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Monitoring of transient cavitation induced by ultrasound and intense pulsed light in presence of gold nanoparticles

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ABSTRACT

One of the most important challenges in medical treatment is invention of a minimally invasive approach in order to induce lethal damages to cancer cells. Application of high intensity focused ultrasound can be beneficial to achieve this goal via the cavitation process. Existence of the particles and vapor in a liquid decreases the ultrasonic intensity threshold required for cavitation onset. In this study, synergism of intense pulsed light (IPL) and gold nanoparticles (GNPs) has been investigated as a means of providing nucleation sites for acoustic cavitation. Several approaches have been reported with the aim of cavitation monitoring. We conducted the experiments on the basis of sonochemiluminescence (SCL) and chemical dosimetric methods. The acoustic cavitation activity was investigated by determining the integrated SCL signal acquired over polyacrylamide gel phantoms containing luminol in the presence and absence of GNPs in the wavelength range of 400–500 nm using a spectrometer equipped with cooled charged coupled devices (CCD) during irradiation by different intensities of 1 MHz ultrasound and IPL pulses. In order to confirm these results, the terephthalic acid chemical dosimeter was utilized as well. The SCL signal recorded in the gel phantoms containing GNPs at different intensities of ultrasound in the presence of intense pulsed light was higher than the gel phantoms without GNPs. These results have been confirmed by the obtained data from the chemical dosimetry method. Acoustic cavitation in the presence of GNPs and intense pulsed light has been suggested as a new approach designed for decreasing threshold intensity of acoustic cavitation and improving targeted therapeutic effects.

1. Introduction

Acoustic cavitation is defined as the acoustically induced activity of gas-filled bubbles (involving nucleation, oscillation and collapse) that can occur at stable and transient modes [1]. In the stable mode, the bubbles oscillate around an equilibrium radius during a considerable number of acoustic cycles without collapsing. In transient cavitation, the bubbles grow rapidly and expand up to several times of their original size, and suddenly collapse during a single acoustic compression cycle [2]. In fact, very high shear stresses and shock waves are produced during the collapse. Moreover, high pressure and temperature at the collapse region can produce free radicals [3]. Transient cavitation can be fatal to cells, and is utilized to destroy tumors [4].

The existence of particles in a liquid provides a nucleation site for cavitation as its surface roughness leads to decreased threshold intensity of the cavitation, and is also responsible for increasing the quantity of bubbles when the liquid is irradiated by ultrasound [5].

Absorption of the energy of laser by the particles causes rapid heating, leading to vaporization of the surrounding medium and formation of transient vapour cavities [6].

Gold nanoparticles (GNPs) have been introduced as a novel nano-material applied in the field of cancer therapy because of their specific optical properties [7]. Low toxicity, good uptake by mammalian cells and induction of anti-angiogenesis properties by linking these agents on the surface make GNPs highly attractive for medical applications [8].

In this study, generation of vapour around intense pulsed light irradiated GNPs has been investigated as a procedure providing nucleation sites for ultrasound induced cavitation.

There are several methods for determining and quantifying cavitation, including sonochemiluminescence (SCL) [9] and chemical dosimetry [10]. We estimated the cavitation rate by two methods at different levels of ultrasound and laser radiations in the presence and absence of GNPS, and compared the results.

In the first method, SCL detection was performed within an ultrasonic transparent phantom [3]. Farny et al. proposed a polyacrylamide gel phantom for soft tissue simulation, the acoustic behaviour of which against ultrasound was similar to soft tissues

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[6]. The velocity of sound (1544 m/s) and acoustic impedance (1.6 MRayls) into the gel phantom have been reported to be similar to the values in soft tissue [11]. Therefore, the present study was performed on the polyacrylamide gel phantom.

Acoustic cavitation occurs when a polyacrylamide gel phantom is irradiated with high-intensity and low-frequency ultrasound. In transient cavitation, the bubbles grow rapidly and expand to several times their original size, and suddenly collapse during the acoustic compression cycle. Very high pressure and temperature at the collapse region can produce free radicals. The extreme conditions in the interior side of the bubble also lead to the emission of light, a phenomenon referred to as sonoluminescence [6]. A more intense light is emitted upon adding luminol to the polyacrylamid gel. This emission is known as SCL, and results from the chemical reaction of luminol molecules with OH radicals produced within the bubbles [9]. Luminol in the presence of sodium carbonate and OH radicals generates SCL at an emission peak of 425 nm.

The second method was performed on a water medium. When water is sonicated, OH radicals are formed on thermolysis of H_2O. Simplified equations for production of free radicals by collapse of cavitation in water solutions are as follows [12]:

$$H_2O \rightarrow H^{\cdot} + {}^{\cdot}OH \quad (1)$$

$$H_2O \rightarrow H_2 + {}^{\cdot}O \quad (2)$$

$$H^{\circ} + H_2O \rightarrow H_2 + {}^{\cdot}OH \quad (3)$$

$$O + H_2O \rightarrow {}^{\cdot}OH + {}^{\cdot}OH \quad (4)$$

Such chemical products may also be used to measure cavitation activity [12]. It has been shown that terephthalic acid [benzene-1,4-dicarboxylic acid; TA] is suitable for detecting and quantifying free hydroxyl radicals generated by the collapse of cavitation bubbles in ultrasound irradiations. During this process, TA as a dosimetric solution reacts with a hydroxyl radical generated by sonolysis of water. Therefore, 2-hydroxyTA is produced and can be detected using fluorescence spectroscopy with excitation and emission wavelengths of 310 and 420 nm, respectively [13].

2. Materials and methods

2.1. Synthesis and characterization of GNPs

GNPs were synthesized according to standard wet chemical methods using sodium borohydride as a reducing agent [14]. In this experiment, 50 ml of aqueous solution containing 4.3 mg of solid sodium borohydride was added to 100 ml of 100 μM aqueous solution of tetracholoroauric acid under vigorous stirring, which was continued overnight. GNPs thus formed were filtered through 0.22 μm filter paper, and were used for the experiments [14]. Transmission Electron Microscopy (TEM) showed the formation of spherical GNPs of approximately 5–9 nm by this method. The size histogram curve of GNPs obtained by counting at least 300 particles showed that 70% of GNPs were in the size range of 5–9 nm [15].

2.2. Ultrasound generator

In this pilot study, ultrasound irradiation was provided by a therapeutic ultrasound unit (215A; a coproduct of Novin Medical Engineering Co., Tehran, Iran; and EMS Co., Reading, Berkshire, England) in continuous and pulsed modes at a frequency of 1 MHz with an intensity of 1 and 2 W/cm^2 (I_{SATA}). The frequency range of therapeutic ultrasound was 1–3 MHz.

Acoustic calibration for the power of the device was performed in a degassed water tank using an ultrasound balance power meter (UPM 2000, Netech Corporation, Grand Rapids, MI) with uncertainty of ±1 W.

The ultrasound transducer (Coproduct of Novin Medical Engineering Co., Tehran, Iran; and EMS Co., Reading, Berkshire, England) was plane with a surface area of 7.0 cm^2 (Effective Radiation Area ; ERA = 5 cm^2) and was submerged in the bottom of a glass container filled with degassed water.

2.3. Light source

Intense pulsed light (IPL) radiation was applied by a therapeutic light unit (Lumenis one, Heinrich-Hertz Co., Germany) through 4 IPL pulses at 35 J/cm^2 fluence, 5 ms duration with a 560 nm long cutoff wavelength filter (long-pass filter).

2.4. Cooled CCD spectrometer

The SCL signal was detected using a cooled electro-optic spectrometer (Thermo-Electric cooled and regulated CCD, Avantec Co., NL-6961 RB Eerbeek, the Netherlands). The AvaSpect-2048 × 14 Fiber Optic Spectrometer was a back-thinned type CCD spectrometer with high quantum efficiency and high UV sensitivity. The spectrometer was equipped with a fiber optic entrance connector (standard SMA), collimating and focusing mirror and a diffraction grating. A 400-micron fiber optic equipped with specific connectors was used to transfer light from the phantom to the spectrometer.

2.5. Gel phantoms and SCL detection of luminol

The luminol stock solution was prepared by dissolving 0.041 g of luminol (5-amino-2, 3,-dihydrophthalazine-1,4-dione) (Sigma, Aldrich, Munich, Germany) and 85.23 g of sodium carbonate (Sigma, Aldrich, Munich, Germany) in 500 ml of deionized water, and was allowed to stand for approximately 24 h before use [9]. 100 μl of luminal solution was added into each gel sample. The polyacrylamid gel was prepared by the procedure stated by Khokhlova et al. [16]. 9.735 g of acrylamide and 0.265 g of bis-acrylamide (Sigma, Aldrich, Munich, Germany) were dissolved in 50 ml of deionized water. Adding 0.02 g of ammonium persulfate and 0.2 ml of TEMED (Sigma, Aldrich, Munich, Germany) as polymerizing initiator created a 7% acrylamide gel. The gel was poured into a mould (4 × 4 × 8 cm^3) and allowed to polymerize under vacuum.

In addition, a container with 10 × 8 × 16 cm^3 dimensions was prepared from black Perspex (PMMA) slabs of 5 mm thickness. A quartz window was designed in one side of the container in order to transmit emitted light. A band-pass filter (FWHM = 375–475 nm) was put on the quartz window.

The ultrasound probe was placed under the phantom through an especially designed hole in the bottom of the container. The container was filled with degassed distilled water thermostated at 18 °C [17]. In order to prevent successive reflections of ultrasound waves, a layer of foam was pasted inside the container on the floor and walls [17]. Black Perspex slabs sealed the phantom from background light. Cavitation detection was conducted in the phantoms made of a transparent polyacrylamide gel in the presence and absence of GNPs (0.22 mg/ml) [17]. It should be noted that GNPs were injected into the central axis of the gel's blocks 5 min before the measurements. The same volume of water was injected into the control phantom instead of GNPs. The experimental set-up is shown in Fig. 1.

The gel's block was put in a specific location in the back of the quartz window into the container. The ultrasonic probe was inserted beneath the gel, and the SCL spectrum was taken for 20 s. These steps were repeated three times for each gel in ultrasonic

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