LOWER POWER ULTRASOUND APPROACH FEASIBILITY VARYING MICROBUBBLE CONCENTRATION

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Abstract: In recent years in vitro studies have improved the understanding of the behaviour of microbubble ultrasound contrast agents (UCAs). However in several studies there has been a tendency to use high concentrations of UCAs, that can reduce the safety use of microbubbles in the clinical application. In this study we investigate a possible strategy to improve the safety of microbubbles by reducing UCA injection dose and insonifying the solutions at low frequency and low mechanical indices (MI). We measured the contrast signal enhancement varying the concentration of a new phospholipidic UCA at constant frequency (3.15 MHz) and very low MI (0.08) using the last technological innovations in the field of signal processing and radiofrequency (RF) spectrum analysis. The microbubble solutions were pumped into the vessel cavities of a new developed tissue-mimicking phantom. The stored RF data were processed by algorithms just developed by a new software in order to evaluate the average backscatter intensity inner the vessel cavities and to calculate and extract the Fast Fourier Transform components. Our results showed that the UCA backscatter depends on the concentration and the highest backscatter intensity is obtained for the lowest concentration using a low frequency and low mechanical index approach.

Introduction

The quantitative evaluation of contrast enhancement is one of the main aims of ultrasound (US) contrast imaging. In order to optimize contrast imaging modalities, it is needed a full knowledge of the contrast microbubble (MB) behaviour when placed inside an ultrasound field, so in vitro experiments are required to better understand the interactions existing between microbubble and ultrasound waves and to determine the factors that affect contrast enhancement.

Many discussions have been raised concerning safety in the use of microbubble contrast agents [1-3]. It is common practice in much of the literature to use a MB density less than that required to cause the shadowing. But the concentrations used are relatively high and they might not be safe. Therefore these concentrations might be expected to affect measurements in a complex manner. Multiple scattering and bubble interactions have usually been assumed to be insignificant but it has been demonstrated that sometimes they become dominant [4]. Another consideration is about bubble-bubble interaction. Theoretically, at high bubble densities, bubbles are close enough to affect each other's oscillatory motion, and consequently display a different acoustical behaviour than that encountered when the bubbles are isolated. The implications of such interactions to the behaviour of individual bubbles have not been investigated in contrast bubble clouds, and therefore their significance is unknown. This adds a further dimension to the problem of characterizing scatter from a population of bubbles, and also requires a complete knowledge of the behaviour of individual bubbles.

In this study we show the differences in contrast enhancement by varying concentration conditions of last generation experimental phospholipid intravenous ultrasound contrast agent microbubbles (supplied by Bracco Research SA, Geneva, Switzerland) at low frequency and low mechanical index (MI) in order to optimize the injection dose. We use an in vitro system that can be developed and modulated in order to be able to study the ultrasonic signal behaviour in almost all kind of human tissues, being able to cover all vessel sizes and to reproduce the different vascular system conditions in terms of spatial and geometrical configuration and flow velocity ranges of human body. The last technological innovations in the field of signal processing and radiofrequency spectrum analysis enable to bring together multiple efforts to solve a common problem of the safety.

This approach could provide the experimental bases for developing new analytical and numerical models for flowing microbubble behaviour, having many parameters under a possible easy control, such as tissue attenuation, flow velocity, pressure, MB concentration, temperature, US pulse parameters, spatial distribution of MB, interaction of MB with wall surface, etc.

Materials and Methods

The contrast agent used in this experiment belongs to the last generation; it was composed by perfluorocarbon gas encapsulated with phospholipidic shell. We used the highest concentrations belonging to

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the range indicated by the manufacturer. The contrast agent concentration was obtained diluting the initial contrast agent suspension with different saline solution volumes. At the beginning 1.3 µL of microbubble suspension was introduced in a beaker containing 100 mL of 0,9 % NaCl saline solution and mixed at low velocity with a magnetic stirrer. In the same way the other concentrations were prepared. In this study we used 0.013 µL/mL, 0.025 µL/mL, 0.033 µL/mL, 0.050 µL/mL, 0.10 µL/mL solutions.

The suspensions were pumped into the phantom vessels through a peristaltic pump (Peri-Star Model 500304, WPI Inc., FL, USA) at constant flow rate of 8 mL/min and at room temperature (25 °C).

The tissue-mimicking phantom was based on an hydrogel having a sound propagation velocity similar to that of the human liver (1560 m/s). It was enough rigid to obtain some vessels whose walls were composed of different hydrogels. The phantom was 6 cm deep, 5 cm long, 8 cm wide and it had two 1-mm diameter vessels, both placed at 2 cm from the upper surface.

The flow phantom was insonified with 0.08-MI US pulses produced by a linear transducer (LA 532, Esaote, Florence, Italy) with a nominal centre frequency of 3.15 MHz and 3-dB bandwith of 0.75 MHz (and at MI of 0.08). The US transducer was positioned on the top of the phantom, so that the imaging plane resulted perpendicular to the vessels and it was fixed using a shaped Plexiglas© connector as reference and the coupling gel.

The transducer was linked to a digital ecograph (Megas GPX, Esaote, Florence, Italy) which was connected through an optical fibre to a prototype for radiofrequency (RF) spectrum analysis (FEMMINA, developed by Florence University), able to get the full raw signal of the probe with no hardware nor software filtering of the ecograph itself [5].

RF signals were sampled at 40 MHz and the number of data points was 3200 for each track, which is equivalent to 6 cm depth. This information was acquired for 180 tracks in each frame. A sequence of at least 180 frames was acquired for each concentration, digitising one frame per second and storing it in FEMMINA hard-disk. The prototype software (Fortezza, supplied by Florence University) was used to reconstruct the image data.

Quantitative off-line analyses were performed using a new Fortezza algorithm (Fig. 1) developed by the Biomedical Engineering Division of Lecce Clinical Physiology Institute. The raw data were not actually filtered, but only enveloped with the highest possible cut frequency (20 MHz), in order to obtain the absolute value of the signal. A first assessment of the regions of interest (ROIs) was gained through visualization of all data in order to spatially locate the vessel cavities, avoiding reflections and artefacts due to vessel walls. The appearance and real time behaviour of echoes, which were present during pure saline solution flow without contrast agents, were used for guidance for data selection. The selected ROI covered exclusively the microbubble flow and had 3 tracks and 25 data points per track, which is approximately equivalent to a square of less than 0.5 mm in side.

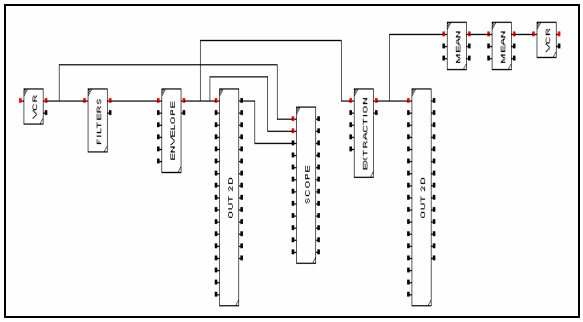

Figure 1: Fortezza algorithm scheme used to spatially locate the ROI and to calculate the correspondent average intensity for each acquired frame.

The mean backscatter intensity of the absolute valued raw data in the defined ROI was calculated for each acquired frame and recorded in a Fortezza proprietary format file, then converted in XLS format by an ad hoc implemented MATLAB® program (The Mathworks, Inc., Natick, MA). The data were analyzed with a significance level of $p<0.05$. The mean and the standard deviation have been calculated for each sequence of significant data by Origin® software (OriginLab Corporation, Northampton, MA) and plotted versus the contrast agent concentration.

Then we calculated the Fast Fourier Transform (FFT) curves and extracted the harmonic component values from them by another developed Fortezza algorithm (Fig. 2).

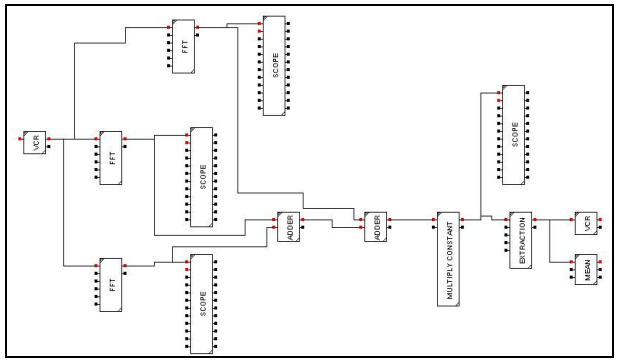

Figure 2: Fortezza algorithm scheme used to calculate the average FFT of the ROI for each acquired frame.

Before FFT calculation, the raw data corresponding to the defined ROI and selected by means of a 25-point "Rect" window were zero-padded to 4096 points to increase the frequency resolution of the correspondent spectra. FFT was calculated in this way for all the three tracks of the selected ROI and the resulting curves were averaged to obtain the FFT representative of the ROI of the specific frame.

Subharmonic, fundamental, second harmonic backscatter values have been extracted from mean FFT

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curves and averaged over the corresponding frame sequence. These values were plotted in an histogram versus the contrast agent concentration.

Results

Figure 3 displays the relationship between contrast agent concentration and average pixel intensity calculated in the defined ROI. The highest intensity was observed for the lowest microbubble concentration (0.013 µL/mL). Then it decreases linearly (r = 0.995) in the range 0.013-0.033 µL/mL and reaches a minimum (0.050 µL/mL). Finally, at 0.100 µL/mL, signal intensity arises again.

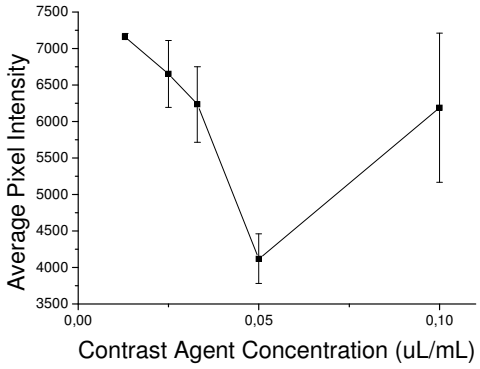

Figure 3: Plot of Average Pixel Intensity versus Contrast Agent Concentration. (nominal centre frequency 3.15 MHz and 3-dB bandwidth of 0.75 MHz, MI = 0.08, ROI = 3 tracks, 25 points per track; $p<0.05$; error bars represent standard deviations of measured data)

This trend was confirmed by the subharmonic, fundamental, second harmonic FFT component intensity values calculated inner the vessel cavity. The single harmonic component average backscatter values were plotted against contrast agent concentration for the designed ROI (figure 4).

The highest value of signal intensity was found at 3.15 MHz component (first harmonic) for all the concentrations. We can also notice that the highest value of the first harmonic is at 0.013 µL/mL; it decreases till 0.033 µL/mL and then rises again for 0.10 µL/mL.

The sub-harmonic component has its maximum value for concentration 0.033 µL/mL and a similar trend is found for the second harmonic component.

The fundamental component trend confirms what observed in figure 3 and it seems to be the component that mainly influence the intensity values.

Discussion

In our study the phospholipidic contrast agent shows the highest intensity when microbubble number inner the solution is the lowest. Then the intensity value decreases till reaching a minimum at 0.050 µL/mL and arises again at 0.100 µL/mL (fig. 3). Average signal

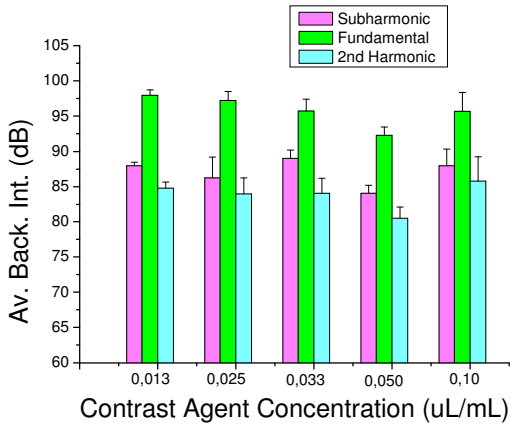

Figure 4: Histogram of Average Backscatter Intensity versus Contrast Agent Concentration (nominal centre frequency 3.15 MHz and 3-dB bandwidth of 0.75 MHz, MI = 0.08, ROI = 3 tracks, 25 points per track; $p<0.05$; error bars represent standard deviations of measured data).

intensity presents a strong linear correlation (r = 0.995) with contrast agent concentration in the range of 0.013-0.033 µL/mL, while for higher microbubble concentrations this linear relationship disappears.

The average pixel intensity trend is confirmed by the values of extracted FFT components (extracted FFT component intensity values). Figure 4 shows that the component that gives a greater contribution to the signal intensity is the fundamental component which has the highest value at 0.013 µL/mL; then it decreases until a minimum and then arises again at the highest microbubble concentration.

The obtained results demonstrate that the microbubble signal intensity is not linearly dependent on concentration and it is not possible to foresee what is the behaviour of a defined microbubble concentration. In literature it is known that the signal intensity of multiple scatterers within a volume increases with frequency to the fourth power and with scatterer size to the sixth power [7]; therefore backscattering and attenuation are interrelated and both depend on the concentration of the contrast agent. This dependence is, however, by no means linear [8-9]. Scattering increases with low concentration, while attenuation caused by multiple scattering dominates when the concentration is high. This poses a limit concentration beyond which the agent cannot be used.

In our case this relation is valid till in the range 0.013-0.050 µL/mL and it might be due to increasing destructive signal interference effects [10-12]. Finally, signal intensity arises again; probably concentration is so high that microbubbles tend to flow in agglomerates rather than as single bubbles. This kind of flow could also explain the high value of the corresponding standard deviation [11].

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This preliminary study has some limits due to several factors that might influence the measurements. For example the measurements might be influenced by the presence of impurities as air bubbles which are not easy to eliminate. Therefore it has been shown that some UCAs have limited lifetime in the vial or deteriorate rapidly in suspension [13], and reproducibility tests have to be performed in order to assess the reproducibility of results in suspension [14].

A parameter that influence the reproducibility of the results is the microbubble distribution that probably might be considered uniform only for solutions at the lowest concentrations. In our study microbubble solutions flow in vessels and probably at the highest concentrations they tend to flow as clouds and not as single microbubbles. So the microbubble contribution to the backscatter is different for every concentration.

In general many studies assume that the microbubbles' scatter belongs to a normal distribution and as a result the scatter from a contrast suspension is statistically well described by an average behaviour. But these studies consider single microbubbles kept in static solutions.

Conclusions

We have investigated a wide range of microbubble concentration in order to evaluate the backscatter intensity at constant frequency and mechanical index. The highest backscatter intensity has been found to be at the lowest concentration. It decreases arising the concentration until reaching a minimum and then arises again. It indicates that there is an optimized concentration that lets to obtain the best contrast enhancement and this is the lowest. For this reason the injection dose might be reduced using the low frequency of 3.15 MHz and low mechanical index.

It is reasonable to contemplate a theoretical model able to determine the minimum microbubble concentration to be used to obtain a maximum signal intensity and therefore maximum information and contrast imaging content, after accurate measurements of the local blood velocity in the targeted tissue and the morphology of the investigated human site.

Further investigations and measurements are needed in order to obtain the complete behaviour of microbubble at lower concentrations, in order to cover all possible concentration ranges to explore the possibilities offered by last generation US contrast media and yet unknown.

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