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Investigation of Inertial Cavitation of Sonosensitive and Biocompatible Nanoparticles in Flow - Through Tissue-Mimicking Phantoms Employing Focused Ultrasound

Abstract: A promising approach to drug delivery applications for chemotherapeutics is the use of drug carriers to reduce the total amount of cytostatics, minimizing side effects. In addition, the carriers, loaded with the drug, can be guided to the tumorous tissue via the vascular system, which enables a local drug release (LDR). In our case, LDR is activated due to the sonosensitive behavior of the nanocapsules by inertial cavitation (IC) caused by focused ultrasound (FUS). Thereby, IC is excited by employing sound pressures within the recommended limit allowed for diagnostic ultrasound. In order to verify this drug delivery approach for its clinical suitability, a tissue-mimicking flow-through phantom, containing a small vessel, is used. Investigations have shown that the drug releasing cavitation effect associated with the sonosensitive and biocompatible nanocapsules also occurs in fine vessel structures, even in the case of moving particles and vessel diameters d_c smaller than the wavelength λ .

Keywords: Inertial cavitation, nanoparticles, tissue - mimicking phantom, flow - through.

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1 Introduction

The article Global Cancer Statistics 2020 illustrates that cancer remains one of the deadliest diseases. Although there have

been great advances in the treatment of cancer as well as research into new methods in recent years, the mortality rate is still high [1]. Common treatment methods include chemotherapy by applying active pharmaceutical ingredient (API), radiation therapy and surgery. The last two medical approaches aim to treat the tumor locally, whereas chemotherapy is usually applied systemically, i.e., affecting the whole body. The advantage of this systemic treatment is that the administered agents are dispensed in the different organs. Thus, scattered tumor cells and metastases can be reached and destroyed.

Due to occurring side effects, systemic chemotherapy is limited in terms of dosage [2]. In some circumstances, the amount of administered API may not be sufficient to treat large tumors that have overgrown their vascular supply, e.g., breast cancer tumors [3]. For this reason, research focusses on drug delivery concepts to on the one hand decrease the systemic concentration of API inside the body and on the other hand to increase local concentration at the tumor while minimizing adverse side effects.

One possibility is to administer the API intra-arterially followed by chemofiltration to purify the blood. This treatment is limited to the tumor, but catheters for blood purification must be placed by means of a surgical procedure. This surgical intervention can be avoided and high concentrations can be achieved at the tumor by use of nanoparticles for local drug delivery, which can be employed as carriers for APIs [4-6].

In this study, we utilize newly designed sonosensitive and biocompatible poly(DL-lactide-co-glycolide) (PLGA) spheres, which are planned to serve as drug carriers. These API-loaded particles are intravenously applied to the body and they accumulate close to the tumorous tissue via the vascular system. Drug release is initiated by FUS via the effect of IC. Depending on the vascularization, blood vessels can exhibit very small diameters in the sub-millimeter range [7], which are smaller than the commonly used ultrasound wavelength λ in FUS applications. As drug release is not supposed to take place by diffusion processes, but rather via IC, one needs to verify the excitation of IC at these small vessel diameters. For

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this investigation, a flow through tissue-mimicking phantom is used for simulating realistic conditions.

Since ultrasound is known to cause severe tissue damage at large peak rarefaction pressures (PRFP), our requirement for this therapeutic application is to comply with the limit value for diagnostic ultrasound applications recommended by the FDA. This limit value is represented by the Mechanical Index (MI), which reads as

$$MI = \frac{\hat{p}}{\text{MPa}} / \sqrt{\frac{f}{\text{MHz}}} \quad (1)$$

Currently, the FDA restricts the MI to a maximum of 1.9 for peripheral vessels in order to prevent damage in the human tissue.

In this study, we utilized a monofrequent burst signal at frequencies $f = (550 \text{ kHz}; 650 \text{ kHz}; 750 \text{ kHz}; 850 \text{ kHz}; 950 \text{ kHz})$ with a PRFP amplitude $\hat{p}_{\text{PRFP}}$ corresponding to a $MI = 1.4$. The results prove that the sonosensitive and biocompatible nanocapsules show IC, even when they are pumped through a very small vessel-imitating canal ($d_c = 1 \text{ mm}$) in the phantom.

2 Experimental

2.1 Measurement Setup

The cavitation measurement setup, controlled by a computer using MATLAB, can be divided into three subgroups, and additional peripheral devices (see Figure 1).

The emitter unit consists of an arbitrary waveform generator (33522B, Agilent) to provide the burst sequence, which is amplified by an RF power amplifier (1140LA, ENI) to then being transferred to the broadband FUS transducer via an RF impedance matching network.

Figure 1: Schematic measurement setup.

The main components FUS transmitter (H-231, Sonic Concepts), flow through tissue-mimicking phantom as well as the broadband passive cavitation detector (PCD) (Y-107, Sonic Concepts) are placed in a cylindrical basin, which is filled with deionized and degassed water. Both the transmitter and the PCD are spherically focussing. The PCD is placed in the central opening of the FUS transducer leading to a superimposition of their focal region. At this point, it should be mentioned that this measurement setup could also be employed in a clinical application, since the transducers' rotational axes are collinear and these are not positioned perpendicular to each other as it is commonly known from various passive cavitation detection setups.

The receiver unit includes a passive 2nd order highpass filter to suppress the low order harmonics of the received broadband IC signal, before it is digitized by a digital oscilloscope (DSO7014B, Agilent).

Additional peripheral devices include a PID temperature control unit to guarantee a constant water temperature of 30 °C. Throughout the measurements, the water temperature is monitored using a temperature sensor (Pt 100, RS Pro). Four heating rods (Schego 548 300 W, Schego), equally distributed in the basin, are utilized for heating the water. A pump circulates the water during the measurement to maintain a constant water temperature of 30 °C.

Besides these devices, we used a peristaltic pump (15KS 4 roller, Boxer) to pump the nanoparticles at a constant flow rate from a beaker through the phantom in a collection container. To prevent any sedimentation and therefore a non-uniformly nanoparticle concentration, the beaker is placed on a magnetic stirrer. For simulating blood flow at a certain velocity, the rotation speed of the pump is controlled via a 0 – 5 VDC signal, which is generated by a multifunction I/O device (USB 6212, NI).

Figure 2: Sectional view of the phantom.

2.2 Measuring Objects

In order to investigate this measurement procedure for its clinical suitability, we developed a flow - through tissue - mimicking phantom. The phantoms' material consists of a polyvinyl alcohol (PVA)-water mixture whose sound velocity ($c_{PVA} = 1521 \text{ m/s}$) and acoustic wave impedance ($Z_{PVA} = 1,60 - 1,65 \times 10^6 \text{ kgm}^{-2}\text{s}^{-1}$) are comparable to the corresponding values of biological tissue. Appropriate molds were designed for the production, into which the PVA compound is cast. Both the phantoms' widths w_p and the canal diameter d_c were varied to imitate various tissue thicknesses and different blood vessel diameters. The values were altered from $w_p = 21 \text{ mm} - 63 \text{ mm}$ and from $d_c = 1 \text{ mm} - 3 \text{ mm}$, respectively. Only the height of the phantoms is constant at $h_p = 100 \text{ mm}$ (see Figure 2). Figure 2 also shows that stabilizers are cast in the phantom and that hose connections are attached which enables the imitation of a perfusion.

However, in former cavitation investigations, we used a

Figure 3: a) Photo and b) sectional view of the cuvette with drawn to scale -1 dB focal zone and hatched cavitation area.

cuvette with routed windows covered by an latex layer to minimize sound reflections at the cuvette wall as well as to match the acoustic impedance (see Figure 3 a)). Figure 3 b) depicts a sectional view of the cuvette, in which the position of the -1 dB focal zone is drawn to scale. In this representation, the focal length l_f extends across the entire width of the cuvette $w_c = 12 \text{ mm}$, but frequency-dependent sound field measurements at $f = (550 \text{ kHz; } 650 \text{ kHz; } 750 \text{ kHz; } 850 \text{ kHz; } 950 \text{ kHz})$ have revealed, that the length becomes $l_f = (12.7 \text{ mm; } 11.5 \text{ mm; } 10.2 \text{ mm; } 9.5 \text{ mm; } 7.9 \text{ mm})$. Corresponding to that, the diameter of the focal zone d_f alters by frequency, it becomes $d_f = (1.62 \text{ mm; } 1.49 \text{ mm; } 1.33 \text{ mm; } 1.17 \text{ mm; } 1.04 \text{ mm})$. Hence, the focal volume, in which cavitation can be excited, changes. This zone is displayed by the hatched area. If one rotates this zone around the horizontal axes, one obtains the -1 dB cavitation volume V_C .

In contrast, the cavitation zone inside the phantom reduces to a fraction of the zone inside the cuvette (Figure 4). For this reason, it is investigated, if the nanoparticles also cavitate at these geometric dimensions.

Figure 4: Sectional view of the phantom with drawn to scale - 1 dB focal zone and hatched cavitation area.

2.3 Nanoparticles

Nanoparticles can be divided in nanospheres (NS) as well as in nanocapsules (NC). In this study, we employed NC. The NC used for these investigations were produced at the Division of Pharmaceutics of the University of Erlangen. We designed new sonosensitive, biocompatible NC, which have dimensions from $200 \text{ nm} - 250 \text{ nm}$ and also show IC in a well focusable frequency range. For the production of the NC, an emulsion-diffusion-evaporation method was applied, followed by freeze-drying. It is assumed that the NC have rough surfaces produced at the freeze-drying procedure, which yield to small gas pockets acting as IC bubble nuclei. The PLGA-NC were rehydrated with degassed pure water, before IC was investigated.

2.4 Measurement Procedure

In this investigation, an established burst signal $u_s(t)$ ($f = 750 \text{ kHz}$, $\hat{p}_{PRFP}(MI = 1.4)$, $M = 50$, $T_B = 0.6 \text{ ms}$, $T_P = 2 \text{ s}$) was applied, which has already induced IC of the nanocapsules in previous measurements, according to

$$u_s(t, M) = \sin(2\pi ft) \cdot W(t, M) , \quad (2)$$

with

$$W(t, M) = \sum_{m=1}^M \hat{p}_{PRFP}(MI = 1.4) \cdot \text{rect}\left(\frac{t - T_B/2}{T_B} - \frac{(m-1) \cdot T_P}{T_B}\right). \quad (3)$$

The digitized signal $u_D(t, m)$ of the hydrophone's output of every burst m was spectrally analyzed via FFT to calculate the

Table 1: Measurement parameters and measurement results.

Measurement object	v_f in mm/s	f in kHz	$\hat{p}_{PRFP}(MI = 1.4)$ in MPa	V_c (-1 dB) in mm ³	S_{rm} (pure water) in $\mu V/\sqrt{Hz}$	S_{rm} (talcum water) in $\mu V/\sqrt{Hz}$	S_{rm} (nano-capsules) in $\mu V/\sqrt{Hz}$	S_{rm}/V_c (nano-capsules) in $\mu V/\sqrt{Hz} \text{ mm}^{-3}$
Cuvette	0	750	1.25	9.44	0.76	5.07	5.20	0.55
P30-3	100	750	1.25	3.96	0.19	4.63	3.60	0.91
P30-1	50	550	1.04	1.21	0.049±0.0035	2.10	2.30	1.74
		650	1.13	1.10		2.22	2.65	2.02
		750	1.25	0.96		1.95	2.08	2.03
		850	1.29	0.82		1.75	2.03	2.13
		950	1.36	0.70		1.66	1.68	2.37

discrete frequency amplitudes $U(i)$. We determined the voltage spectral density (VSD) $S_r(m)$ as follows from [8]

$$S_r(m) = \sqrt{\sum_{i=i_s}^{i_E} U(i)^2} / \sqrt{B} \quad (4)$$

Finally, the mean value of the VSD S_{rm} is calculated in order to compare the measurement results

$$S_{rM} = \frac{1}{M} \sum_{m=1}^M S_r(m) \quad (5)$$

Individual parameters for the measurements are listed in Table 1. The flow velocity v_f in each canal corresponds to the blood flow velocity in small arteries (1 mm – 3 mm) and small veins (1.5 mm – 2.4 mm), respectively [7]. In addition, we set an $MI = 1.4$ to ensure that the phantom, imitating human tissue, is exposed to the same acoustic conditions at each frequency.

3 Results and Conclusion

For classifying the nanocapsules' VSD, we tested a talc-water mixture as a positive sample (indicator of cavitation) and pure water as a negative sample for IC.

First, we used the phantom (P30-3), which has a width $w_p = 63$ mm and a canal diameter $d_c = 3$ mm, representing a blood vessel beneath a tissue thickness of 30 mm = $(w_p - d_c)/2$. Since cavitation could be excited in this phantom, we then employed the phantom (P30-1) with the same width, but with the smallest canal diameter $d_c = 1$ mm, which is smaller than the wavelength λ at each frequency. As the results in Table 1 demonstrate, the NC show IC at each employed frequency. It is also became obvious, that IC is weaker than in the previous measurements using the cuvette and the phantom P30-3 with a larger canal diameter, indicating the relevance of the size of the cavitation volume. Moreover, the VSD of the NC

(phantom P30-1) declines in spite of an increasing sound pressure $\hat{p}_{PRFP}$.

If one would normalize the VSD of the NC to the cavitation volume V_c , IC would be strongest at $f = 950$ kHz. At this frequency, focusing of the sound wave is strongest and the risk of damaging surrounding tissue due to IC is minimized which is beneficial for a clinical application.

In summary, we were able to show that IC of the NC can also be excited in vessels, which are smaller than the wavelength of FUS.

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