Comparson between the effects of ultrasound on chicken and human erythrocytes

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Abstract: Under the 1.17 MHz continuous-wave ultrasound exposure, we make experimental comparisons of hemolysis, osmotic fragility and lipid peroxidation for both human erythrocyte and chicken erythrocyte dilute suspensions. Results demonstrate that ultrasound exposure at low sound intensities leads to slight increases in hemolysis, osmotic fragility and lipid peroxidation due to the effect of shear stress. However, a significant increase of hemolysis and even rupture of cell can be observed as the ultrasound intensity exceeds the cavitation threshold. The level of the cavitation threshold for chicken erythrocyte suspensions is higher than that for human red cell suspensions, suggesting that the cavitation threshold is associated with not only acoustic irradiation parameters, but also cell size and structure.

Key words: ultrasound; erythrocytes; biomembrane permeability; cavitation

超声对鸡血与人血红细胞作用的比较研究

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摘要: 实验研究了 1.17MHz 的连续超声波作用下, 人及鸡的血红细胞的溶血率、渗透脆性及膜脂过氧化水平的变化。结果表明, 在声压较低时, 流体间的剪切张力使得红细胞的溶血率、渗透脆性、脂质过氧化水平随作用时间及作用强度增加缓慢增长; 但当声压超过空化阈值时, 红细胞的溶血率随作用时间及作用强度的增加而显著增大, 直至血红素完全释放细胞完全破裂; 鸡红细胞的空化阈值明显高于人红细细胞, 空化阈值不仅与声学参数有关, 还与细胞体积和结构有关。

关键词: 超声; 红细胞; 膜透性; 空化效应

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1 INTRODUCTION

With the wide applications of the ultrasound diagnosis and therapy, its safety issue has attracted much attention in recent years. It has been demonstrated that ultrasound-induced bio-effects lead to the changes of the biomembrane permeability and cell structure due to cavitation.[1-3] There are two categories of the cavitation, i.e. stable cavitation and transient cavitation. The former is generated by shear

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stress due to microstreaming caused by periodical oscillating resonant bubbles. It can enhance membrane permeability without damaging cellular structure and applications in drug delivery and DNA transfection. The latter induces powerful shock waves and jet derived from the implosive collapses of cavitation bubbles at a higher ultrasound intensity, which may lead to cell structural damage and even cytolysis. Therefore, studying the mechanism of ultrasound-induced bio-effects and optimization of the ultrasound exposure parameters is helpful for information on safety issue of biomedical therapy.

Erythrocyte is usually used as an experimental model because of its simple structure but possessing preliminary characters of most cells[4-6]. Hemolysis is a cavitation indicator in studies of nucleation and evolution of cavitation[7-8]. Lipid peroxidation and osmotic fragility are used to analysis changes in plasma membrane. Extensive lipid peroxidation in biologic membranes causes loss of fluidity, decrease in membrane potential, and increased permeability to ions and eventual rupture[9]. The osmotic fragility test of erythrocytes is another important indicator for evaluation of the membrane permeability and the sensitivity of erythrocyte toward hypotonic saline[10]. However, much attention has been focused on the studies of mammalian erythrocytes such as mice, dogs, humans, rabbits and cows, which are structurally simple without nucleus and mitochondria.[11-12]. Most cells such as tumor cells and somatic cells have complex cellular structure including mitochondria, endoplasmatic reticulum. As a result, study of effects of ultrasound exposure on both the chicken and adult human erythrocytes is of importance.

This article experimentally studies the change of membrane permeability of both the human (non-nucleated) and chicken (nucleated) erythrocytes in the presence of 1.17 MHz continuous ultrasound exposure. Hemolysis, lipid peroxidation and osmotic fragility for both erythrocytes are examined associated with ultrasound intensity and exposure time.

2 MATERIALS AND METHODS

2.1 Cell preparations

Human erythrocytes and chicken erythrocytes are structurally different. Human erythrocytes are flatted without nucleus and mitochondria. Its diameter is about 7 μm. Chicken erythrocytes are nucleated and ellipsoidal with 10.7-15.8 μm long diameter and 6.1-10.2 μm short diameter. The mean corpuscle volume of chicken erythrocyte is larger than that of human erythrocyte.

The fresh blood from mature human or chicken anticoagulated with ACD (citric acid 4.8g, sodium citrate 13.2g and glucose14.7g /1000mL) is drawn into a 50 mL vial. After centrifugation at 1000 *g* for 20 min and removing the supernatant of plasma, erythrocytes are washed 4 times with the isotonic buffer 0.9% and 0.75% NaCl solution. After washing, erythrocytes are diluted in the incubation medium phosphate-buffered saline (PBS) to 1% hematocrit.

2.2 Ultrasound exposure

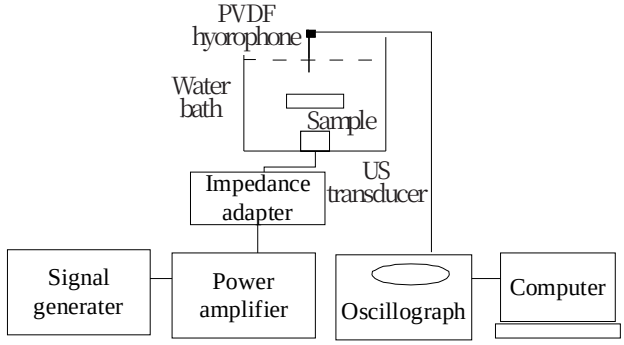

Fig.1 Ultrasound exposure system

The arrangement of the ultrasound exposure system is shown in Fig.1. A 1.17 MHz continuous-wave generated by a function generator (Agilient-33250A, USA) is amplified by a power amplifier (AG 1012, T&C, USA) and drives a planar PZT transducer (1 MHz, diameter 2 cm, China). Take 8ML processed erythrocytes suspensions and pack it into a cylindrical sample container with diameter

2cm and height 2cm. The bottom of the container is covered with an acoustically transparent film. The sample container is placed in a distilled water tank, and located at a distance of 3cm from the surface of the transmitter. The transmitter aims upward at the exposure container, and its intensity is calibrated by a needle hydrophone. During the experiment, we should keep the temperature in the water bath at 20℃±3℃. Each trail is conducted in six independent measurements and finished in 8 hours.

2.3 Principles and methods

Hemolysis: 1.5 ML ultrasound exposed samples are centrifuged at 1000 rpm for 10 min, and 20 mL supernatant is drawn into an optical cell. The fraction of hemolysis cells is calculated from the optical absorption in the supernatant, which is measured at 540 nm by means of a Spectrofluorometer. Hemolysis with 100% is calibrated by the cells lysed with pure water (osmotic lyses).

Lipid peroxidation: After being stored at 4℃ for 2 hours, 1.5ML ultrasound exposed erythrocytes are centrifuged at 9000 rpm about 30 min, then the supernatant liquid is removed. The process is repeated for 3 times and a milky red blood membrane suspension is obtained for test. The amount of hemoglobin is measured according to the guide of Coomassie brilliant blue. Lipid peroxidation is determined by the thiobarbituric acid-reactive substances (TBARS) expressed as MDA that reacts with thiobarbituric acid (TBA) to form a colored complex that has maximum absorbance at 532 nm.

Osmotic fragility: After ultrasound exposure, 200 mL erythrocytes suspension is added into 1mL NaCl solutions at concentrations ranging from 0.1 to 0.9%, respectively. After 60 minutes incubation, the sample is centrifuged at 1000 rpm for 10 min. The degree of hemolysis is estimated from the absorbance at 540 nm of the hemoglobin in the supernatant. The control value is 100% hemolysis by adding water.

3 RESULTS AND DISCUSSION

3.1 Dependence of hemolysis on intensity and time

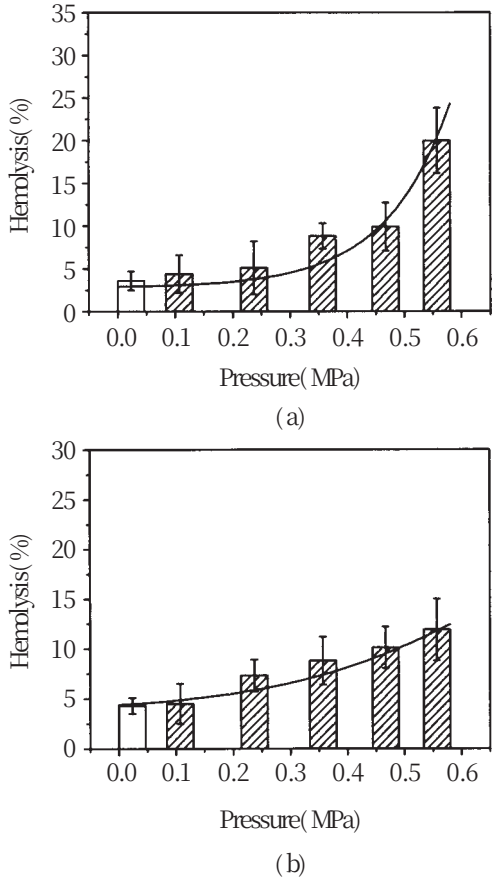

Fig.2 The hemolysis of human (a) and chicken (b) vs. ultrasound exposure pressure

The dependence of hemolysis on sound pressure is shown in Fig.2, where the exposure time is 5 min, and the sound pressure changes in the range of 0.13 to 0.58 MPa. As shown in Fig.2(a), hemolysis of human erythrocyte gradually grows along with the increase of the sound pressure. When the sound pressure is increased to 0.49MPa, the hemolysis is observed to have a considerable rise from 9.87% to 19.98%. It suggests that this intensity arrives at the threshold where cells are largely destroyed. Fig.2(b) shows the chicken erythrocyte hemolysis with the increase of sound pressure. It is observed that the hemolysis gradually grows from 4.5% to 11.9% without the sharp rise in the measured range of sound pressure.

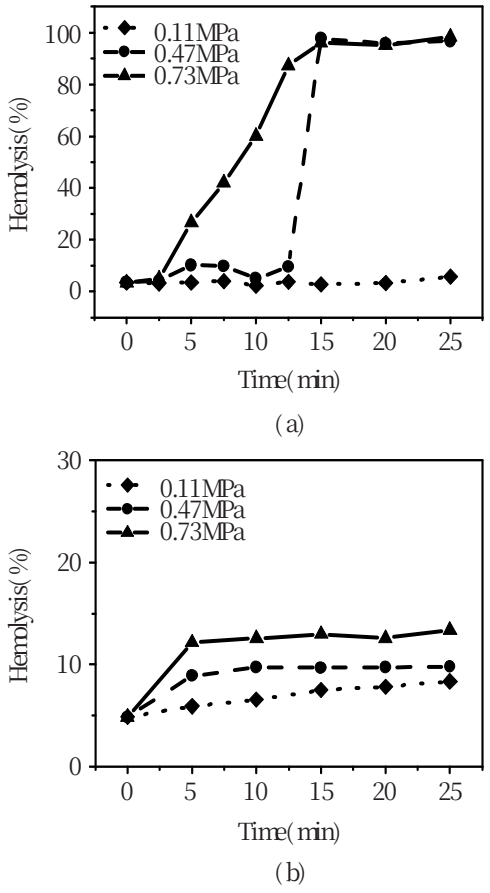

Fig.3 The hemolysis of human (a) and chicken (b) vs. ultrasound exposure time.

Figure.3 shows the dependence of both hemolysis on the exposure time. At the sound pressure 0.11 MPa, there is no obvious change in the human erythrocyte hemolysis with the increase of the exposure time as shown in Fig.3 (a). When the sound pressure is 0.47 MPa, a gradual rise in hemolysis is found before 12.5 min, after that a rapid rise is observed, finally a saturation stage is reached at 15 min. For the sound pressure 0.73 MPa, the hem-olysis increases rapidly with the increase of time and exceeds 20% in 5 min and reaches the saturation in 15 min. The rapid rise of hemolysis demonstrates the generation of cavitation. As a contrast, though the hemolysis for chicken erythrocyte increases with increasing exposure time, it keeps low level even long exposure time at the three sound pressures. It suggests that the used sound pressure is much lower than the threshold of the cavitation for chicken erythrocytes.

It has been demonstrated that the threshold of the cavitation for the mammal erythrocytes is associ ated with the cell volume, and that the lower thres-hold value corresponds to the larger cell volume. However, this study finds that the threshold for the chicken erythrocyte is higher than that for the hu man erythrocyte, though the volume of the former is larger than that of the latter. The reasons may be as follows: (1) The karyon's inhomogeneous distri-bution in chicken erythrocyte suspension results in the mismatch of acoustic impedance of chicken red cells with the surrounding medium, thereby ultraso-und is greatly scattered and attenuated; (2) Cellular endoplasmic reticulum that associates the cellular karyon with cellular membrane is equal to an ul-trasonic vibration isolation mounting under ultra sound exposure. As a result, the chicken red blood cell membrane is relatively less frangibility for ul-trasonic disturbing; (3) Compared with the non-nu-cleated cells, cell damage induced by ultrasonic mic-rostreaming inside cells and eddy flow of intracel-lular organelle is difficult to be observed inside the nucleated cells, because the karyon occupies large proportion in both space and mass of nucleated cells.

3.2 Lipid peroxidation and osmotic fragility

Figure 4 compares the lipid peroxidation changes with the sound pressure between human and chick en erythrocytes after ultrasound exposure for 5 min, where the sound pressure changes ranging from 0.13 to 0.58 MPa. It is shown that lipid peroxidation for either human or chicken erythrocyte grows with the increase of the sound pressure, which is similar to the behavior of the hemolysis. As the sound pre-ssure is increased to 0.58 MPa, the value of MDA for human erythrocyte grows from 360.5 nmol/g HB to 550.5 nmol/g HB, increasing by about 52.7% compared with control value. While for the chicken erythrocyte, the increase is from 597.6 nmol/g HB to 807.1 nmol/g HB, about 35.1%. Results indi-cate that the lipid peroxidation for chicken erythro-cyte is less obvious than that of human erythrocyte.

Lipid peroxidation results from the accumulated

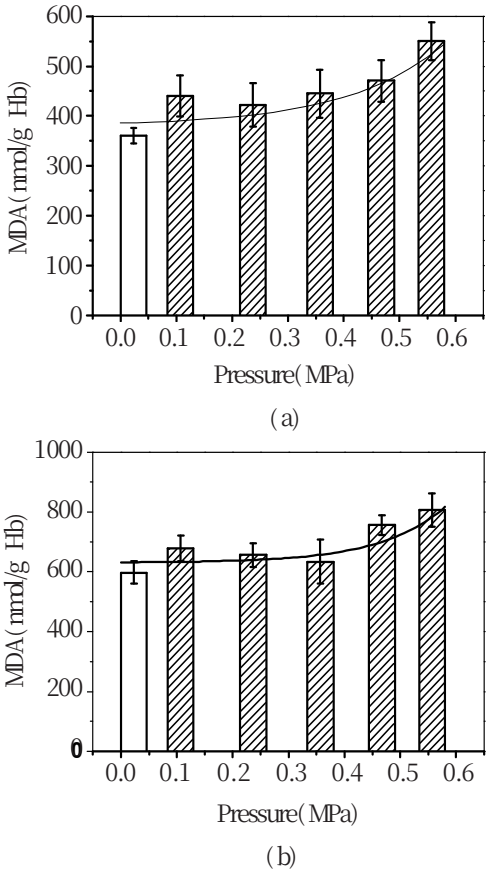

Fig.4 The product of lipid peroxidation of human (a) and chicken (b) MDA amount vs. ultrasound exposure pressure

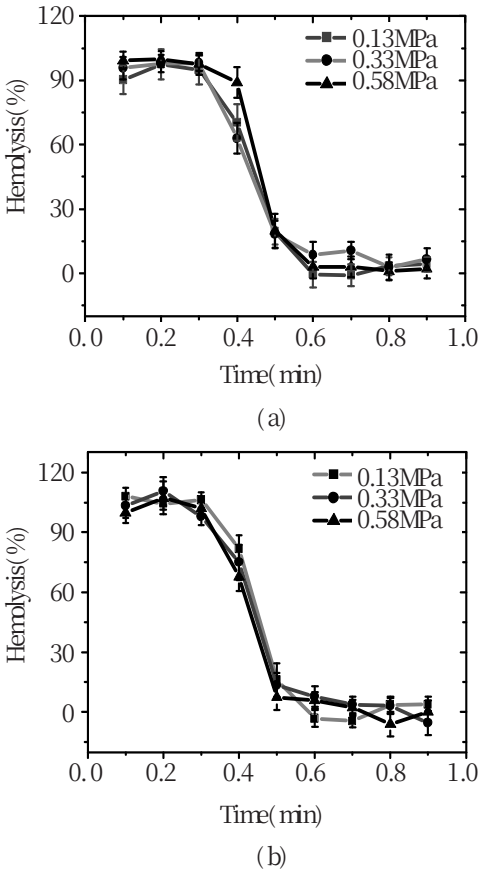

Fig.5 The osmotic fragility of human (a) and chicken (b) vs. ultrasound exposure pressure

amount of oxidized free radical that is associated with ultrasound exposure energy. Compared with non-nucleated human erythrocyte, the karyon inside chicken erythrocyte contains some important DNA genetic materials, such as antioxidase gene which can organize and conduct the antioxidase synthesis that suppresses the lipid peroxidation. On the contrast, antioxidase is difficult to generate in human erythrocyte. for lacking of karyon structure and integrated enzyme system. As a result, the human erythrocytes are easier to be attacked by oxi dized free radical, and their changes of lipid peroxidation are relatively apparent than chicken erythrocytes.

Figure 5 plots the osmotic fragility for human and chicken erythrocytes as a function of saline concentration, where the ultrasound exposure time is 5 min. Results show that the osmotic fragility changes at the NaCl concentration from 0.3% to 0.6%. From Fig5, it is observed that the osmotic fragility of human erythrocyte is more fragile than that of chicken erythrocyte after sonication. As an example, at the concentrate 0.4% of hypotonic saline, the percentage of human erythrocyte hemolysis increased from 62.9% to 89.0%; while the percentage of chicken erythrocyte hemolysis increases from 67.7% to 81.8%. It indicates the lower resistance to hypotonicity for the human erythrocytes.

4 CONCLUSION

In conclusion, this article experimentally studies the ultrasound-induced effects on membrane per-meability and cell structure for human and chicken erythrocytes. Hemolysis, lipid peroxidation, and osmotic fragility are discussed associated with the ultrasound exposure parameters (sound pressure and exposure time). Results show

that, for the case of human erythrocytes, hemolysis values grow with the increase of sound pressure and a significant rise and even rupture are observed as the sound pressure exceeds a threshold. On the contrast, although there is a trend of the increase in chicken erythrocytes hemolysis, the values keep low and less than 15%. It is very likely that chicken erythrocytes have a higher cavitation threshold than human erythrocytes. Furthermore, the measurements of lipid peroxidation and osmotic fragility demonstrate that kayron may be effect on ultrasound-induced cells changes. The ultrasound-induced effects depend on not only ultrasound irradiation dose, but also cell size and structure.

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