The procedure for the preparation of the proposed blood-mimicking fluid (BMF) started with just water and glycerol as the components of the fluid, mixed with polystyrene microsphere as scatter particles. Polyethylene glycol was first used as part of the mixture fluid but the acoustic and physical properties were not suitable, leading to its replacement with D(+)-Glucose, which is soluble in water and has a higher density. A common carotid artery wall-less phantom was also produced to assess the flow properties. The prepared blood-mimicking fluid with new scatter particles has a density of 1.067 g/cm³, viscosity of 4.1 mPa.s, speed of sound 1600 m/s, and attenuation of 0.192 dB/cm at 5 MHz frequency. Peak systolic velocity, end diastolic velocity and mean velocity measurements were gotten to be 40.2 ± 2.4 cm/s, 9.9 ± 1.4 cm/s, and 24.0 ± 1.8 cm/s, respectively. Based on the results obtained, the blood-mimicking fluid was found suitable for ultrasound applications in carotid artery wall-less phantoms because of its good acoustic and physical properties.

Keywords
blood-mimicking fluid, cholesterol scatter particles, acoustic properties, Doppler ultrasound

Introduction
A blood-mimicking fluid (BMF) is made up of ultrasound scattering particles that stay neutrally buoyant in a fluid. For these particles to be able to remain suspended in the fluid, their density must be very close or equal to the density of the mixture fluid. In addition, the BMF must also have acoustic properties (speed of sound, attenuation and backscatter power) and physical properties (density and viscosity) that are close to the internationally acceptable standard(1–3). The research on BMF started with just water and glycerol as the components of the fluid, mixed with polystyrene microsphere as scatter particles(4,5). Orgasol (nylon) was then introduced as scatter particles, mixed with water, glycerol, dextran, a surfactant and sodium azide(6–12). Other substances that have been used as scatter particles include sephadex(13–16), cellulose pulver(17–18), and hard red blood cells(19).

Real human blood is composed in approximately 55% of plasma and 45% of formed elements. The formed elements consist of red blood cells, white blood cells, platelets, and other insoluble substances such as cholesterol; while plasma is made up of about 90% serum and 10% other soluble substances including proteins and glucose(20,21). Because cholesterol is an insoluble particle in the blood, its movement within blood vessels such as arteries makes it a risk factor for cardiovascular disease known as atherosclerosis or hardening of the artery wall by cholesterol deposits (stenosis)(22).
In the present study, we prepared a blood-mimicking fluid consisting of water, propylene glycol and glucose as a ternary mixture fluid, with cholesterol serving as the scatter particle. The choice of cholesterol as scatter particles is informed by the knowledge that it is practically insoluble in water and has a density (1.067 $g/cm^3$) close to the acceptable limit, as seen in Tab. 1\(^{(1,2,3)}\). This BMF was used to determine the effect of the presence of cholesterol on in-vitro flow velocity in the common carotid artery wall-less phantom.

**Material and methods**

**Procedure for preparing BMF**

The BMF mixture fluid was first prepared by varying the percentage compositions of distilled water, propylene glycol (Sigma Aldrich, Germany), and polyethylene glycol (Merck KGaA, Darmstadt, Germany) in order to find the correct combination that gives an acceptable ternary fluid. The process was carried out as follows:

(i) First, a glass beaker the size about twice the volume of the BMF was washed with distilled water and wiped clean with a tissue paper. The beaker of this size was selected to avoid the overflowing of the fluid while stirring. This was followed by placing a magnetic stirrer inside the beaker.

(ii) 90% weight/weight (w/w) of distilled water was measured using a measuring cylinder, and 5% w/w of propylene glycol (PG) was weighed as well as 5% w/w of polyethylene glycol (PEG) in a fume cupboard using an electronic balance (multi-function balance, A&D Company Ltd, Japan). Distilled water was first poured into the beaker, followed by PG and PEG. The combined components were then placed on a magnetic stirrer hot plate (Favorit, 41200 Klang, Malaysia) for stirring.

(iii) The stirring plate was set to operate at 700 revolutions per minute (rpm) for 20 minutes at a temperature of 37°C, after which a vacuum pump was used to degas the fluid for about 30 minutes.

(iv) The procedures above were repeated four more times but with different percentages of distilled water and PEG, while the amount of PG remained fixed at 5% w/w composition. The five (5) samples as well as 100% w/w distilled water were then tested for their densities, viscosities, speed of sound, and attenuation properties. The results are tabulated in Tab. 2.

The serial number 8 in the table has a density (1.065 $g/cm^3$) close to that of cholesterol. However, it was not suitable as a mixture fluid because the speed of sound (1670.2 m/s) and viscosity (12.40 mPa.s) diverged considerably from the internationally acceptable values (Tab. 1), even though the attenuation value was within a good range. This led to the replacement of PEG with D(+)-Glucose (DG) (Merck KGaA, Darmstadt, Germany) with a higher density (1.54 g/cm\(^3\)), which the steps in (i) to (iv) above were repeated, with the results shown in Tab. 3.

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**Tab. 1. Specifications of the BMF defined as the IEC standard.** $f$ is the acoustic frequency (Hz) (IEC, 2001)

| Acoustic and physical properties of BMF | Values |
|----------------------------------------|--------|
| Viscosity ($\times 10^{-1}$ Pas)        | 4.0 ± 0.4 |
| Sound speed (m/s)                      | 1570 ± 30 |
| Attenuation (dB/cm/MHz)                | $<0.1 \times 10^{-4} \times f$ |
| Density ($g/cm^3$)                     | 1050 ± 40 |

---

**Tab. 2. Physical and acoustic properties of the mixture fluid for the preparation of a blood-mimicking fluid with propylene glycol and polyethylene glycol at a temperature of 37°C**

| S/No. | Distilled water % (w/w) | Propylene glycol % (w/w) | Polyethylene glycol % (w/w) | Density (g/cm\(^3\)) | Viscosity (mPa.s) | Speed of sound (m/s) | Attenuation (dB/cm/MHz) |
|-------|-------------------------|--------------------------|----------------------------|----------------------|------------------|---------------------|------------------------|
| 1     | 100                     | 0                        | 0                          | 0.997                | 0.90             | 1506.7              | 0.044                  |
| 2     | 90                      | 5                        | 5                          | 1.012                | 2.50             | 1512.1              | 0.050                  |
| 4     | 80                      | 5                        | 15                         | 1.031                | 6.80             | 1568.4              | 0.056                  |
| 6     | 70                      | 5                        | 25                         | 1.044                | 5.80             | 1692.0              | 0.057                  |
| 7     | 60                      | 5                        | 35                         | 1.059                | 10.60            | 1652.5              | 0.059                  |
| 8     | 55                      | 5                        | 40                         | 1.065                | 12.40            | 1670.2              | 0.061                  |
| 9     | 50                      | 5                        | 45                         | 1.078                | 14.50            | 1696.3              | 0.062                  |

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**Tab. 3. Physical and acoustic properties of the mixture fluid for the preparation of a blood-mimicking fluid with propylene glycol and D(+)-Glucose at a temperature of 37°C**

| S/No. | Distilled water % (w/w) | Propylene glycol % (w/w) | D(+)-Glucose % (w/w) | Density (g/cm\(^3\)) | Viscosity (mPa.s) | Speed of sound (m/s) | Attenuation (dB/cm/MHz) |
|-------|-------------------------|--------------------------|----------------------|----------------------|------------------|---------------------|------------------------|
| 1     | 100                     | 0                        | 0                    | 0.993                | 2.8              | 1513.4              | 0.0460                 |
| 2     | 90                      | 5                        | 5                    | 1.016                | 3.4              | 1549.3              | 0.0650                 |
| 3     | 85                      | 5                        | 10                   | 1.035                | 3.8              | 1566.4              | 0.105                  |
| 4     | 80                      | 5                        | 15                   | 1.057                | 4.4              | 1588.4              | 0.190                  |
| 5     | 75                      | 5                        | 20                   | 1.079                | 5.2              | 1614.1              | 0.194                  |
| 6     | 70                      | 5                        | 25                   | 1.100                | 5.8              | 1634.3              | 0.198                  |
A blood-mimicking fluid with cholesterol as scatter particles for wall-less carotid artery phantom applications

J Ultrason 2021; 21: e219–e224

A blood-mimicking fluid with cholesterol as scatter particles for wall-less carotid artery phantom applications

of benzalkonium chloride solution was added to 2

TM

, a value required to match the density of cho-

lesterol particles were added to the mixture fluid until the

ing them with the fluid. Different concentrations of cho-

lesterol scatters. This allows the scatter particles to remain

eutrally buoyant in the fluid mixture.

Fig. 1. Measurement of the acoustic properties of solutions using the A-scan GAMPT technique

(v) A linear relationship was established using the statisti-
cal package for social sciences (SPSS, Version 21) software
between the density and the amount of DG in the fluids,
resulting in the equation:

\[
\text{Density} = 0.00426 \times \text{(amount of DG)} + 0.993
\]

With equation 1, 17.37% w/w of DG by weight was required
to mix with 5% w/w of PG and 77.63% w/w of distilled
water to produce exactly a mixture fluid with a density of
1.067 g/cm³, a value required to match the density of cho-

lesterol. This allows the scatter particles to remain

neutrally buoyant in the fluid mixture.

(vi) Finally, a sieving net with the mesh size of approxi-

mately 10 was used to sieve the cholesterol particles (≥90% purified,
Merck KGaA Darmstadt, Germany) before mix-

ing them with the fluid. Different concentrations of cho-

lesterol particles were added to the mixture fluid until the

right amount required for the particles to stay suspended

in the fluid was achieved. The BMF thus prepared was

then mixed with about 4 drops (0.2 ml) of benzalkonium
chloride (Sigma Aldrich, Germany) solution to serve as a

preservative.

Preparation of tissue-mimicking material (TMM) wall-less phantom

A TMM wall-less phantom of the CCA was prepared
with a lumen diameter of 8.0 mm, as described by
Ammar et al.\textsuperscript{24}. The TMM composition was made up of
84% w/w distilled water, 0.53% w/w of silicon carbide
(Logitec, Glasgow, UK), 0.96% w/w of aluminum oxide,
3.0 μm size (Sigma Aldrich, Germany), 0.89% w/w of
aluminum oxide, 0.3 μm size (Sigma Aldrich, Germany),
0.92% w/w of gelatin (gel strength 300, Type A, Sigma
Aldrich, Germany), 1.5% w/w of konjac powder (dietary
supplement, NOVA Nutritions, Scotch Plains, New
Jersey USA), 1.5% w/w of carrageenan powder (Sigma
Aldrich, Germany), 0.7% w/w of potassium chloride
(Sigma Aldrich, Germany), and 9.0% w/w of glycerol
(Vetec\textsuperscript{TM} reagent grade, Sigma Aldrich, Germany). About
5 cm\textsuperscript{3} of benzalkonium chloride solution was added to 2
liters of the TMM as an anti-fungal agent. The prepared
TMM was cast inside a plastic (acrylic) rectangular box
with a straight metal rod placed horizontally inside at
a depth of 15 mm. When the TMM had cooled to about
40°C, the metal rod was removed gradually to provide
a lumen which was attached to rubber tubes for connec-
tion to a pump.

Measurements of density and viscosity

The densities of the BMF fluids were measured using a por-
table density meter (DMA 35, Anton Paar GmbH, Austria)
at 37°C. The strip attached to the meter was pressed down
and dipped inside the fluid, and then released to draw up
the fluid inside the strip by suction pressure, while the den-
sity reading was recorded automatically by the meter in
just few seconds.

About 700 cm\textsuperscript{3} of each BMF was required for the viscosity measurements using the electronic rotational viscometer (ERV) (Software Version 1.2, Fungilab, Barcelona, Spain). The spindle L1 was selected for viscous liquid; it was fixed
to the ERV and then lowered into the fluid, while the ERV
was switched on. The spindle rotated inside the liquid for
a few minutes until a steady value of viscosity was recorded
due to the viscous resistance from the fluid\textsuperscript{25,26}.

Measurements of speed of sound, attenuation and backscatter power

The speed of sound was measured by the pulse echo (PE)
method using the ultrasonic echoscope GAMPT-scan
machine (German Society for Applied Medical Physics
and Technology, GAMPT Ultrasonic Solutions, Hallesche
Straße, Merseburg, Germany). The arrangement for this
measurement is shown in (Fig. 1), where the time of
flight (TOF) between the highest two peaks of the trans-
mitted and about 90% of the received signal (energy) at
a frequency of 5 MHz was measured. For good accuracy,
the protective layer thickness of the probe was first cal-
culated and added to the distance between the walls of
the vessel containing the liquid. This was done by using
two acrylic plates, 40 mm and 80 mm thick, with the
same speed of sound of 2700 m/s. The pulse echo method
using A-scan GAMPT technique was able to provide time
of flight for the two plates which was used to calculate
the thickness of the protective layer of the probe from
the equation:

\[
dp = \frac{\left(\frac{t_1}{t_2} - da\right) - da_1}{2 \left(1 - \frac{t_1}{t_2}\right)}
\]

where \(dp\) is the thickness of the transducer protective
layer, \(da\) is the thickness of the acrylic plate (40 mm), is
the thickness of the acrylic plate (80 mm), \(t_1\) is the time
of flight through the plate (40 mm), and \(t_2\) is the time

of flight through the plate (80 mm). The values for the speed of sound for the liquids were calculated from the equation:

\[
\text{speed of sound} = \frac{2s(x+dp)}{t}
\]

where \(x\) is the distance between the walls of the vessel containing the mixture liquid and \(t\) is the time of flight.

The amplitudes of the highest two similar peaks were also measured, and the attenuation coefficients (\(\alpha\)) were calculated from the attenuation equation:

\[
\alpha = \frac{2 \times 0.868}{x} \ln \frac{A_2}{A_1}
\]

where \(A_1\) and \(A_2\) are the amplitudes of the two highest but similar wave peaks, respectively, and \(x\) is the distance.

The backscatter power of the BMF was measured at different radio-frequency signals by calculating the average power spectrum through applying the fast Fourier transform (FFT) generated by the A-scan GAMPT at 5 MHz. This was done to find out if the BMF simulates real human blood.

\[\begin{align*}
\text{Results} \\
\text{Discussion}
\end{align*}\]

of the lumen and the BMF were displayed on the screen of the scanning machine. The measurements of peak systolic velocity (PSV), end-diastolic velocity (EDV), mean or average velocity (\(V_m\)), pulsatility index (PI) and resistivity index were carried out by color Doppler and pulsed-wave (PW) Doppler systems. The angle of beam (insonation angle) was set at the center of the flow at 60° with the required sample volume and entrance length of about 8 cm. To obtain a good image of the phantom and BMF flow, the transmitting frequency was adjusted on the scanner and set at 5 MHz, while the display depth, focus position, image size, sample gate, pulse repetition frequency (PRF), sample volume and color brightness settings were all adjusted until the normal settings were achieved.

Fig. 2. PW-mode ultrasound measurements of flow indices using the Hitachi Ultrasound Machine

The results obtained when a fixed percentage composition of PG (5% w/w) was combined with different percentages of PEG and distilled water can be seen in Tab. 2. A second trial of the same procedure with DG also showed a linear relationship between DG with the physical and acoustic properties (Tab. 3). This mixture fluid produced a BMF with 0.7% of cholesterol as scatter particles, viscosity of 4.1 ± 0.04 mPa.s, speed of sound 1600 ± 2 m/s, and attenuation of 0.192 ± 0.0002 dB/cm at 5.0 MHz. The results of velocity measurements (Fig. 2) at a flow rate of 1500 ml/min through the wall-less lumen of the phantom revealed that the PSV, EDV and the \(V_m\) were (40.2 ± 2.4 cm/s, 9.9 ± 1.4 cm/s, and 24.0 ± 1.8 cm/s, respectively). The ± sign values attached to the physical and acoustic measurements represent the estimated standard deviation values.

The measured attenuation, \(\alpha\) (dB/cm), was found to vary with frequency, \(f\) (MHz), as 0.08\(f\) – 0.00832\(f^2\). Using this polynomial, the value of the attenuation at a frequency of 5 MHz was estimated to be 0.192 dB/cm.

Doppler ultrasound measurement of flow velocities

A digital clinical Hitachi ultrasound scanning machine (HI VISION Avius, Hitachi Medical Corporation, Tokyo, Japan) connected to a linear array transducer (probe) EUP-L74M with frequencies ranging from 5 to 13 MHz was used to obtain an image of the wall-less flow phantom. The phantom was placed on a flat table and connected to a centrifugal multi-flow pump (German Society for Applied Medical Physics and Technology, GAMPT mbH, Hallesche Straße, Merseburg, Germany) with the aid of plastic tubes to pump the BMF. The fluid was pumped through the lumen at a flow rate of 1500 ml/min (for both steady and pulsed flow), and B-mode images

Discussion

The amount of PG was fixed because its density (1.036 g/cm³) is lower than that of PEG (1.124 g/cm³), but greater than that of water (0.998 g/cm³). Therefore, increasing the amount of PG alongside those of water and PEG resulted in decreasing the density of the resulting mixture fluid. Increasing the amount of PEG while decreasing that of water led to increases in the values of density, viscosity, speed of sound, and attenuation. This result could not be considered suitable for selection because the combinations required to produce a density equal or close to that of cholesterol (1.067 g/cm³) had higher values for speed of sound and viscosity even though attenuation was within an acceptable range. The need to replace PEG with a substance of higher density led to the introduction of DG with a density of 1.50 g/cm³, with another advantage of being soluble in water.

To obtain the exact percentage combination which yielded the same density with the cholesterol scatter particles,
A blood-mimicking fluid with cholesterol as scatter particles for wall-less carotid artery phantom applications

A mathematical equation from the linear relationship (Fig. 3) between percentage DG and density was established (equation 1). The use of equation 1 resulted in a ternary mixture fluid prepared by combining 77.63% w/w distilled water, 5% w/w PG, and 17.37% w/w DG with a density of 1.067 g/cm$^3$. With these properties, the BMF is suitable for use in Doppler ultrasound pre-clinical applications and studies. The backscatter measurement of the BMF at different percentage concentrations of cholesterol showed that backscatter increased with an increase in the particle ratio (Fig. 4)$^{22,27}$. Cholesterol scatter lowers the flow velocity of the BMF within the arteries when compared with normal standards, which requires more pressure from the pump to achieve the required volume flow to the desired organs. The high pressure makes these cholesterol particles have more contact with the walls of the artery, thereby attaching them to the walls, which leads to the formation of plaques (atherosclerosis) in the real human artery$^{22,28}$. The PI and RI values for the BMF flow are within the normal range of 20 cm/s to 32 cm/s for EDV, 0.98 to 1.94 for PI, and 0.72 to 0.84 for RI$^{31}$.

**Conclusion**

In this study, we introduced a new scatter particle (cholesterol) to prepare a BMF with acceptable acoustic and physical properties. The BMF is made up of 77.63% distilled water, 5% PG, 17.37% DG, and 0.7% cholesterol as scatter particles. It has acceptable backscatter properties and hemodynamic parameters; therefore, it is recommended for use in ultrasound phantom applications and research studies.

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**Conflict of interest**

There are no conflicts of interest.

**Author contribution statement**

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