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ON COMPARISON BETWEEN POLYMER- AND PHOSPHOLIPID-SHELLED MICROBUBBLES FOR CONTRAST-ENHANCED ULTRASOUND MEASUREMENTS OF CAPILLARY MICROCIRCULATION.

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Abstract

The focus of contrast-enhanced ultrasound research has developed beyond visualizing the blood circulation to new areas such as perfusion and molecular imaging, drug and gene therapy. This work compares the application of polymer- and phospholipid-shelled ultrasound contrast agents (UCAs) employed for characterization of the capillary microcirculation. To quantify microcirculation destruction/replenishment technique with varied time intervals between destructive and monitoring pulses is used. The dependence of the peak-to-peak amplitude of backscattered wave versus pulse interval is fitted with an exponential function of the time $\gamma = A(1 - e^{-\beta t})$, where A represents capillary volume and the

time constant β represents velocity of the flow. Working under assumption that backscattered signal is linearly proportional to the microbubble concentration, for both types of the UCAs it is observed that capillary volume, A, is in linearly relationship with the concentration, and the flow velocity, β , remain unchanged. Using 500 µm diameter microtube as a vessel phantom a delay of about 0.25 s in evaluation of the perfusion characteristics is found for the phospholipid-shelled UCA, while polymer-shelled UCA provide response immediately. In conclusion, these results suggest that the novel polymer-shelled microbubbles have a potential to be used for perfusion evaluation.

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Introduction

Medical ultrasound produces reliable visual and quantitative information of anatomical and physiological conditions of the internal structures. Introduction of ultrasound contrast agents (UCAs) further improves the diagnostic outcome of this technique.

The ideal UCAs should be manufactured from the biocompatible material; be easily injected into the cardiovascular system; be stable during the ultrasound examination; do not cause any obstruction of the flow; remain within the blood pool or have well-defined specific tissue distribution; after destruction the residues should be safely processed and removed from the body. From the technical point of view UCAs should modify the acoustic properties of the tissue, for instance by increasing ultrasound backscattered efficiency, or introducing harmonic component to the echo, or combination of both. [1-3]

The latest generation of commercially available UCAs have a thin shell of phospholipid monolayer (SonoVue, Bracco Diagnostics Inc., Princeton, NJ, USA), or protein shell (Optison, Mallinckrodt Inc., St. Louis, MO, USA). Extremely thin shell of about 10 nm would allow incorporated air to diffuse out from the microbubble (MB) and dissolve into the blood. In order to increase the time of effective image acquisition the air is exchanged with less soluble gases, for instance, perfluoropropane in Optison and sulphurhexafluorane(SF6) in SonoVue.

The blood flow in the microcapillaries can be estimated by contrast enhanced perfusion study. This technique is based on destruction of MBs under insonification with high intensity ultrasound burst. After destroying all MBs in the area of interest, perfusion could be assessed by monitoring the reflow into this area with non-destroyed MBs circulating in the blood volume. Perfusion study has been used in the clinical routine to estimate changes in myocardial microcirculation during stress-echocardiography in order to diagnose ischaemic heart disease.

There are however some fundamental problems when using UCAs in perfusion study. Problems arise from the fact that the self-resonance of the phospholipid MBs gives intense reflections. This reflection introduces shadow artefacts destroying visualization of the deeper structures. In addition the resonance of the bubbles creates a non-linear reflection of the returning signal leading to a distortion of the returning wave from cosines shape towards saw-tooth shape [4]. This results in errors in the velocity measurements in Doppler mode.

Some of these limitations could partly or completely be overcome by applying the new type of MBs. The resonance frequency of the polymer MBs is on the order of 100 MHz [5]. Such high value can be partially explained by the thickness of the polymeric shell which is about 0.7 μ m or 30% of the MBs radius. Insonification with a frequency 2.2 MHz, that is far from the resonance, introduce less nonlinearity to the backscattered signal. Nevertheless, considerable backscattered enhancement up to 25 dB has been observed [5].

Material and methods

In this investigation two different types of UCAs are investigated. The first one, which is suspension of polymer-shelled MBs filled with air. They are synthesized from PVA aqueous solution kept at room temperature (RT) and pH = 5 and is labelled as MB-pH5-RT has average diameter $4.1 \pm 0.7 \,\mu\text{m}$ and shell thickness $0.7 \pm 0.1 \,\mu\text{m}$ [6]. The second type of studied MBs is commercially available UCAs – SonoVue, with the average diameter and shell thickness of 2.5 μ m and 2.5×10⁻³ μ m [7].

Experimental setup

Figure 1 shows a schematic representation of the experimental setup used in this work. Two transducers with focal length equals to 50 mm and diameter equals to 12 mm are employed. The 2.2 MHz focused transducer (Krautkramer, Gamma Series, Lewistown, PA, USA) is used as emitter while the 5 MHz focused transducer (Panametrics, V309, Waltham, MA, USA) is employed as receiver. The -6 dB bandwidth for 2.2 MHz transducer ranges between 1.8 MHz and 3.4 MHz, while for 5 MHz transducer it ranges from 3.43 MHz up to 6.77 MHz, respectively. This experimental set-up has as its object to simulate a harmonic imaging technique, where detection is specific for the second or higher harmonic of the excitation frequency. For the evaluation of the capillary microcirculation emitting transducer is excited by 10 cycles square modulated sinusoidal burst of 2.2 MHz frequency which provides in a focal region peak negative pressure equals to 2.344 MPa or mechanical index (MI) equal 1.58.



Figure 1. Schematic representation of the experimental set-up.

MBs are injected into the acoustical and optical transparent microtube with an inner diameter of 500 μ m and a wall thickness of 50 μ m (Zeus Orangeburg, SC, USA). Such diameter corresponds to the diameter of a small artery. The flow of the microbubbles within the microtube has been controlled using syringe pump (Chemyx Fusion 200, Chemyx Inc., Stafford, TX, USA). The volume flow rate is set to 5 ml/h, which in terms of average velocity over cross-section of the microtube is equal 7 mm/s.

Results and Discussion

The perfusion study based on destruction/replenishment technique described by Wei et al. [8] was carried out at 4 different values of the MBs concentration. For each value the mean peakto-peak amplitude of the backscattered signal, A_{pk-pk} , is evaluated with respect to the pulse interval between destruction and monitoring pulse. The results for polymer-shelled MBs are presented on Figure 2a. As predicted by the perfusion model by increasing the time interval more and more intact MBs penetrate into the investigation volume. The amplitude of the backscattered signal increases and reaches the plateau when the duration of the pulse is long enough for the new bubbles occupy investigation volume completely. With the average velocity of the flow over cross-section equals to 7 mm/s in the microtube of diameter 0.5 mm after 1 sec complete replacement of the destroyed bubbles with a new one is achieved.

The same experimental setup is used to perform contrast enhanced perfusion study with phospholipid-shelled MBs. Figure 2b illustrates the funding for commercial available UCAs – SonoVue. Similar to the polymer-shelled MBs increase of backscattered signal versus pulse interval between ultrasound pulses is detected for each concentration levels. The plateau peak-to-peak amplitude also increases with concentration. Worth notice that investigated volume

should be filled in with at least 10 folds more phospholipids-shelled MBs than with polymershelled UCAs in order to acquire similar backscattered enhancement.



Figure 2. Mean peak-to-peak amplitude of backscattered signal versus time between destruction and monitoring pulses for increasing value of the concentration for polymer- (A) and phospholipid-shelled (B) microbubbles.

For the phospholipid-shelled MBs, Figure 2b, diluted to the concentration 3.5×10^6 MB/ml decrease of the backscattered signal was detected at the maximum pulse interval equal to 3 sec. As was mentioned earlier after 1 sec all investigation volume is refreshed with new MBs. In this sense the decrease might be caused by two factors: 1.attenuation of the backscattered signal from the inner part of the microtube occurs; 2.multiple reflection and interaction between the MBs in a solution of high concentration takes place. In contrary, no saturation effect in a backscattered signal was detected for polymer-shelled UCAs.

In order to recalculate the perfusion characteristics, which are capillary volume and flow rate, the mean values of peak-to-peak amplitude for each concentration were fitted with the exponential function of a time $\gamma = A(1 - e^{-\beta t})$. Table 2 presents the values of the coefficients for 4 concentrations of polymer MBs (MB-pH5-RT) and phospholipid MBs (SonoVue).

MB-pH5-RT			SonoVue		
Concentration	A	ß	Concentration	A	β
3.6×10^{5}	2.837 ± 0.057	3.405 ± 0.519	3.5×10^{6}	2.346 ± 0.087	9.903 ± 1.295
3.6×10^{4}	2.124 ± 0.076	2.174 ± 0.143	3.5×10^{5}	2.334 ± 0.085	1.547 ± 0.177
3.6×10^{3}	0.956 ± 0.078	2.103 ± 0.309	3.5×10^4	0.843 ± 0.115	1.588 ± 0.517
3.6×10^2	0.682 ± 0.038	2.101 ± 0.214	3.5×10^{3}	0.493 ± 0.051	1.627 ± 0.445

 Table 2. Coefficients defining peak-to-peak amplitude of backscattered signal from polymer (MB-pH5-RT) and phospholipid (SonoVue) shelled microbubbles.

Common for both types of MBs behaviour was found. This is increase of capillary blood volume (A) with increase of the concentration of circulating MBs. Since the average velocity of the flow is constant during the experiments the rate constant (β) should stay on the same level independently on the concentration. The sharp raise of the rate constant indicates the incorrect estimation of the flow because of the overload of the investigation volume with MBs.

Worth to note from results presented on Figure 2b that for the phospholipid-shelled UCAs a delay of about 0.2-0.3 sec in evaluation of the microcirculation is found. When the time interval between the pulses is very short no statistically significant backscattered signal can be detected. Only after considerable amount of MBs is presented in the investigation volume the

perfusion study can be performed. On the contrary polymer-shelled UCAs provide response immediately without any statistically significant delay, attenuation or saturation of the system.

The possible explanation of the reported delay in evaluation of perfusion characteristics using commercially available UCAs is hidden in the parameters of the experimental set-up itself. The peak negative pressure of the ultrasound pulse might be too high, fracturing the phospholipid-shelled MBs even with the side lobe of the pulse. As a result, the bubbles are destroyed even before they reach the detection region. For instance, the -6db beamwidth of emitting 2.2 MHz transducer is 3.6 mm, which means that in a region ± 1.8 mm around the focal point the pressure will be as high as 1.2 MPa. This pressure level is enough to destroy all phospholipid-shelled MBs, which pressure threshold for fracturing is approximately 300 kPa [9]. On the contrary, more robust polymer shelled MBs at a pressure level of 1.2 MPa oscillate nonlinearly or just start to fracture. The pressure threshold for fracturing the MB-pH5-RT in a bulk volume is found to be equal 1.1 MPa [10].

In addition, the delay observed for phospholipid-shelled MBs can partially be explained by the spectral content of the backscattered signals. In figure 3 the power spectra of the detected signals at pulse interval of 2 sec is shown for polymer-shelled MBs (a) and for phospholipid-shelled MBs (b). For polymer-shelled MBs the second and third harmonic lies within 10 and 20 dB from the fundamental harmonic, respectively. For phospholipid-shelled MBs the amplitude of the second harmonic of the signal is 20 dB lower than fundamental one. Because the detection system is specific to the second harmonic, a greater amount of phospholipid MBs will have to reach the investigated volume in order to give the same signal as for the polymer analogues.



Figure 3. Power spectra of the backscattered signal detected at pulse interval of 2 sec for polymer-shelled (a) and phospholipid-shelled (b) UCAs for different values of concentration.

Conclusions

This paper discusses the application of the polymer-shelled UCA for evaluation of perfusion characteristics, such as capillary volume and velocity of the flow in comparison with commercially available phospholipid-shelled UCA. Using destruction/replenishment technique operating at high excitation pressure is found that approximately 10 fold lower concentration of the polymer UCA is needed for investigation compared to phospholipid-shelled analogues. For both types of the UCAs capillary volume is linearly proportional to the concentration, whereas flow velocity is independent on the concentration variations. For the

phospholipid-shelled UCA a delay of about 0.2-0.3 sec in evaluation of the perfusion characteristics is found while polymer-shelled UCA provides response immediately without attenuation or saturation of the system. In conclusion, special attention should be paid when implementing the high MI destruction/replenishment technique in collaboration with phospholipid-shelled MBs. On the contrary, these results suggest that the novel polymer-shelled MBs have more potential for perfusion evaluation performed under high acoustical pressure.

References

- 1. Qin S., Caskey C.F., Ferrara K.W., 2009. Ultrasound contrast microbubbles in imaging and therapy: physical principles and engineering. Phys. Med. Biol., 54, R27-R57.
- 2. Schutt E.G., Klein D.H., Mattrey R.M., Riess J.G., 2003. Injectable microbubbles as contrast agents for diagnostic ultrasound imaging: the key role of perfluorochemicals. Angew. Chem. Int. Ed., 42, 3218- 3235.
- 3. de Jong N, Ten Cate F.J., Lancee C.T., Roelandt J.R.T.C, Bom N., 1991. Principles and recent development in ultrasound contrast agents. Ultrasonics, 29, 324-330.
- Ressner M., Brodin L.A., Jansson T., Hoff L., Ask P., Janerot-Sjoberg B., 2006. Effects of ultrasound contrast agents on Doppler tissue velocity estimation. J Am Soc Echocardiogr, 19, 154-64.
- 5. Grishenkov D., Pecorari C., Brismar T.B., Paradossi G., 2009. Characterization of acoustic properties of PVA-shelled ultrasound contrast agents: linear properties (Part I). Ultrasound Med Biol, 35, 1127-1138.
- 6. Cavalieri F., Hamassi A. El, Chiessi E., Paradossi G., 2005. Stable polymeric microballoons as multifunctional device for biomedical uses: Synthesis and characterization. Langmuir, 21, 8758–8764.
- 7. Greis C., 2004. Technology overview: SonoVue (Bracco, Milan). Eur Radiol Suppl, 14, 11-15.
- Wei K., Jayaweera A.R., Firoozan S., Linka A., Skyba D.M., Kaul S., 1998. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. Circulation, 97, 473-483.
- 9. Marmottant P., Meer S. van der, Emmer M., Versluis M., de Jong N., Hilgenfeld S., Lohse D., 2005. A model for large amplitude oscillations of coated bubbles accounting for buckling and rupture. J. Acoust. Soc. Am., 118, 3499–3505.
- 10. Grishenkov D., Pecorari C., Brismar T.B., Paradossi G., 2009. Characterization of acoustic properties of PVA-shelled ultrasound contrast agents: ultrasound-induced fracture (Part II). Ultrasound Med Biol, 35, 1139-1147.