Review Article

A Review of Suspension-Scattered Particles Used in Blood-Mimicking Fluid for Doppler Ultrasound Imaging

Ammar A. Oglat[1], Nursakinah Suardi[1], M. Z. Matjafri[1]*, Mohammad A. Oqlat[2], Mostafa A. Abdelrahman[3], Ahmad A. Oqlat[3]

[1]Department of Medical Physics and Radiation Science, School of Physics, Universiti Sains Malaysia, 11800 Penang, Malaysia, [2]Department of Biological Sciences, School of Science, Yarmouk University, Irbid, Jordan, [3]Department of Emergency, Faculty of Medicine, JUST, Irbid, Jordan

Abstract

Doppler ultrasound imaging system description and calibration need blood-mimicking fluids (BMFs) for the test target of medical ultrasound diagnostic tools, with known interior features and acoustic and physical properties of this fluid (BMF). Physical and acoustical properties determined in the International Electrotechnical Commission (IEC) standard are specified as constant values, the materials used in the BMF preparation should have values similar to the IEC standard values. However, BMF is ready-made commercially from a field of medical usage, which may not be appropriate in the layout of ultrasound system or for an estimate of novel imaging mechanism. It is often eligible to have the capability to make sound properties and mimic blood arrangement for specific applications. In this review, sufficient BMF materials, liquids, and measures are described which have been generated by utilizing diverse operation mechanism and materials that have sculptured a range of biological systems.

Keywords: Acoustical properties, blood-mimicking fluid, physical properties, suspension scatter particle materials

Introduction

Blood that precisely mimics real human blood systems in all its features is often referred to as blood-mimicking fluid (BMF). BMF consists of blood-mimicking materials with simple and similar physical properties. Chemical fluid or powder materials are occasionally mixed to mimic both physical and chemical properties.[1,2] Furthermore, BMF was applied for calibration and description of Doppler ultrasound imaging systems since the 1980s. BMF is also used to compare the act of ultrasound systems for the practice of Doppler ultrasound technicians, to allow comparison of backscatter properties of Doppler ultrasound and to evaluate it in a Doppler flow test object or diagnostic techniques. Items used in the preparation of BMF that could be constructed with general acoustic properties, particle features, and physical properties are helpful in studying the ultrasound imaging. To achieve this purpose (preparation of a suitable BMF), the acoustical and physical properties of BMF should be close to the International Electrotechnical Commission (IEC) standard with constant values [Table 1]. BMF is available commercially, imitating the properties of human blood such as density, concentration, scattering, the speed of sound, and viscosity. The range price of the commercial BMF differs depending on the volume of required blood. However, commercial BMF is not customizable because it is made for broad markets and specific implementation. For this reason, customization and preparation of BMF are needed for more precise usage.

This article reviews numerous materials and methods used to prepare BMFs, focusing mainly on those advanced for Doppler ultrasound imaging rather than those developed more specifically for alternative ultrasound mechanisms such as high-intensity focused ultrasound or elasticity imaging (electrography).[3] Many of the physical properties, acoustic properties, and other measurements were done with different methods applied to develop general BMF. Although different types of mixture fluids and scatter particle materials

Address for correspondence: Prof. M. Z. Matjafri, Department of Medical Physics and Radiation Science, School of Physics, University Science Malaysia, 11800 Penang, Malaysia.
E-mail: mjafri@usm.my

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Table 1: Specifications of the BMF defined as the IEC standard. f is the acoustic frequency (Hz)

Acoustical and physical properties of BMF	Values
Viscosity ($\times 10^{-3}$ Pas)	4.0±0.4
Attenuation (dB/cm/MHz)	$<0.1\times10^{-4}\times f$
Acoustic speed (m/s)	1570±30
Density ($\times 10^{3}$ kg/m^3)	1.050±0.040

F is the acoustic frequency (Hz)[67]

used in BMF preparation have been reviewed by Hoskins, the current review aims to provide a better understanding of the pros and cons of various techniques in ultrasound research. Artificial or mimic blood is prepared as a particle suspension in fluids, there are many particles used in the preparation of BMF, where many of these particles have a diameter close to the diameter of human's red blood cells. The hardened red blood cells studied by Law *et al*.[4] showed that the backscattered power increased due to the change in the density and compressibility factor of the hardening process. In other words, blood gives the same backscattered power with 4% suspension by volume of hardened red cells in saline, and the viscosity of hardened cells was 1.06 Pc at room temperature which means less than water's viscosity. These hardened red blood cells were used in both pulse wave and continuous wave (CW) studies. However, the main disadvantage of this process is that it takes 8 h for preparation, which is relatively longer than the time compared to other mimic blood fluid preparation. Also, separating the red cells and suspending them again in saline is a significant way to prevent red cell aggregation.[5] In general, the BMF using *in vitro* hemodynamic styles of Doppler ultrasound should preferably have identical properties to real human blood and be prepared in a simple method.

Why the red blood cells (erythrocytes) are responsible for the blood scattering in ultrasound, although that the blood consists of erythrocytes, platelets (thrombocytes), and white blood cells (leukocytes)?

The spectrum of Doppler signal depends on the red blood cells of whole blood components. Blood consists of suspension materials, namely, red blood cells (erythrocytes), platelets (thrombocytes), and white blood cells (leukocytes). Due to the presence of a comparatively low number of platelets and leukocytes, it is typically supposed that the red blood cells (erythrocytes) are responsible for blood scattering in ultrasound imaging. The average diameter of the erythrocyte is 7 μ, which is much less than the ultrasound wavelength that is around 0.2 mm–0.5 mm. Thus, the single erythrocyte works as a point scatter, the combined influence is referred to as Rayleigh scattering. The size of the pulse-echo (PE) from blood is tiny compared to that resulted via specular reflection with tissue interfaces. One result relating to the Rayleigh scatter process is that the power of the scattered signal wave rises with the fourth power of frequency (1-f^4).[6,67]

Physical Properties of Blood-mimicking Fluid

Density

The particle materials used in BMF preparation should be able to remain suspended (not float or ascent) inside a liquid; in other words, it is important to remain neutrally buoyant even at minimum speeds.[1,7-10] The density of particles should approximate as much as possible to the human blood density which is between 1.01 and 1.09 g/cm^3. Some particles were used but were inappropriate in this regard, like Sephadex, which has an announced density of 1.7 g/cm^3. However, some particles are suitable as suspension particles in the mixture fluid such as nylon and polystyrene with a density of 1.03 and 1.05 g/cm^3, respectively.[11] The density of particles should be appropriate to allow them to suspend in the fluid, particularly when these particles flow along the tube or vessel. During steady flow, with a zero-speed zone at the bottom of the tube, the velocity profile should be parabolic. The main problems related to the densities of particles will happen if the density of particle is lower or higher than the fluid density.[1]

Particle size and concentration

Erythrocytes are concave on both sides, their diameter, thickness, and mean volume are 7–8, 2–3, and 87 μm, respectively.[12] Hematocrit (which is the volume concentration of erythrocyte) ranges from 40% to 50% in normal people.[13-15] Particles that should be used in BMF are mostly spherical or shaped like a disk, for example, polystyrene microspheres with particle diameters 10–30 μm,[8-10,16] 3–20 μm for nylon,[1,7,17-20] and 20–70 μm for Sephadex.[21] Low scattering particle with a minimum volume concentration is mostly used because backscatter from erythrocyte is lower than from the particles.

Viscosity

Viscosity has an immediate effect on the velocity in the including vessel.[22] For instance, in the so-called inlet zone to tube, the expansion of the tube after the velocity is steady is inversely proportional to the fluid viscosity. The velocity will increase with a higher viscosity fluid, and this velocity occurs before the beginning of turbulence, and this is an eligible advantage of a flow test object. High velocity occurs due to high viscosity fluid, and this happens before the start of turbulence, which is the primary disadvantage of a flow test object.[23] The measurements of dynamic viscosity for overall human blood with high shear rate are reported as 3.5–4.5 mPas[24,25] and with a different shear rate at 2.25–4.5 mPas.[26-28] Pedley stated that we could consider blood as Newtonian in large vessels (>0.5 mm) with viscosity measured under situations of high shear rate, that is, 4 mPas.[29]

Viscosity coefficient is constant for a Newtonian fluid, and this viscosity is not based on the shear rate at a specific temperature. When the viscosity depends on PCV and shear rate, we can consider that blood is non-Newtonian.[30,31] The viscosity that depends on the shear rate is displayed at most tiny vessels of arterial measure. Blood acts as a Newtonian fluid when treating with the major vessels of the body, and in this case, there is benefit for Doppler ultrasound with major vessels

with a viscosity of 0.004 kg/ms.[29] When the red blood cells flow, the signal of Doppler ultrasound appears. Also, several theoretical models have progressed to a better understanding of viscosity depending on the shear rate method.[32-34] The strength of the Doppler signal does not linearly rise with PCV.[33,34] Furthermore, the main disadvantage of Doppler signal is that it produces noise, which is the product of the vast difference between the numeric and geometric order of the sample volume within the scattering elements, the occurrence of this noise causes many problems. For example, the exhibited spectrum of Doppler ultrasound has a speckle, the casual difference in the frequency being of similar size to the spectrum itself.

The correlation between shear rate and viscosity is inversely proportional because the blood is a non-Newtonian fluid. Thus, the viscosity increases with the decreased shear rate. A shear rate of 23/s measured at 37°C with natural range is 0.84–0.56 Pas (mean value = 0.7 Pas) and 0.51–0.38 Pas (mean value = 0.44 Pas) at a shear rate of 230/s.[23] The main cause that makes the blood behave like a non-Newtonian is an aggregation of red blood cells created in fixed blood and at low shear rates (<50/s) [Figure 1].[35] It is affected by the existence of fibrinogen in the blood (a soluble protein in blood plasma).[36] The aggregation will separate when the shear rate rises, which gives a change in viscosity. This separation of aggregates also minimizes the size of the scattering centers so that it will result in a decrease in the backscattered power.[32,33,35] In small vessels (<0.6 mm diameter), the non-Newtonian differences in viscosity become significant.[37,38] By applying high frequency and color flow mapping, we can see the small vessels on scanner.[39] The model of a non-Newtonian fluid should work with small vessels accurately. However, in the large anatomical structures such as heart chambers or vessel bifurcations, the effect of flow will increase by a non-Newtonian flow.[40-42] The large anatomical structure will be clear in any vessels with the pulsatile flow.[38,43] The flattening of the velocity profile occurs in vessels with the laminar flow when the shear rate over the streamlined center is zero.[36]

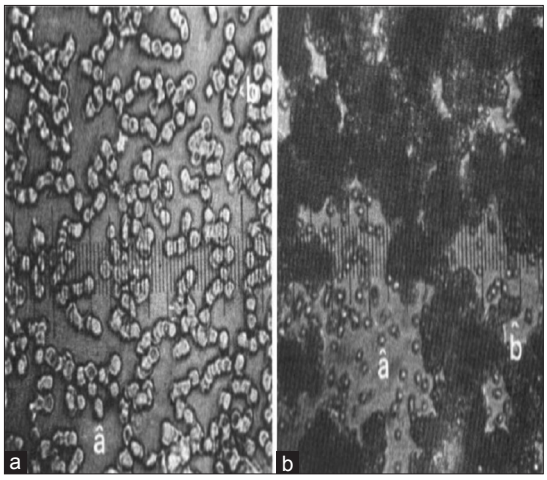

Figure 1: (a) Erythrocytes showing – a^ : One cell and b^: a gross cell. (b) Orgasol™ or Nylon particles showing – a^: solo particle and b^: an aggregate of particles. Small divisions equal 2.2 μ[38]

Hence, the best fluid to use is the blood itself.[4] However, there are some obstacles related to using blood and its ingredients. There is a possibility of biohazard and attention must be taken to reduce this hazard. Red blood cells can easily damage *in vitro* because the expiration date of erythrocytes is limited, and this prevents the use of blood as a normal fluid in long-range studies of measurement and quality monitor. Blood properties such as physical and acoustical properties differ according to temperature, humidity, and atmospheric pressure. Furthermore, the complexity of flow models occurs at higher temperature. However, non-Newtonian model fluid should work with small vessels accurately. But, in the large anatomical structures like heart chambers or vessel bifurcations, the effect of flow will be non-Newtonian flow.[40-42] The large anatomical structure will be apparent in any vessel with the pulsatile flow.[38,43]

Acoustical Properties of A Blood-mimicking Fluid

Ultrasound backscattering of blood

One of the essential features of a possible suspension in the BMF is constancy and the ultrasound backscatter.[44,45] The theoretical and experimental studies of scattering ultrasound from blood have been done extensively. Theoretical models depend on both of discrete Rayleigh scattering[46] and the continuum approach.[32,47,48] Some studies have used an integrated approach, they have presented that the backscatter coefficient is proportional to both the product of backscattering cross-section and packing factor.[33] The physical properties (compressibility, size, and density) and the packing factor of the individual scatter determine the distribution and interaction of scatters. However, the mixture fluid is determined by the backscattering cross section.[49]

In general, two main factors affect the scattering properties of blood: first, physical properties of the erythrocyte (distribution of size, compressibility, acoustic properties, and density) and second, distribution properties (flow disturbance, hematocrit, and plasma proteins that encourage red cell grossing). However, when the proportion of particle diameter for an erythrocyte to ultrasound wavelength is in the field (0.009–0.036) and with an ideal frequency of 2–8 MHz at minimal scatter concentrations, the Rayleigh scattering with a frequency reliance of 1-f^4 will occur.[50,51] The rate based on the scattering overtake 1-f^4 when the scatter concentration is more than 50%.[52] The difference of backscatter measurement with hematocrit is linear up to nearly 8%, arrives a top that is hugely based on flow conditions around 12%–26%, and then reduces with the rising hematocrit. Due to the backscatter based on flow conditions, the measurements should be carried out under regular flow and at physiologically closely connected shear rates. Backscatter and the suspended red cells have been used in many studies. In 1977, the IEC had commented that a standard BMF should have similar backscatter to that of real human red cell suspensions under regular flow (draft IEC 1685 standard), Shung *et al*. presented a brief review about that.[53]

The backscatter measurements depend on exchange method.[44] backscattered is defined as a reflected power of sample surface. The backscatter power measurement is affected by two main system parameters: first, the magnitude of the useful scattering (beam zone, spatial sensibility response of the detector, and time section forms) and second, the capacity of the system (sensibility, earn and receiver area, signal-to-noise ratio, and incident intensity). For any real value of backscatter coefficient, an adjustment process is required to remove system-particular parameters.[54] Measurements of the backscatter are usually carried out using a single plane transducer works as sender and receiver. Furthermore, focused transducers have been used, but they demand additional complex normalization procedures.[55,56] Despite different backscatter values of measurements, there is no final backscatter scale test object. This is necessary to evaluate mistakes and find the precision of real backscatter measurements between several laboratories. In this case, because of the hardness of actual backscatter measurements, relative measures are necessary, so the measurements of backscatter of the BMF should be compared with human blood flow measurements by applying the same measurement system.

When using the backscatter power of BMF in a Doppler flow test, the object should be steady, reproducible, and entirely described. The measurements of penetration deepness and sensibility are necessary because they should be similar to backscatter from the BMF and flow human blood.[57] Sometimes, the BMF has an inappropriate characterization of scattering properties and appears to be frequently higher than human blood scattering. For instance, fresh pig blood may have similar scatter to that of real human blood, but for experimental researches and high-range firmness, a proper synthetic blood mimic is more desirable. The backscatter should be recognized by the draft IEC 1685, with relative mistakes not more than a factor of 2 ± 3 dB. In some experiments, for example, this will participate to slip lower than ± 7% in the measurement of penetration deepness using a standard flow Doppler test object.

The Doppler signal should be shaped like a Gaussian method when the value of scatters in the sample volume is very high,[58,59] which is the situation of human red blood cells with the count of $5 \times 10^6/mm^3$. However, the scattering cannot be Gaussian, when the number of red blood cells is incomplete or deficient. For comparing the statistical properties of BMF and human blood, the small data is required. Two methods are used to scan the statistical kind of the Doppler signal: first, calculation of the first and second demand statistics after spectral analysis from the acoustic Doppler signal and second, testing the radiofrequency (RF) spectrum of the received Doppler signal. Using this process, Hoskins *et al*.[21] explained that there is no variation in the statistical properties of human blood and particles like a Sephadex for concentrations as minimal as 1% by volume. Furthermore, the vessel itself will impact on the scaling of Doppler frequencies within the Doppler spectrum because of the difference in backscattered power over a vessel such as ultrasound beam shape. The scaling of Doppler signals will also be affected by the attenuation from different parts of the vessel lumen. A small value of attenuation in real red blood cells is 0.9 dB/cm at 3.5 MHz.[36]

Backscattered power rises when flow becomes turbulent because of the difference in density between the erythrocytes and plasma.[32,60] During the turbulence, the flow will be highly accelerating because the erythrocyte and plasma have to be "drawn apart" to form larger scattering position. When the hematocrit is >0.10, this effect will be visible. Thus, it is essential to apply a fluid with a good physiological hematocrit.

The speed of sound, attenuation, and backscattered power are the acoustic properties of interest. The important point is having a correct speed of sound in applications like measurements of volume flow wherever the vessel's cross-sectional area is being measured by ultrasonic means. The shear rate,[35] turbulence, and hematocrit[60] can affect the backscattered power.

Speed of sound and attenuation

The acoustic speed in the BMF should be identical to the tubes and tissue-mimicking material (TMM) to prevent refraction artifacts.[19] The speed of sound in BMF and TMM is usually 1540 m/s.[61] The refraction artifacts can be noticed when using tubes with a high velocity of sound.[62] The speed of BMF in the draft IEC 1685 standard is 1570 ± 30 m/s. This vast range permits the speed to correspond vessel wall, blood, and the TMM of a flow test object. The rate of speed of sound and attenuation has been studied for human blood.[14,15] The attenuation of the BMF must be <0.1 dB/cm MHz, as recommended by the draft of IEC 1685 standard. Hence, to reduce inhomogeneity of the sound scope into the tube, the attenuation of the BMF must be minimal. The acoustic speed of the BMF was measured by PE signal technique while the attenuation was measured by proceeding a Fast Fourier Transform on the RF signal from the reflector.[1,7,9,10,16,17,19-21,45,57]

Effect on velocity profile and distribution of particles

Typically, the red blood cells distribute in large vessels. The particle moves toward the center of the vessel due to its force which results from the presence of a shear gradient.[63] Therefore, it is not suitable to use large particles in small vessels due to the particles' inability to take over a small proportion of the diameter, and this can have effects on both particle distribution and the velocity profile. Also, this effect can be an unnoticeable effect. It is essential to utilize particles that are tiny and similar to red blood cells for many reasons. One, to make sure that the concentration of particle is rising even for the small sample volume in the most narrowing focused Doppler beam. Two, to supply a BMF that may probably be helpful at higher frequencies (non-Rayleigh scattering increase due to the high proportion of diameter to wavelength for massive particles). Finally, to provide a BMF that may probably be helpful in tiny or small vessels. However, one of the main cons of using large particles in small vessels is the aggregation or clotting of these particles inside the flow track and then causing flow obstructions and obstacles.

The rheological or physical parameters of BMF are the viscosity, the density, and the particle concentration or hematocrit. Blood behaves like a Newtonian liquid at high shear rates; in other words, its viscosity is not based on velocity gradient when the direct and equivalent flow is determined. However, the velocity of improving turbulent flow is based on hematocrit because the presence of red blood cell rises the constancy of the flow.[64]

Types of Suspension Particles Used for Blood-mimicking Fluid Preparation

Blood-mimicking fluid using Orgasol™ (nylon) particles

BMF based on the use of the smooth powder of Orgasol™ (nylon) suspended in a mixture of water and glycerol is used as an alternative to blood.[20] However, there is a disadvantage of applying nylon particles despite having densities and diameters close to red blood cell that these particles aggregate at low shear rates to give non-Newtonian manner which are the physical features of real blood.[65] This method does not only affect the flow in small diameter vessels, but also in large structures [Figure 1].[38]

Some studies have used different diameters of nylon particles such as 5, 10, and 20 μm with 1.03 g/cm^3 as density. The reliance of attenuation, speed or velocity of sound, and backscatter power with nylon particle diameter, at a constant particle concentration by weight 1.82%, is tabulated in Tables 2 and 3.[42] Small effect on the speed of sound and attenuation from nylon diameter can be observed when the scattered concentrations are deficient. This was set fundamentally by the glycerol-to-water ratio. The outcome of backscatter measurements on BMF with particle scattering of 5, 10, and 20 mm diameter is explained in Figure 2 as a mission of scattering particle concentration.[1, 42]

A suitable BMF for utilizing with Doppler ultrasound was applied with (5-μm-diameter nylon particles) as a scatter particle. This BMF has the velocity of sound of 1547 m/s; attenuation of 0.26 dB/cm at 5MHz, density of 1037 kg/m^3, and viscosity of 4.1 ± 0.1 mPa/s. In addition, a new type of surfactant called household surfactant is used to reduce air bubbles.[17] For testing the influence of "red blood cell density," the basic structure of the BMF was preserved. The BMF was established on a suspension of nylon or Orgasol™ particles. The norm solution was made by mixing pure water, surfactant, dextran, and glycerol at concentrations of 83.86%, 0.9%, 3.36%, and 10.06%, respectively, with 1.82% 5-μm Orgasol™ particles by weight.[18]

Table 2: Dependence of backscatter, attenuation and speed of sound for the plasma base, BMFs with Orgasol™ concentration (1.82% and diameter 5, 10 and 20 mm) and human red blood cells resuspended in saline[43]

	Backscatter power at 5 MHz (dB)	Attenuation at 5 MHz (dB/cm)	Speed of sound (m/s)
5 μm Orgasol™	0	0.26	1547
10 μm Orgasol™	7.9	0.3	1547
20 μm Orgasol™	13	0.3	1548
Human blood	0	0.73	1580

For inspecting the influence of red blood cell density, the basic structure of the BMF was preserved. The BMF was established on a suspension of nylon or Orgasol™ particles for examining the blood flow in flow phantom. The norm solution was made by mixing pure water, surfactant, dextran 185,000 D, and glycerol at concentrations of 83.86%, 0.9%, 3.36%, and 10.06%, respectively, with 1.82% 5-μm Orgasol™ particles by weight. Magnetic stirrer device was used for mixing the items for preparation BMF and then filtered it to eliminate any residual mass clumps by vacuum pump; this filtering was done through a 32-μm sieve. Ultimately, before measuring the acoustical and physical properties of BMF, it was mixed and degassed by vacuum pump technique until removal of the air bubbles.[18]

BMF is necessary for the vessel flow phantom, for reflected ultrasonic sound waves, and for assessing the speed of flow in the same method as in arteries. Utilizing 5-μm-diameter Orgasol™ particles (same size to red cell) is more prevalent in the literature and also cited in IEC 61685.[1] Although this fluid (BMF) is a Newtonian, it has extremely similar features to human blood, with a sound speed of 1548 m/s, the viscosity of 4.1 ± 0.1 MPa/s, density of 1037 ± 2 kg/m^3, and attenuation coefficient of 0.05 ± 0.01 dB.cm/MHz.[19] However, the BMF was prepared in a similar method to the previous methods used to prepare BMF. First, a plastic beaker was used with a size of two times the fluid needed to prepare BMF to avoid overflow of the components during stirring, components of the fluid which are required for the sample are weighed in a fume hood and poured into the plastic beaker, and the stirrer was turned on for 2 h. Second, a vacuum pump device was used to degas the fluid mixture for 2 h. Finally, both the acoustical

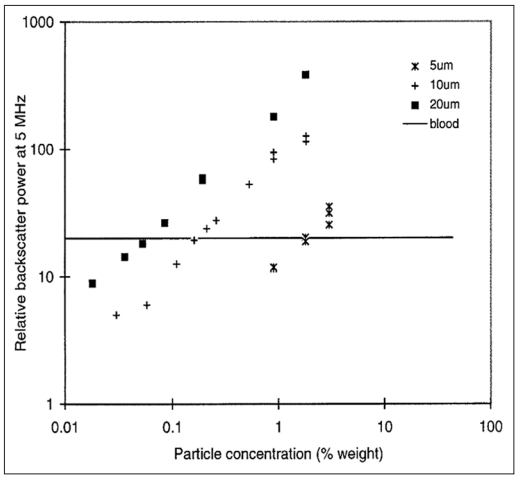

Figure 2: Regular flow from blood-mimicking fluid with relative backscatter plotted versus nylon particle ratio with different particle diameters of 5, 10, and 20 μm[43]

Table 3: Comparing between human blood and recommended blood-mimicking fluid that used Orgasol™ particles through their physical and acoustical properties[43]

Properties	IEC 1685 draft specifications	Human blood (37°C) Red blood cells	Recommended BMF (22°C) Orgasol™ (nylon)
Scatterer size (μm)		7	5
Hematocrit (percentage volume)		45	<5
Density (kg m^{23})	1050±40	1053	1037±2
Viscosity (mPas)	4±0.4	3	4.1±0.1
Velocity (m/s)	1570±30	1583	1548±5
Attenuation (dB/cm MHz)	<0.1	0.15	0.05±0.01
Backscatter (f^4/m/sr)	cf human blood	4×10^{-31}	cf human blood
Fluid properties	Newtonian	Non-Newtonian	Newtonian

BMF: Blood-mimicking fluid, IEC: International Electrotechnical Commission

(speed of sound) and physical (density and viscosity) properties of fluids of each sample were measured.[19]

For studying the thermal and acoustic properties of high intensity-focused ultrasound, the BMF has been advanced. The BMF relies on a degassed water solution scattered with polyethylene microspheres, Gellan gum, glycerol, and Orgasol™ particles. A wide range of physical properties are inclusive of thermal conductivity, viscosity, attenuation coefficient, the velocity of sound, and diffusivity.[3] Moreover, the BMF utilized for the tortuous vascular wall with fewer flow phantoms was a standardized model with acoustic and viscosity properties agreeing to those of human blood. The added materials to prepare BMF substances are a mixture of Dextran 185000D, Tergitol™ surfactants, Orgasol™, glycerol, distilled water, and potassium sorbate with specific weight amounts of 3.3%, 0.9%, 1.8%, 10.0%, 83.7%, and 0.3%, respectively. With adding 5-μm-diameter of Orgasol™ as a scattering material, the physical and acoustical properties matched the real human blood cells.[66]

Several research studies used the Orgasol™ material as suspension particles for preparing BMF. For example, BMF was utilized for the flow test object phantoms, and it was a standardized model with acoustic and viscosity properties consistent with those of human blood. The materials added for the preparation of BMF substance mixture were dextran of average molecular weight 185,000 D, Synperonic N surfactants, Orgasol™, pure glycerol, and distilled water, the mixture's ratios of weights were 3.363%, 0.9%, 1.82%, 10.06%, 83.86%, and 0.3%, respectively. With adding 5-μm-diameter Orgasol™ as a scattering material, the physical and acoustical properties matched the real human blood cells.[7] Another example was that a BMF was prepared to make Doppler spectral waveforms precisely with those which would be gained in real blood. They have used different types of Orgasol™ (nylon) powder with different densities and with the same diameters (5–15 mm), Orgasol™ 2001, Orgasol™ 3501, and Orgasol™ 1009. The densities of three different types are 1.02, 1.06, and 1.13 g/ml, respectively. Finally, the Orgasol™ (nylon) powder 2001 was used for preparing BMF and it was mixed with mixture fluid (water and glycerol). Acoustical and physical properties of BMF were suitable and agreed the values at IEC standard.[20]

Blood-mimicking fluid using Sephadex particle

Law *et al*. briefly studied and used nonbiological blood.[4] The used particles in BMF preparation should regularly be distributed in the fluid. When the density of particles is more than that of the surrounding liquid, the settling may happen, this can surely be visible at low speeds for particles such as Sephadex,[1] The Sephadex particle has been studied by many researchers.[67-70] Sometimes, the BMF is prepared with the powder material Sephadex, which is a scattered particle and suspended in water. Sephadex particles have a diameter and density almost similar to the human blood. However, according to past studies, although the Sephadex's density and size are much greater than the real human density and size, the backscattered measurements and other measurements of Sephadex are not too much different from that of human blood such as 20–50 μm at a concentration of l. 5 g/L.[68] Moreover, they aggregate or clot at low shear rates to give non-Newtonian manner which is the physical feature of blood.

The backscattered power of the scatter suspension is based on several factors such as the size of scattering, concentration volume of scattering, and variation in size modulus and density between the suspending fluid and the scatter. When the erythrocyte concentration increases, the backscattered power will increase, but the backscattered power reduces with plateaus at PCV percentage with 15%–30%.[33,51] The backscattered power of Sephadex particle suspension in fluids follows an identical style of human blood when the particle concentration increases,[21] and this is possible to be an event for other particles. The backscattered power of any size and strength will be nearly relative to the third power of particle diameter. Furthermore, the backscattered power will increase with the variance between the size elastic modulus and the density of liquid suspension and the scatter. The particles' diameter and density distribution of Sephadex usually are 20–70 μm and 1.7 g/cm^3, respectively.[20,21] Some researchers discovered that when using Sephadex particles with identical volume concentration to blood, the backscattered power will be massive. For example, dialysis tubing phantom used a Sephadex

particle suspension of 2 g/l and has similar backscatter power to blood *in vivo* of carotid artery.[67] Measurements utilizing Sephadex particles and blood pointed out no variation in the speckle pattern, or any reliance of this pattern on Sephadex concentration.[71] There was no influence on speckle pattern as an outcome of scattered bulk. When the dimensions of scattered are large compared with the ultrasonic wavelength and when the number of scatters is small, an unsuitable speckle pattern of Sephadex particles will appear.

The diameter of red blood cells on the order of 5–7 μm is suitable for Rayleigh scattering of ultrasonic frequency between 1 and 10 MHz because the scattering of red blood cells is large enough, so the ultrasonic backscattering signal considers a Gaussian random process.[32,72] Different concentrations of Sephadex particles have been applied in water,[68,73] also in glycerin mixture.[21] The size of Sephadex particles is in the range between 20 and 80 μm and its diameter is greater than red blood cells. However, it is not considered a Rayleigh scattering at a particular frequency which is 5 MHz. The backscatter power of Sephadex particles is based on its concentration.[21]

Blood-mimicking fluid using polystyrene particle

As we said before, when the density of particles is more than the surrounding fluid, the settling might happen. Thus, when preparing the BMF, we should take into consideration that the fluids and scatter particles should be suitable and simulate the acoustical and physical properties of real human blood. Numerous studies used polystyrene as a scattering material for BMF preparation since the polystyrene density and particle size are close to that of the red blood cells. A BMF with polystyrene has been studied and used by Kimme-Smith, he prepared BMF and used it with Doppler phantom. BMF was prepared with the total amount of 500 mL, composed of glycerol (96 mL), polystyrene divinylbenzene microspheres (11 mL), and degassed distilled water (393 mL). The diameter of microsphere was 29.4 mm, and also the viscosity, density, and scattering properties of blood were suitable to be used in Doppler flow.[10] The liquid is a mixture of glycerol and degassed water in a proper ratio to give a gravity density of 1.043 g/cm^3. This specific density was chosen to minimize deposition of the third element of the BMF. Polystyrene microspheres with 30 μm as a particle size were used. The polystyrene microspheres supply scattering from the liquid; their concentration and size distribution were chosen to provide an equal level of backscatter and to the actual blood. The velocity of sound of this liquid was 1546 m/s and the attenuation coefficient was 0.1 dB/cm.[58]

Since polystyrene microspheres are suitable to scatter particles in BMF, many of the liquids are prepared with proper density and viscosity which is close to polystyrene density of 1.05 g/cm^3. For example, a preparation of BMF with a density of 1.05 g/ml that was complies with the density mentioned in the IEC standard, however, this BMF prepared by a suitable mixture fluid that made of glycerin aqueous solution and water-soluble and dispersed with polystyrene microsphere particles.[10]

The BMF was utilized for the flow test object phantoms, and it was a standardized model with acoustic and viscosity properties agreeing to those of human blood. The materials added for the preparation of BMF substance mixture were, silicone oil, poly (ethylene glycol) 400, and distilled water, with the specific amount of wt% at 25.0%, 18%, and 57%, respectively. With 5-μm-diameter polystyrene as a scattering material, the physical and acoustical properties were matched to those of the real human blood cells.[11]

A specific instrument measured the physical properties (the viscosity and the density) and the acoustical properties (the speed of sound and the attenuation) of BMF. For example, the electronic rotational and U-tube viscometers were used for the viscosity measurement, pycnometer and densitometer were used for density measurement, and signal PE by A-scan GAMPT ultrasonic device was used for the acoustical measurements (attenuation and speed of sound). However, several types of particle scatters are used in BMF preparation and in several methods.[74-77] The most common BMF materials and preparation methods are shown in Table 4.

Conclusion

Many BMF materials have been described with various properties such as attenuation, viscosity, concentration, the speed of sound, density, and backscatter. Hardened red blood cells and cellulose Pulver were used rarely. Their features include simple to prepare, can be fitted to change their acoustic properties, and are eligible to help a normal distribution of scatters. However, the Orgasol™ particles or any other particles

Table 4: Most common blood-mimicking fluid items and their preparation methods

Material number	Material	Method	Application	References
1.0	Orgasol™ (nylon)	Nylon particles of three different sizes (5, 10, and 20 μm) were mixed with four materials of fluids (dextran, surfactant, pure water, and glycerol) based on weight by the magnetic stirrer and then the mixture was filtered by sieve and degassed by a vacuum pump	Speculating the achievement of Doppler ultrasound tool in the test object by using artificial blood	[1,3,5,11,12,17,40,49]
2.0	Polystyrene microspheres	BMF was prepared with the acoustic speed and density with polystyrene particles as a scattered material suspension in the fluids and it was mixed with water-soluble silicone oil	Using Doppler ultrasound for measurement of blood flow	[2,8,9,13,15]

BMF: Blood-mimicking fluid

used as scatters and suspension in fluid should be with a diameter close to the erythrocyte diameter, at least between 5 and 10 μ and with a density of 1.05 ± 0.04 g/ml to give the same physical and acoustical properties, these properties must be close to the values which were determined by the IEC standard.

The density measurement of BMF in most of the research studies was done either by using the pycnometer method or by the densitometer to produce accurate measurements of the liquid mixture in unit g/ml or g/cm^3. Furthermore, several previous studies measured the viscosity of BMF using U-tube viscometer and electronic rotational viscometer. Finally, the speed of sound of mixture fluid and BMF was measured in all experimental studies using PE technique.[1,7,9,10,16,17,20,45,57]

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Conflicts of interest

There are no conflicts of interest.

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