoelectric transducer[11], further leading to an acoustic radiation force and a drag force induced by acoustic streaming[12]. The BAW-induced acoustic streaming is a secondary steady flow, generated by the nonlinear acoustic wave-fluid interaction, when the piezoelectric transducer induces a high-frequency oscillation of a compressible fluid[13]. According to the wave-fluid interaction mechanisms, acoustic streaming can be categorized into two main categories: Eckart and Rayleigh streaming[14]. The Eckart streaming is typically generated in traveling BAW-based devices, while the Rayleigh streaming is usually generated in BAW-based resonators[14]. In this study, we used the traveling BAW-induced Eckart streaming to assist the self-assembly of collagen molecules into a hydrogel layer containing collagen fibers. We also compared the results with and without (control group) acoustic streaming. The comparison shows that acoustic streaming plays a critical role in the arrangement of collagen fibers during the process of collagen self-assembly. Our results show that collagen fibers can be generated compactly along with the streaming flow direction and aligned in the same orientation with respect to the streaming flow.

2. MATERIALS AND METHODS

2.1 Acoustic device design and operation

Fig.1 shows a test setup for studying acoustic streaming effects on collagen assembly. The test device is composed of a piezoelectric transducer fixed on a customized holder and a glass bottom petri dish (MatTek, 35 mm dish with 14 mm diameter glass bottom). The used transducer (Steiner & Martins, Inc.) has dimensions of 26 × 8 mm × 0.7 mm and a thickness-mode resonance frequency of 3.09 MHz. To excite the transducer for traveling BAW generation, a continuous sinusoidal signal at the resonance frequency of 3.09 MHz was generated by a function generator (Tektronix, AFG3022C), amplified using a power amplifier (E&I, A150), and then sent to the piezoelectric transducer. This test setup with a transducer can generate traveling BAWs in the Petri dish, as illustrated in Fig.1. The traveling acoustic waves further induce acoustic streaming, which can drive the flow of a collagen solution in the Petri dish and further affect the collagen self-assembly process.

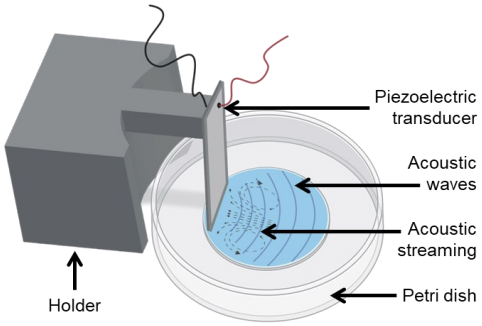

Figure1. Schematic of the device design, which is composed of a piezoelectric transducer fixed on a customized holder and a glass bottom Petri dish containing a solution with collagen molecules.

2.2 Collagen solution preparation

Rat tail type I collagen (Bio-Techne) is stored in a 0.06 mM acetic acid solution, and its stock concentration is 5 mg/mL. The stock solution was diluted to a concentration of 1 mg/mL for our experiments. To get 500 µL collagen solution with a concentration of 1 mg/mL, 100µL 5 mg/mL type I collagen stock solution was combined with 340 µL deionized water and 10 µL 0.1 N NaOH, followed by adding 50 µL 10× PBS to keep the pH in a range of 7.2-7.4. Note that this process should be performed in a low temperature environment, such as inside an ice bucket.

2.3 Microscopic imaging

During the acoustic experiment, the entire test device was placed on the stage of an inverse microscope (Nikon, T-DH) for monitoring the acoustic streaming field, as well as the phase change from a collagen solution to a collagen hydrogel. After experiments, for characterizing the collagen hydrogel, we used confocal laser scanning microscopy (Zeiss, LSM 880) to obtain both bright-field and fluorescence-field images of the fabricated collagen hydrogel layers.

3. RESULTS

3.1 Acoustic streaming characterization

The attenuation of acoustic energy during the traveling wave propagation leads to a steady net flow, *i.e.*, acoustic streaming. This streaming is governed by both the continuity and the Navier-Stokes equations.[15] To characterize the acoustic streaming field generated by our device, particle imaging velocimetry (PIV) experiments[16] were performed using a test setup illustrated in Figure 2a. A solution containing 5 µm-diameter fluorescent polystyrene particles was used as the tracing particles for PIV, and the piezoelectric transducer was excited at its thickness-mode resonance frequency of 3.09 MHz.

Figure 3 gives a stacked fluorescent microscopy image showing the flow pattern in a region close to the front side of the piezoelectric transducer. The stacked image shows a directional flow pattern in the middle and two streaming vortices on the left and right sides. To further characterize the streaming velocity field, the acquired time-sequential fluorescent microscopy images were processed by a PIVlab toolbox in MATLAB[16–18].

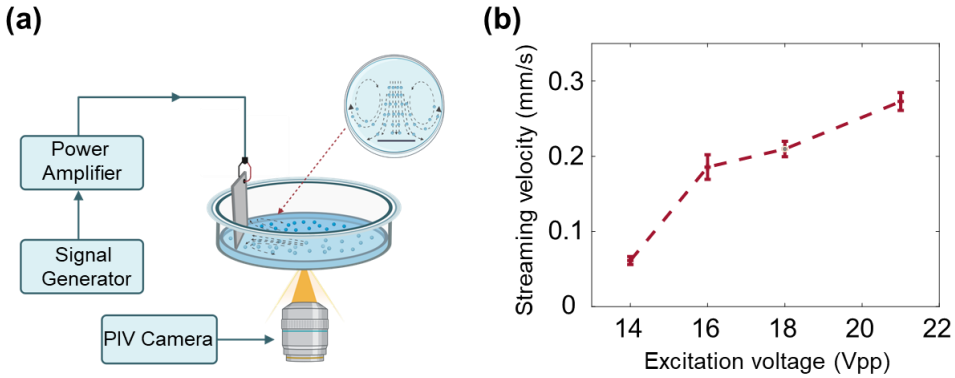

Figure 2. Setup and result of acoustic streaming characterization. (a) A schematic of the test setup for characterizing acoustic streaming. (b) Experimental streaming velocity in the middle region, near the front side of the transducer (n=5). The error bar shows streaming velocity tolerance.

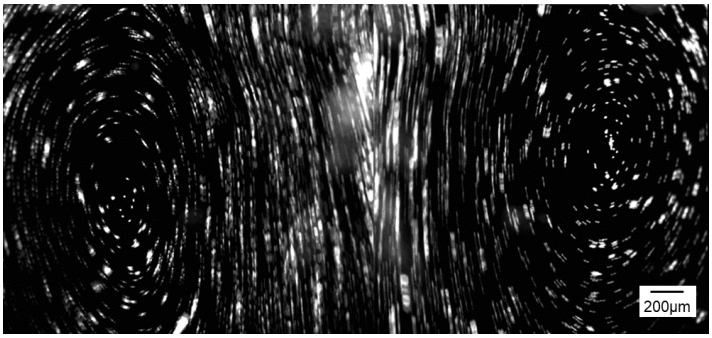

Figure 3. A stacked fluorescent microscopy image showing traveling acoustic wave-induced streaming field.

3.2 Acoustic streaming effects on collagen self-assembly

After characterizing the acoustic streaming field, we investigated the impact of our device on collagen self-assembly. In this experiment, a 1 mg/mL collagen solution was utilized, and we compared collagen self-assembly with and without continuous acoustic streaming. Following the self-assembly process, collagen fibers in the control group exhibited curved shapes and a randomly arranged pattern within the collagen hydrogel (Fig. 4a). In contrast, collagen fibers in the experimental group were clearly oriented in the direction of the streaming flow, appearing longer and more compact within the collagen hydrogel (Fig. 4b), compared to the control test. Fig. 4c demonstrates that nearly all collagen fibers were aligned and elongated, distributed at various heights within the acoustically fabricated collagen hydrogel. During the collagen self-assembly process, collagen fibers were stretched by the acoustic streaming flow. We gradually adjusted the excitation voltage for the piezoelectric transducer, while monitoring the process of forming a collagen hydrogel. Initially, the excitation voltage was set to approximately 20 Vpp to drive the collagen molecules flowing with the acoustic streaming. Once collagen fibers formed, the excitation voltage was reduced to approximately 14 Vpp to prevent alterations in the shapes of the aligned collagen fibers. Additionally, Fig. 5 provides a view in a large region of the collagen hydrogel that was fabricated adjacent to an acoustic streaming vortex. Within this region, most collagen fibers exhibited an aligned arrangement within the collagen hydrogel.

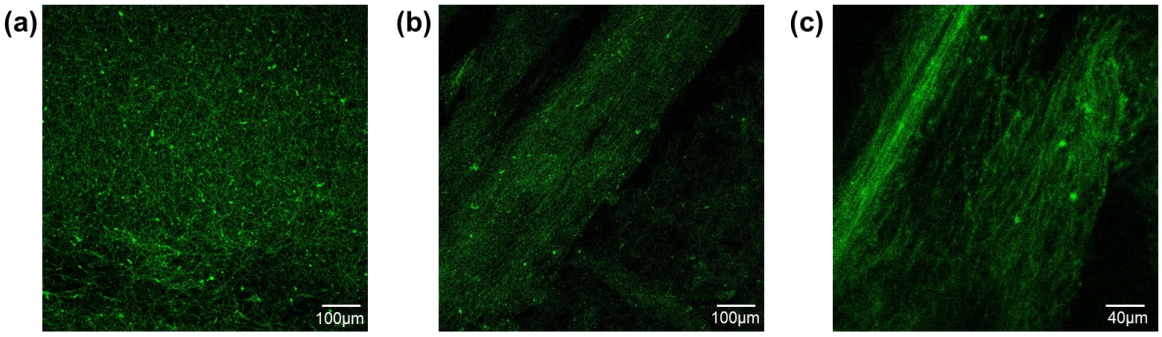

Figure 4. Fluorescent microscopy images of control and experimental groups. (a) Microscopic image of the control group showing random-distributed collagen fibers. (b and c) Aligned collagen fibers observed in the experimental groups under 25x and 10x objectives, respectively.

Figure 5. Microscopic image of a region in the aligned collagen hydrogel. The region is next to an acoustic vortex.

3.3 Standing waves effects on collagen self-assembly

Recently, multiple studies have shown that acoustic waves can modify collagen arrangement[19,20]. For instance, standing surface acoustic waves generated by interdigital transducers were shown to align collagen molecules[21]. The

acoustic radiation force and acoustic streaming are primary factors influencing the arrangement of micro/nano-particles in acoustic waves. In this study, we examined and compared the effects of both factors during collagen self-assembly. Significantly, there is a notable difference between the effects of these two acoustic factors. As illustrated in Fig. 6a, the diameter of collagen molecules is considerably smaller than the working region of an acoustic potential well at a node. Consequently, a group of collagen molecules can be trapped along an acoustic pressure node line of standing acoustic waves, during the collagen self-assembly process. Moreover, the standing wave-induced acoustic radiation force facilitated the aggregation of several bundles of high-density collagen fibers, as proven in Fig. 6b. The boundaries of these collagen fiber bundles are straight and in parallel with the wavefronts of standing acoustic waves. Therefore, standing acoustic waves tend to capture collagen molecules in specific regions (*e.g.*, areas with pressure nodes), whereas acoustic streaming enables the alignment of collagen fibers along the streaming flow direction. The streaming can also create collagen hydrogels containing larger bundles of aligned collagen fibers.

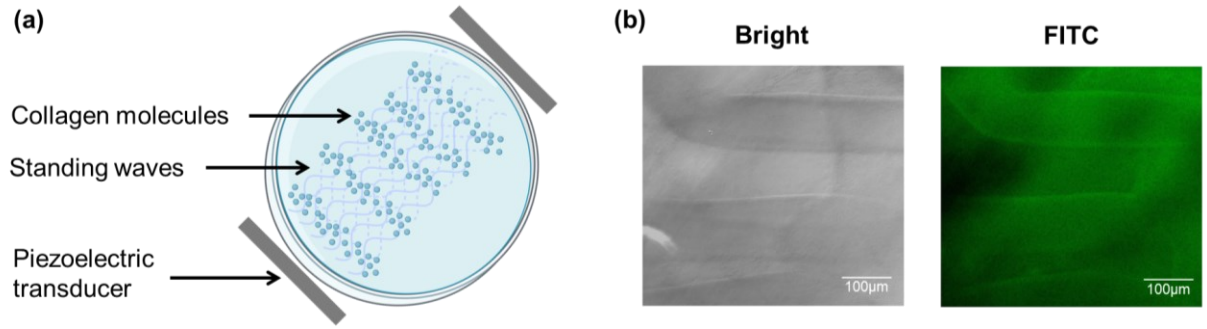

Figure 6. Effects of standing waves on collagen self-assembly. (a) The schematic illustrating the effects of standing acoustic waves on collagen molecules. (b) Microscopic images showing collagen fibers in a standing wave-based fabricated collagen hydrogel (left: bright field image, right: FITC fluorescent image).

4. CONCLUSION AND DISCUSSION

In this study, we employed an acoustic device to influence collagen self-assembly and obtain aligned collagen fibers within collagen hydrogels. Our device comprised a piezoelectric transducer and a glass-bottom Petri dish, operating with the traveling BAW-based mechanism capable of generating Eckart streaming through high-frequency acoustic wave interaction with collagen solutions. Specifically, the acoustic streaming generated by our device exhibited a main flow and symmetrical vortices. Collagen molecules within the collagen solution were driven along the direction of the acoustic streaming flow during the self-assembly process. This method not only promoted collagen self-assembly but also facilitated a customized arrangement of collagen fibers within the fabricated collagen hydrogel. PIV was utilized to visually reveal the fluid flow, demonstrating that collagen fibers exhibited an oriented arrangement consistent with the streaming flow direction. Additionally, the aligned collagen fibers exhibited elongated and aligned morphologies, while collagen fibers in control groups exhibited curved and randomized arrangements.

In our result, standing acoustic waves can trap collagen molecules at the acoustic pressure nodes, while acoustic streaming drives collagen molecules along the direction of the acoustic streaming flow. Furthermore, compared to other collagen-aligning methods, our method can complete the fabrication of aligned collagen hydrogels within minutes and perform this process in a Petri dish that is widely used in biomedical labs. Our acoustic device is easy to operate, contactless, label-free, and highly biocompatible. To further explore the potential influence factors on collagen self-assembly and acoustic streaming, such as collagen solution concentration, flow direction, and acoustic wave frequencies, we aim to conduct subsequent studies. We anticipate that the acoustic streaming-based collagen arrangement approach can lead to a valuable tool for quickly fabricating anisotropic collagen hydrogels, thereby contributing to tissue engineering, regenerative medicine, and other biomedical applications.

5. ACKNOWLEDGEMENTS

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