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HIFU induced particles redistribution in polymer matrix via synchrotron radiation X-ray microtomography

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ABSTRACT

High-intensity Focused Ultrasound (HIFU) was used to stimulate the embedded copper sulfate ($CuSO_4$) particles to release from the crosslinked poly (methyl methacrylate-co-butyl acrylate) copolymer solid matrix. In order to better understand the ultrasound release mechanism for drug/polymer delivery systems, the synchrotron radiation X-ray computed microtomography (SR-CT) was used to non-destructively investigate the structure of drug/polymer delivery systems after different HIFU treatment time. For the first time, we clearly demonstrate that ultrasonic waves can overcome the constraints of the polymer chain and drive the filler to move from the strong region to the weak region in the solid polymer matrix, thus resulting in a change in distribution of the filler in solid polymers. This result also demonstrates that SR-CT is a powerful technique which can be used to quantitatively study the 3D structure of fillers/polymers composite as it can take a broader and overall view than the conventional localized two-dimensional analysis method such as SEM, TEM.

1. Introduction

High intensity focused ultrasound (HIFU) is a technique that focuses high energy ultrasonic wave with a frequency range of 0.8 to 3.5 MHz to a millimeter size area [1], achieving very high spatial intensities in range of 1000–10,000 W/cm^2 [2], leading to remarkable mechanical effects, cavitation, and thermal effects without harming overlying and adjacent structures within the path of the beam [3]. HIFU originally developed as an extracorporeal tool for the treatment of tumors [4,5]. In recent years, HIFU also has been developed as a potential means of on-demand drug delivery due to its strong spatial and temporal capabilities, triggering drugs release from solid polymer matrix [6–9], polyelectrolyte microcontainers [10], multilayered capsules [11,12], and block copolymer micelles [13–21].

In the area of minimally invasive surgical techniques, important research efforts are being made in designing drug embedded interventional devices [22–24]. The material of interventional therapy device at the same time as a drug carrier, can precisely release drug to desired site, and thus promote the healing of diseased tissue, avoid causing chronic, repeated infection [25,26]. Furthermore, if this technique is combined with HIFU triggered release, “controlled or on-off release” at target site is possible be achieved [6–9].

Polymer-drug delivery system as the most commonly used drug delivery system, its drug release behavior induced by HIFU has attracted much attention. Langer et al proposed the concept of releasing drugs entrapped in a solid polymer matrix under ultrasound irradiation [6]. Bruinewoud et al. demonstrated an ultrasound controlled on-off release behavior of Ibuprofen loaded in a poly (butyl methacrylate-co-methyl methacrylate) P(BMA-MMA) random copolymer matrix [7]. As copper can regulate the activity of hypoxia-inducible factor (HIF-1α), and induce the regression of cardiomyocyte hypertrophy, thus facilitating tissue reconstruction and vascular regeneration [27–29], copper sulfate embedded shaped memory polyurethanes and cross-linked PMMA/BA have been developed in our previous work [8,9] as potential materials of cardiovascular stent, and their shape memory and drug release behavior under HIFU stimulation have been studied.

In the past, the research on ultrasound triggered drug release behavior was mainly focused on the relationship between ultrasound parameters and drug release rate [6–9]. However, no one has studied the distribution change of drugs in the polymer matrix induced by ultrasound. As we know, the drug distribution in the drug delivery systems [30] has an important effect on its release behavior, so the study of changes in drug distribution after ultrasound stimulation not only has practical value, but also has theoretical value for improving understanding of the mechanism of HIFU triggered drug release behavior.

X-ray computed microtomography is a powerful non-invasive investigative technique, which can investigate the internal three dimensional (3D) structures of various objects. Compared with the

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conventional X-ray micro-CT, the synchrotron radiation light source usually provides 2 higher orders of photon flux in relative parallel beam morphology, permits the rapid acquisition of data with high intensity and strong collimation via a high specification detector, and the resulting resolution of the synchrotron radiation X-ray computed microtomography (SR-μCT) can reach micron level and shows great possibilities for quantitative evaluation and design for solid drug delivery systems [31]. Especially, SR-μCT is an ideal method to obtain the spatial distribution of drugs when there is a high contrast resolution between the polymer and the drug, especially metal-contained compounds. In previous studies, this novel technology was successfully used to investigation the controlled release kinetics of monolith osmotic pump tablets [32], and study the mixing and separation process of drug particles [33], as well as the quantification of swelling and erosion in the controlled release of a poorly water-soluble drug [34]. SR-μCT has also been employed to investigate tablet swelling in real time by following the movements of embedded glass microsphere tracers [35].

In this study, the 3D distribution and $CuSO_4$ particle morphology change in the cross-linked PMMA/BA after different time of HIFU stimulation were visualized using a synchrotron radiation X-ray microtomography technique. This not only helps understand the drug release behavior triggered by HIFU in solid polymer/drug delivery systems, but also leads to a deep insight in the effect of ultrasound on the filler distribution in polymer composites, which may expand the field of application of ultrasound in material field. Moreover, it also demonstrates a new method for nondestructive quantitative characterization of the filler structure in polymer composites.

2. Experimental

2.1. Materials

Copper sulfate ($CuSO_4$, AR), Methyl methacrylate (MMA, CP) and butyl acrylate (BA, CP) were purchased from Chengdu Kelong Chemical Reagent Factory; Ethylene dimethacrylate (EGDMA, 98%) was purchased from ACROS Organic, Vlaanderen, Belgium; 2,2′-Azobis(isobutyronitrile) (AIBN) was purchased from Shanghai Chemical Reagent.

2.2. Preparation of crosslinked P(MMA-BA) containing $CuSO_4$ particles

After drying under vacuum at 110 °C for 24 h, $CuSO_4$ particles were first milled and sieved with a 500 mesh decimate sift. The monomer MMA and BA were distilled in vacuum prior to use; AIBN was purified by recrystallization from ethanol; EGDMA were used as received. Firstly, MMA (3.96 g), BA (2.04 g), EGDMA (0.06 g), AIBN (0.03 g) and $CuSO_4$ (0.3 g) were mixed and filled into the glass mold. The glass mold (100 mm × 100 mm × 2.5 mm) was placed in ultrasonic bath (KQ-250DE, Kunshan Ultrasonic Instruments Co., Ltd) and the polymerization proceeded at 60 °C for 24 h under ultrasound vibration. Afterward, the sample was taken out from the glass mold and vacuum dried at 80 °C for 24 h to remove unreacted monomers. For thermometric probe-embedded polymer samples, a thermometric probe was placed in the glass mold before the reaction mixture was poured into it, and during the polymerization the probe was fixed inside the polymer. The resulting sample were cut into several small discs with a diameter of about 8.5 mm and a thickness of about 2.5 mm.

2.3. Setup of HIFU

The setup of HIFU is shown in Scheme 1. There are four major components: the HIFU generator, the acoustic lens transducer, the sample fixing device and the water bath. The HIFU transducer (H-101, Sonic Concepts, USA) with an active diameter of 64.0 mm and a geometric focal length of 62.6 mm is mounted at the bottom of a tank filled with water and the beams of ultrasound are pointed upwards and focused on a circular spot with a diameter of about 3 mm. The ultrasound power output can be adjusted in the range of 0–200 W and the frequency of ultrasound is 1.1 MHz.

2.4. Operating procedures

The drug release behavior of copper sulfate-loaded samples under HIFU was investigated by exposing the samples to HIFU beams using the setup in Scheme 1. A NS9500 IR camera (FLIR Systems Inc, Wilsonville, OR, USA) with a sensitivity of 0.12 °C was used to determine the focus point of the HIFU device. The Copper sulfate-loaded samples was fixed on a height-adjustable clip to make sure the sample is fixed at the focus point, then 200 mL PBS buffer was filled in the container which was sealed with a latex sound permeable membrane and submerged the sample. The HIFU power output was set at 25 W and the environmental water bath temperature was kept constant at 37 °C. The samples were respectively exposed under HIFU beams for 30 min, 60 min, and 90 min, meanwhile the released Cu^{2+} concentration was measured by SpectrAA 220FS Atomic Absorption Spectroscopy.

2.5. Synchrotron radiation X-ray microtomography (SR-μCT)

SR-μCT tomographic images were acquisited at beam line BL13W1 of Shanghai Synchrotron Radiation Facility (SSRF) [31]. Briefly, Samples were scanned with synchrotron radiation with beam energy of 16 keV; the exposure time was 3.0 s and the distance between sample and CCD detector was 20.0 cm. Images were recorded by an X-ray digital camera with direct coupled (micro) fiber-optics (Photonics Science Ltd., Robertsbridge, UK), and then magnified by diffraction-limited microscope optics (Multiple of PCO = 2) and digitized by a high-resolution 2048 × 2048 pixel CCD camera (pco.2000, PCO AG, Kelheim, Germany), and the pixel size was 5 μm. For each acquisition, 720 projection images over 180° were taken. Bright field images and dark-field images were also collected during each acquisition procedure to correct for the electronic noise and variations in the X-ray source brightness. Reconstructed slices were acquired by using the software PITRE developed by SSRF [36]. The slices images were reconstructed to 3d STL-format models using 3D Viewer (plugin of Fiji, an open-source freeware) [37]. The 3D STL-format model was analyzed, calculated and rendered using Rhino 5.0 software (McNeel & Associates, USA).

3. Results and discussion

3.1. HIFU induced selective heating and drug release

The ultrasound-stimulus responsive behaviors for polymer have been studied for many years. Ultrasonic waves, as one kind of mechanical wave, can make polymer behave in a pattern of a forced vibration, and thus exert alternative stress at every chain of the polymers matrix. For viscoelastic polymer materials, one part of the stress is stored by elastic deformation of polymer chains then released as kinetic energy, other part of stress is transformed into heat due to the internal friction of polymer chain, which is known as ultrasound-induced heating effect [38,39]. In Fig. 1a, once the 25 W HIFU is turned on, the interior temperature of the sample increases rapidly from 37 °C to 103 °C in 10 s, and then remains unchanged. In contrast, the temperature of the surrounding water only has a slight increase from 37 °C to 39 °C and the temperature of the surface of polymer matrix increases from 37 °C to 49 °C. The results suggest that HIFU can be used to selectively heat a polymer while hardly heating the surrounding medium. The selective heating effect is manifested in two aspects: (1) spatial selectivity: selective heating of the focal region of HIFU while having little effect on the surrounding region; (2) material selectivity, different materials have different ultrasonic response behavior. In the liquids or flexible tissues, ultrasound easily propagates in the form of kinetic energy, rarely converting to thermal energy. In contrast, the forced motion of the viscoelastic polymer molecular chain under ultrasound