Development of a Microfluidics-Based Tooth-Pulp Flow Phantom for Validation of Doppler Ultrasound Devices

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Development of a Microfluidics-Based Tooth-Pulp Flow Phantom for Validation of Doppler Ultrasound Devices

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Abstract

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I. Introduction

Doppler ultrasound is generally used in medical diagnostics for measuring blood flow in a wide range of blood vessels. Recently, Doppler ultrasound has been used for measuring pulpal blood flow (PBF). However, the reliability of this method has not been sufficiently addressed. Flow phantoms have been used to evaluate the velocity estimation using Doppler ultrasound devices. However, most of these phantoms are designed to simulate relatively large blood vessels in milli or centimeter scale, which passes through the matrix

material that mimics soft tissue. Therefore, they are not applicable as substitutes for dental hard tissues and pulp. Microfluidics is known as the science of fluid mechanics manipulated at micro or nanometer scales. It has emerged as an important tool in several research fields for microanalytical purposes. There have been a few studies using microfluidics for simulating tissue-vascular network. However, the use of microfluidics for evaluating Doppler ultrasound devices, as well as dental hard tissue and pulp has not been reported.

In this study, we present a microfluidics-based flow phantom developed as a blood flow model of dental hard tissue and pulp for use in experiments involving Doppler ultrasound technique. By using the flow phantom, the accuracy of a Doppler ultrasound device in making velocity estimations was evaluated.

II. Materials and methods

A computer-controlled microfluidic system was constructed to generate triangular pulsatile flow profiles. Blood-mimicking fluid was pumped through a 200×200 μm-sized channel in the microfluidic chip. A Doppler ultrasound device with a 20 MHz-transducer was used for the measurement of fluid flow. The peak, mean, and minimal flow velocities obtained from the flow phantom and the Doppler ultrasound device were compared using linear regression analysis and Pearson's correlation coefficient. Bland-Altman analyses were performed to evaluate the differences of the velocities between the phantom and the Doppler ultrasound device.

III. Results

The microfluidic system was able to generate the flow profiles as intended, and the fluid flow could be easily monitored and controlled by the software program. Using the soft lithography technique, we were able to fabricate a micrometer-sized channel.

There were excellent linear correlations between the peak, mean, and minimal flow velocities of the phantom and the measured velocities from the Doppler ultrasound device ($r = 0.94$, 0.98, and 0.996, respectively, $p < 0.001$). However, it is observed that the Doppler ultrasound device overestimated the flow velocities by 1.69, 2.00, and 2.23 cm/s, with respect to the peak, mean, and minimal velocities.

IV. Conclusions

We believe that this phantom provides opportunities for expanding future researches involving Doppler ultrasound, as well as in the field of hemodynamics and physiology of the dental pulp. Although Doppler ultrasound can be an effective diagnostic tool for quantitative measurement of PBF, it is essential to validate and calibrate the system prior to clinical use.

Key words: dental pulp; blood flow velocity; Doppler ultrasound; flow phantom; microfluidics; microfluidic chip;

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I. INTRODUCTION

1. Assessment of pulpal blood flow in a tooth

Dental pulp is a loose connective tissue surrounded by avascular and highly calcified hard tissues—enamel, dentin and cementum. It receives blood supply from arterioles which enter the tooth through small apical foramina located at the end of the root. While the rigid hard tissue shell provides protection for the pulp tissue from outer oral environment, it makes the clinical assessment of pulp status difficult, because the pulp is inaccessible

unless a hole is made through the hard tissues (Matthews and Andrew 1995). Although measurement of PBF would be an ideal tool for determining the pulp vitality, only sensitivity tests such as thermal or electric pulp testing, which examine the nerve response to a stimulus, are available clinically. These sensitivitiy tests often produce false results, particularly in healthy immature teeth (Fulling and Andreasen 1976), traumatized teeth (Andreasen 1986), or teeth undergoing orthodontic treatment (Sailus et al. 1987).

The vascular architecture of the dental pulp was confirmed in early histological studies. The arterioles run axially in the center of the pulp, whereas venules are located in the periphery (Kramer 1960). Shunt vessels such as arteriovenous anastomoses, venovenous anastomoses, and U-turn loops, which provide direct communications between the arterioles and venules, are also observed in the pulp (Takahashi et al. 1982). The diameters of blood vessels are less than 100 μm, and vary according to the tooth and location within the same tooth (Cheng and Provenza 1959; Provenza 1958).

In animal studies, researchers have used invasive methods such as the radioisotope clearance test (Hock et al. 1980) and hydrogen gas desaturation test (Tonder and Aukland 1975) to investigate the pulpal blood flow (PBF) in a tooth. It was confirmed that the pulp is one of the most highly vascularized tissues of the body (Vongsavan and Matthews 1992). The PBF is estimated to be 40-50 ml/min/100 g tissue (Kim et al. 1986; Meyer 1993), which is relatively high compared to that of the other tissues (Kim 1985). Kim et al. (1984) used intravital microscopy to study flow profiles in the microvessels of rat incisors. The velocity of red blood cell (RBC) was higher in arterioles (2.56 mm/s) than similar-sized

venules (0.48 mm/s). The regulation of PBF was found to be controlled by systemic blood pressure (BP) (Sasano et al. 1989; Tonder 1980), as well as by neuronal, local, and humoral mechanisms (Andrew and Matthews 2002; Matthews and Vongsavan 1994; Olgart 1996).

In contrast to those in animals, only limited information is available regarding the PBF in human teeth. For a direct assessment of the PBF in human teeth, several non-invasive methods have been introduced such as laser Doppler flowmetry (Musselwhite et al. 1997), pulse oximetry (Munshi et al. 2002), photoplethysmography (Miwa et al. 2002), and dual wavelength spectrophotometry (Nissan et al. 1992). Each of them has its own strengths and weaknesses (Chen and Abbott 2009). Laser Doppler flowmetry has been commonly used for measurement of PBF through the hard tissues (Ajcharanukul and Matthews 2015; Eroglu and Sabuncuoglu 2014; Kijsamanmith et al. 2011). However, the signals can be easily interfered by the backscattered light from the adjacent tissues, and any obstruction of light pathway can make the technique useless (Hartmann et al. 1996). The signals are recorded only with relative proportion, which cannot be calibrated in absolute units (Vongsavan and Matthews 1993). By using the optical methods such as pulse oximetry and photoplethysmography, it is possible to distinguish between vital and nonvital teeth (Schnettler and Wallace 1991). However, the flow rate cannot be measured by this technique. Only experimental results have been reported with spectorphotometry (Nissan et al. 1992). Currently, there still exists significant need for a technique that is able to detect and quantitatively measure the PBF.

2. Fundamentals of Doppler ultrasound for blood flow measurement

For all waves, such as sound or light, there is change in the frequency when it is reflected from a moving object. It is known as the 'Doppler effect' or 'Doppler shift' (White 1982). This change in the frequency—the Doppler frequency (f_d)—is proportional to the velocity of the moving object:

$$f_d = (2\ f_0\ v \cos \alpha)\ c^{-1}$$

, where f_0 is the transmitted frequency, v is the velocity of the moving object, α is the angle between the movement vector and the transmitted wave, and c is the velocity of the wave in the matrix.

Doppler ultrasound can be used to determine blood flow velocity because the Doppler frequency depends on the direction of blood flow and is proportional to the speed of moving RBCs. A number of techniques which make use of the Doppler ultrasound principle have been developed and applied in medical diagnostics. It offers a real-time, non-invasive, painless, simple and apparently safe method for measuring blood flow in a wide range of blood vessels (Gill 1985). Basically, the system comprises a piezoelectric transducer, which consists of two independent elements: the transmitting element, which generates a wave of ultrasound, and the receiving element, which detects the reflected signal. When the transmitted ultrasound is scattered or reflected against a moving structure within the body, it experiences a frequency shift and returns to the receiver. Movement towards the transducer produces an increase in the reflected frequency, whereas motion away gives a

reduction in the frequency. The doppler frequency is small and within the audible range. Doppler ultrasound devices are designed to detect the frequency shift, and process and feed the signal to an audible sound or a visible spectrum (Pozniak and Allan 2013).

3. Doppler ultrasound for measurement of pulpal blood flow

Ultrasound is considered to be well suited for dentistry because it is able to penetrate hard tissues, it has proper range resolution, and it does not expose ionizing radiation. It has been previously investigated as a complemental tool to detect dental caries, tooth fractures, and debonded restorations (Culjat et al. 2003; Culjat et al. 2005; Singh et al. 2007). It has also been used to measure the blood flow in tissue surrounding the tooth (Lustig et al. 2003; Rajendran and Sundaresan 2007). However, direct application of ultrasound to a tooth is limited for a number of reasons (Ghorayeb et al. 2008; Lees and Rollins 1972). The high acoustic impedance of dental hard tissue is not matched to commercially available transducers. The complexity and irregularity of tooth structures cause acoustic scattering. The small dimensions of a tooth and limited spaces obscure proper transducer positioning. The small sizes of vessels and the low blood flow velocities hinder accurate measurement.

These limitations have been partially overcome by the use of high-frequency ultrasound. Christopher et al. (1996; 1997) built 40 MHz-continuous wave (CW) and 50 MHz-pulsed wave (PW) transducers, which can detect blood flow velocities less than 1-5

mm/s in vessels that are 20-35 μm in diameter. Berson et al. (1999) reported that a 20 MHz-PW transducer has the capability to measure blood flow velocities less than 0.5 mm/s in 100-300 μm-diameter vessels. Hughes et al. (2009) reported the use of a 35 MHz-transducer for the measurement of enamel structures within an accuracy of 50 μm.

Recently, researchers have utilized Doppler ultrasound for the measurements of PBF. By using a Doppler ultrasound device, the PBF of vital teeth can be distinguished from that of root canal-filled teeth (Yoon et al. 2010). Changes in the PBF velocity can be identified after infiltration with an anesthetic agent containing epinephrine (Yoon et al. 2012). The mean PBF velocity of normal maxillary anterior teeth was successfully measured and found to be approximately 0.56 cm/s regardless of tooth type (Cho and Park 2015). Although it showed promising results, the device is not generally available for clinical use, and the reliability of this method has not been verified. In our preliminary study, the measured PBF velocity revealed large deviations between examiners. Therefore, the validation and calibration of Doppler ultrasound devices, as well as performance and quality control tests are necessary to determine the accuracy of the method.

4. Flow phantom for Doppler ultrasound devices

Experimental systems, referred to as ‘phantoms’, have been used to simulate human tissues for the characterization and calibration of ultrasound devices (Culjat et al. 2010). They enable the testing of ultrasound devices on targets with known acoustic properties,

dimensions, geometries, and internal features. They are also used to compare the performance of ultrasound devices, for training of technicians, for comparison to computer models, and to assist in the development of new devices or diagnostic techniques.

Flow phantoms consist of tissue substitutes called ‘tissue mimicking materials (TMMs)’, surrounding channels through which blood-mimicking fluid (BMF) is pumped. They also include a pump system, which generates flow profiles—either steady or pulsatile. Flow phantoms for various types of tissues have been developed and employed to evaluate the velocity estimation by using Doppler ultrasound devices (Camfferman et al. 2014; Hein and O'Brien 1992; Hoskins 2008). Several types of BMF have also been introduced (Lubbers 1999; Oates 1991; Ramnarine et al. 1998; Samavat and Evans 2006). Currently, there are numbers of commercially available flow phantoms. However, most of them are designed to simulate relatively large blood vessels, and their matrices resemble soft tissue. Therefore, they are not applicable as substitutes for dental hard tissue and pulp. The development of an effective flow phantom for a tooth and pulp is critical for the use of Doppler ultrasound in dentistry.

A few studies have been performed to explore candidate materials for the replacement of tooth structures (Table 1). Aluminum is partly similar to enamel, whereas copper is a poor substitute for dentin (Blodgett 2003). Singh et al. (2008) suggested that glass and dental composites can effectively mimic the acoustic properties of enamel and dentin. There have been some experimental studies to develop PBF models, which could be considered as potential flow phantoms. Vongsavan and Matthews (1993) used extracted

teeth, nylon tubes, and diluted blood to fabricate a tooth-pulp model for the validation of laser Doppler technique. Although this model performed well, there are some drawbacks. The dimension of extracted teeth cannot be standardized. The blood has short lifespan and unstable acoustic properties *in vitro* (Oates 1991). Niklas et al. (2014) introduced a blood flow model for the optical detection of PBF. However, they only detected the pulse and not the velocity of PBF. Moreover, the inner diameter of the tubes was 1 mm, which is significantly larger than the vessels in human dental pulp.

Table 1. Physical and acoustic properties of tissues and tissue mimicking materials (TMMs)

Material	**Velocity ($m\ s^{-1}$)**	**Density ($kg\ m^{-3}$)**	**Acoustic impedance ($10^6\ kg\ m^{-2}\ s^{-1}$)**
Enamel[a]	6250	3000	18.8
Dentin[a]	3800	2000	7.6
Soft tissue[b]	1561	1043	1.63
Aluminium[a]	6300	7750	33.7
Copper[a]	3300	1740	24.2
Glass[c]	6025	2475	14.9
Dental composite[c]	3350	2200	7.4
Air[b]	330	1.2	0.0004
Water[b]	1480	1000	1.48

[a] Values are cited from Blodgett (2003)

[b] Values are cited from Culjat et al. (2010)

[c] Values are cited from Singh et al. (2008)

5. Microfluidics-based flow phantom

Microfluidics is known as the science of fluid mechanics manipulated at micro or nanometer scales. It has emerged as an important tool in several research fields such as chemistry, physics, optics, biology, and medicine (Arya et al. 2013). Advanced microfabrication techniques offer an accurate guide for the development of microfluidic chips (Abgrall and Gue 2007). These techniques provide a variety of designs and the ability to control flow profiles at microscale. Recently, it has also been incorporated into dental research. Verhaagen et al. (2014) fabricated a root canal model using a microfluidic device to investigate the transport of irrigating solution. Samarian et al. (2014) suggested a method to develop oral biofilms in a microfluidic system emulating human oral cavity. Attempts to integrate microfludics for oral diagnositics are ongoing (Herr et al. 2007).

There have been a few studies using microfluidics for simulating tissue-vascular network. Parthasarathy et al. (2007) constructed a microfluidic phantom to model the capillary network in the cerebral cortex of brain, which contained various channels of different sizes (10-150 μm). Luu et al. (2012) introduced a prototyping method to fabricate phantoms of superficial vascular network for optical monitoring. Stern et al. (2014) used a laser Doppler device to detect flow rates in a microfluidic system, and could measure flow rates of 2 mm/s through a 60 μm-wide channel at a resolution of 0.08 mm/s. From such studies, it can be inferred that microfluidics could be utilized for flow phantoms including small vessels. However, the use of microfluidics for testing of Doppler ultrasound devices, as well as simulating a tooth and pulp has not yet been reported.

In this paper, we present a microfluidics-based flow phantom that we have developed to simulate the PBF of an arteriole in a tooth for use in experiments and educations involving Doppler ultrasound devices. By using the phantom, the accuracy of a Doppler ultrasound device in making velocity estimations was evaluated.

II. MATERIALS AND METHODS

The experimental system consisted of two parts: a flow phantom and a Doppler ultrasound device (Figure 1).

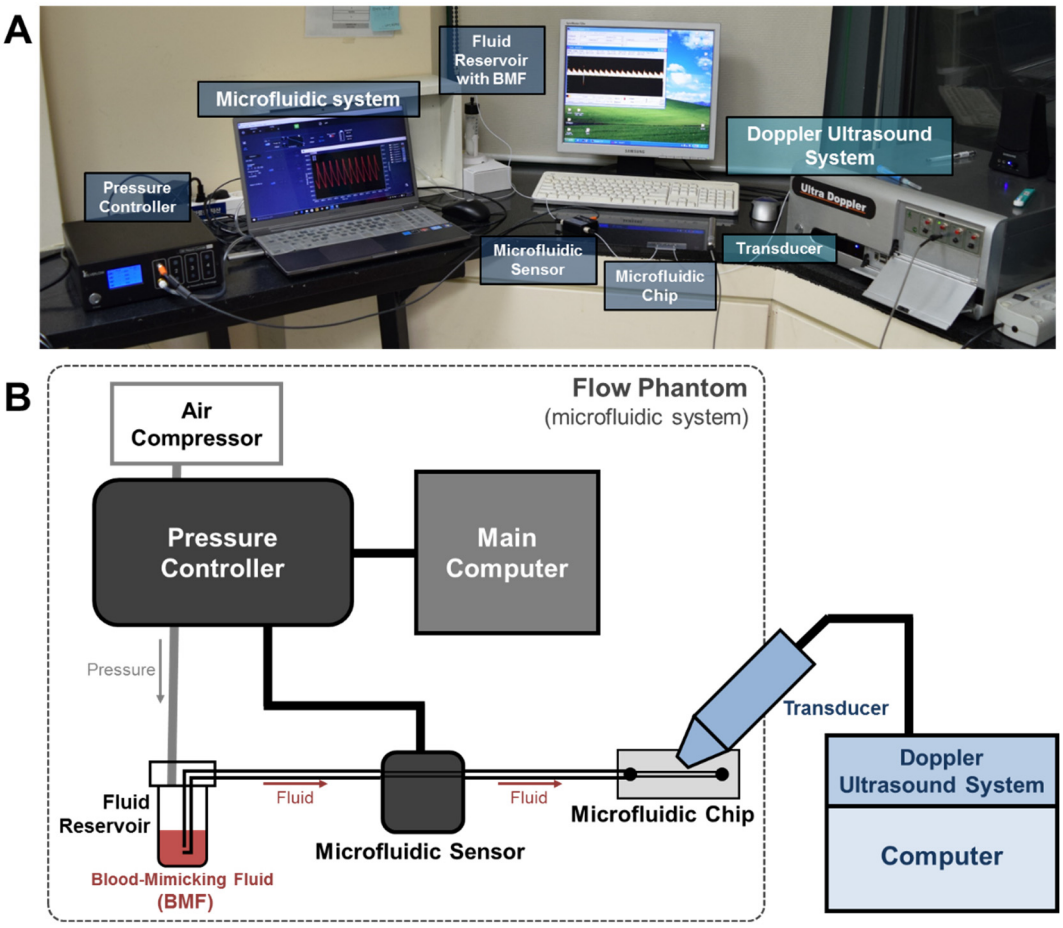


Figure 1. The experimental system

(A) Laboratory setting; **(B)** Schematic diagram of the system

1. Flow phantom: a microfluidic system with blood-mimicking fluid

A computer-controlled microfluidic system was constructed with an air compressor (BBT-001; Yamato Comp, Seoul, South Korea), a pressure controller (OB1-Mk3; Elveflow, Paris, France), a fluid reservoir (Elveflow, Paris, France), a microfluidic sensor (MFS-3; Elveflow, Paris, France), and a custom-made microfluidic chip (Microfit, Gyeonggi, South Korea). Polytetrafluoroethylene (PTFE) tubes (Elveflow, Paris, France) were used to connect each component in the circuit. The specifications of each component are described in Table 2. The fluid reservoir was filled with BMF (Model 046; CIRS Inc., Norfolk, VA, USA), which was pumped and infused through the circuit. The physical and acoustic properties of BMF compared to human blood are presented in Table 3.

The microfluidic chip was fabricated using the soft lithography technique (Qin et al. 2010). A photoresist was coated onto a silicon wafer and exposed to 365 nm-ultraviolet light through a mask to make a mold for a channel. Subsequently, polydimethylsiloxane (PDMS) was casted and bonded on a glass slide. The final product includes a single straight channel with a width and depth of 200 μm (Figure 2).

Table 2. Components of the microfluidic system

Component	**Specifications**	
Air Compressor	Model	BBT-001 (Yamato Comp, Seoul, South Korea)
	Type	Piston, oil-free
	Power	125 W
	Pressure	- 4 bar
	Capacity	23 L/min
	Tank	3 L
Pressure controller	Model	OB1-Mk3 (Elveflow, Paris, France)
	Type	Piezoelectric
	Pressure range	0-200 mbar
	Pressure stability	0.005 %
	Pressure sensor resolution	0.006 %
	Response time	- 9 ms
	Setting time	- 40 ms
Flow sensor	Model	MFS-3 (Elveflow, Paris, France)
	Type	Thermal
	Flow rate range	0-80 μL/min
	Accuracy	2-80 μL/min: 5 %, <2 μL/min: 120 nL/min
	Sensor capillary material	Quartz
	Sensor inner diameter	430 μm
Tube	Tube material	PTFE
	Tube inner diameter	500 μm
	Tube outer diameter	1/16 inch

From the manufacturers' instructions

PTFE, polytetrafluoroethylene

Table 3. Physical and acoustic properties of blood-mimicking fluid (BMF) compared to the human blood

Properties	**Human blood (37ºC)**[a]	**BMF Model 046 (22ºC)**[b] (CIRS Inc., Norfolk, VA, USA)
Scatterer	Red blood cells	Nylon particles (Orgasol™)
Scatterer size (μm)	7	5
Viscosity (mPa s)	3	4±0.5
Velocity (m s^{-1})	1583	1570±30
Attenuation (dB cm^{-1} MHz)	0.15	< 0.1
Fluid properties	Non Newtonian	Newtonian

[a] Values are cited from Ramnarine et al. (1998)

[b] From the manufacturer's instructions

Orgasol ™, ELF Atochem, Paris, France

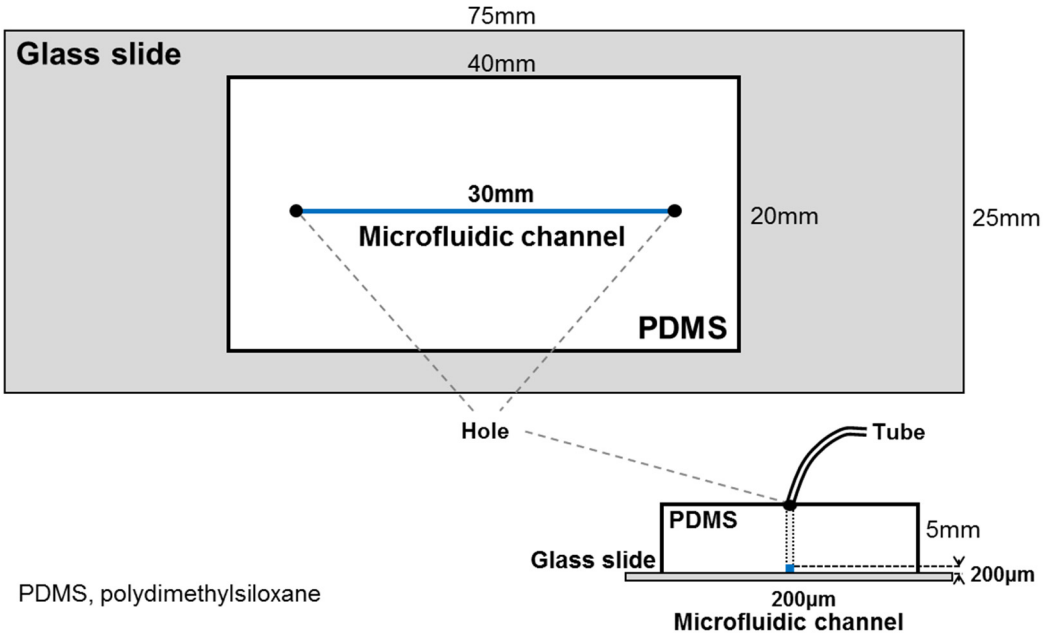


Figure 2. Dimensions of the microfluidic chip and channel

The flow profiles were generated and controlled by pressure and flow rate regulation through the Elveflow Smart Interface (Elveflow, Paris, France) software program (Figure 3). The flow patterns, pressures, flow rates, and pulse repetition intervals were monitored and regulated by the program. The real-time feedback of the flow rates from the sensor was displayed on a graph and the values were exported to a computer data file at 0.01 s intervals.

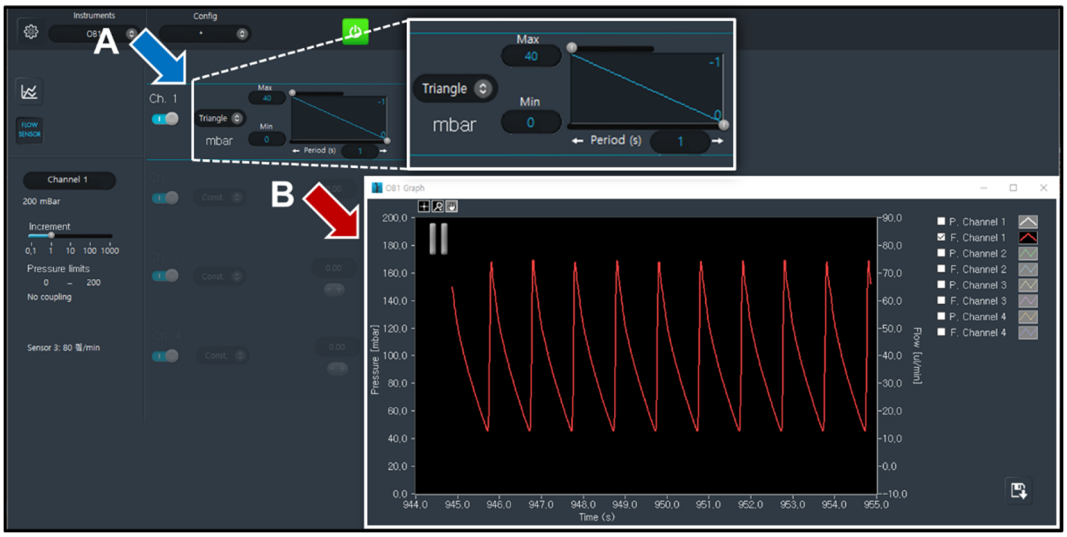


Figure 3. Elveflow Smart Interface software program (Elveflow, Paris, France)

(A) The blue arrow indicates the properties of generated flow profile: triangular shape; pressure range = 0-40 mbar; and pulse interval = 1 s.

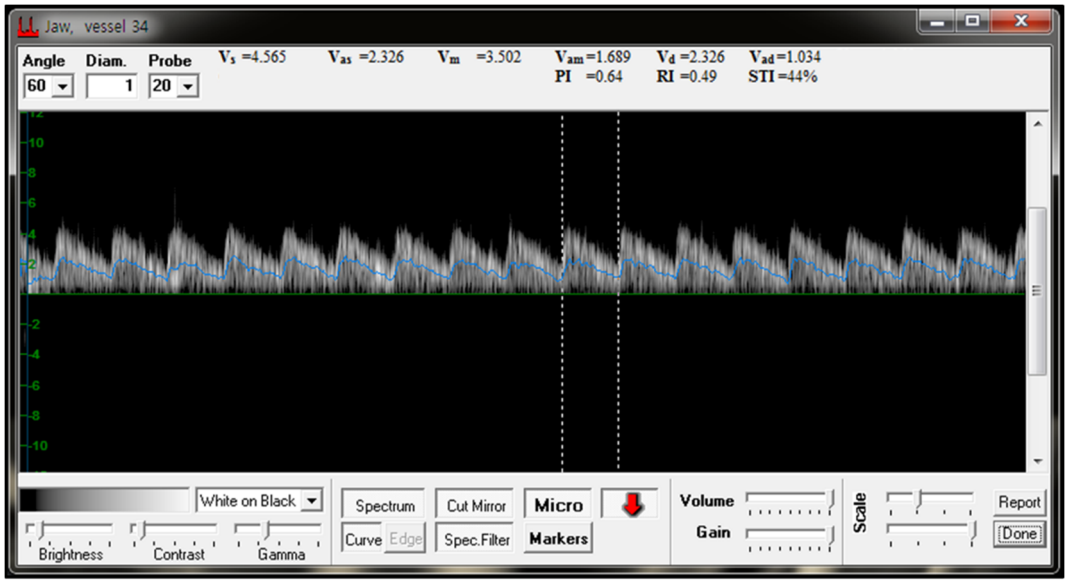
(B) The red arrow indicates the real-time flow rate graph detected by the microfluidic sensor: peak flow rate = 75 µL/min; minimal flow rate = 13 µL/min; and pulse interval = 1 s.

2. Doppler ultrasound device: measurement of flow velocity

A Doppler ultrasound device (MM-D-K; Minimax Ltd., St. Petersburg, Russia) was used for the measurement of fluid flow (Figure 4A). A 20 MHz-CW transducer with a 1.5 mm sensor diameter was connected to a computer (Figure 4B).

Figure 4. Doppler ultrasound device (MM-D-K; Minimax Ltd., St. Petersburg, Russia) **(A)** Main body; **(B)** 20 MHz-transducer

A small amount of coupling gel (Pro-gel II; Dayo Medical, Seoul, Korea) was applied over the glass side of the microfluidic chip prior to the measurement. The transducer was positioned at the center of the channel and at an angle of approximately 60º with respect to the direction of flow. In order to prevent motion artifacts and to obtain consistent measurements, the transducer was firmly fixed on the chip with silicone impression material (O-Bite; DMG, Hamburg, Germany). Once the measurement was carried out, Doppler spectra were recorded and flow indices were calculated by the Minimax Doppler 1.71 (Minimax Ltd., St. Petersburg, Russia) software program (Figure 5).



Vs, systolic peak velocity from the maximum velocity curve; Vas, systolic peak velocity from the average velocity curve; Vm, mean velocity from the maximum velocity curve; Vam, mean velocity from the average velocity curve; Vd, end diastolic velocity from the maximum velocity curve; Vad, end diastolic velocity from the average velocity curve; PI, pulsatility index; RI, resistivity index; and STI, systolic to diastolic ratio

Figure 5. Doppler spectrum and indices from the Minimax Doppler 1.71 software program (Minimax Ltd., St. Petersburg, Russia)

3. Data acquisition and statistical analysis

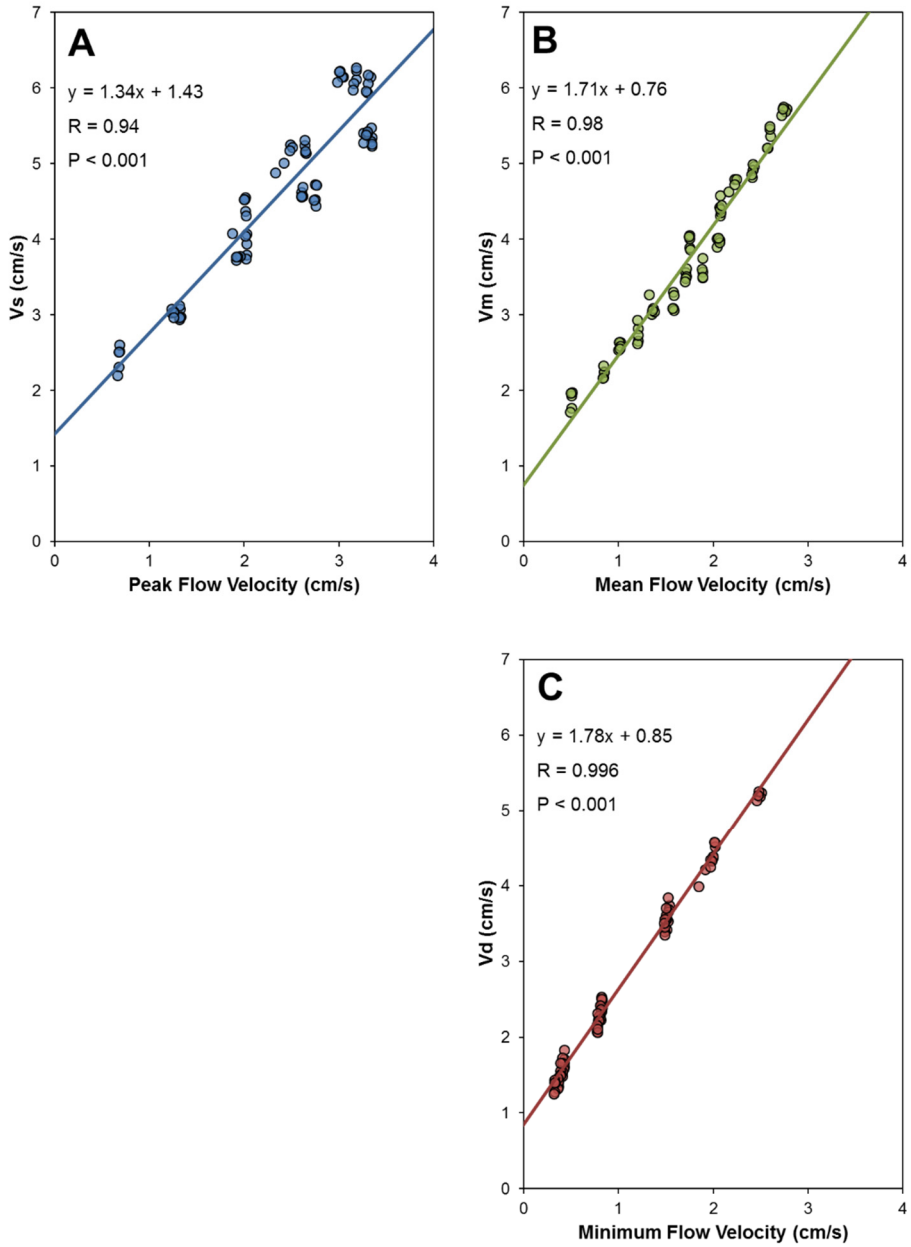
A triangular pulsatile profile was used for fluid flow, based on previous clinical studies demonstrating triangular-shaped Doppler spectra from the PBF in human teeth (Cho and Park 2015; Yoon et al. 2010; Yoon et al. 2012). Considering the resting heart rate in humans, the pulse frequency was set to 1 Hz. Fifteen sets of flow profiles, at different pressure ranges at an interval of 10 mbar, were generated from the microfluidic system. Five measurements for each profile, a total of 75 measurements, were made by the Doppler ultrasound device. Fluid flow datasets of 9 s duration were obtained simultaneously by the microfluidic sensor and the ultrasound Doppler device from each measurement. The linear flow velocity of the phantom was calculated by dividing the volume flow rate by the cross-section area of the channel (0.04 mm^2). The peak, mean, and minimal flow velocities from the flow phantom and the those measured by the Doppler ultrasound device were compared, using linear regression analysis and Pearson's correlation coefficient. Bland-Altman analyses were used to evaluate the differences in the velocities between the phantom and the Doppler ultrasound device. All statistical analyses were performed using the SPSS 23 (IBM Corp., Somers, NY, USA) and Excel 2013 (Microsoft Corp., Redmond, WA, USA) software program, and the level of significance was set at 0.05.

III. RESULTS

The characteristics of the 15 sets of flow profiles used in this study and the peak and minimal flow velocities of each profile are presented in Table 4. The flow profiles generated by the microfluidic system were consistent and reliable. There were excellent linear correlations between the peak, mean, and minimal flow velocities of the phantom and those of the Doppler ultrasound device ($r = 0.94$, 0.98, and 0.996, respectively, $p < 0.001$) (Figure 6). The Bland-Altman plots demonstrate the velocity differences between the phantom and the Doppler ultrasound device (Figure 7). It is observed that the Doppler ultrasound device overestimated the flow velocities by 1.69, 2.00, and 2.23 cm/s, with respect to the peak, mean, and minimal velocities. The differences increased with an increase in the velocities.

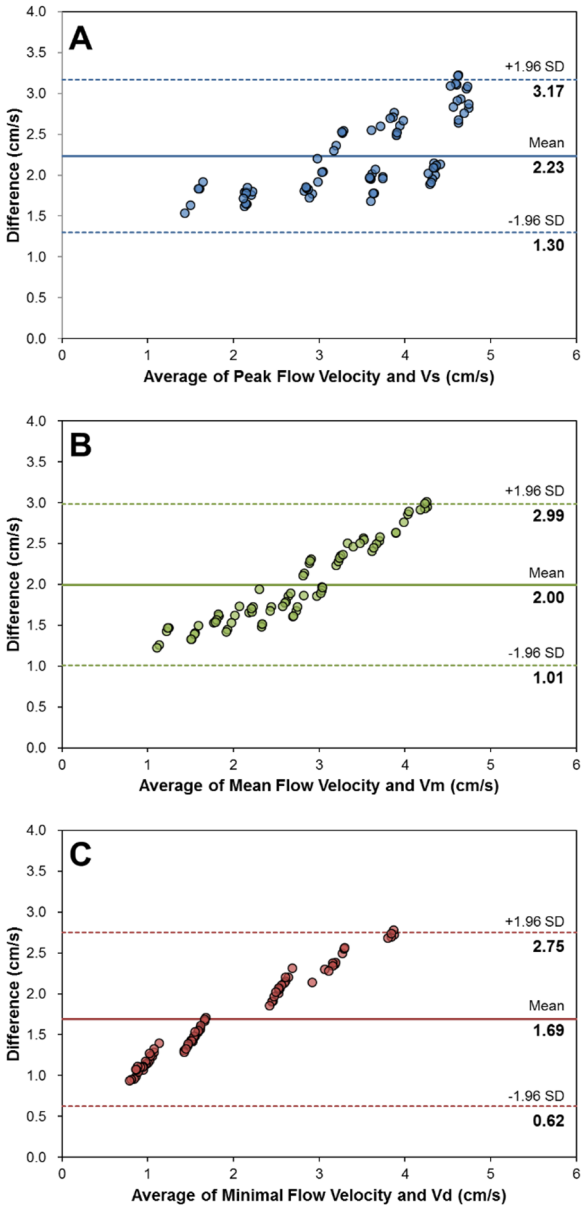
Table 4. Flow profiles and the peak and minimal flow velocities of the flow phantom

Pressure range (mbar)		**Peak flow velocity (cm/s)**		**Minimal flow velocity (cm/s)**	
Min	Max	Mean	SD	Mean	SD
0	50	3.343	0.013	0.425	0.003
0	40	2.748	0.014	0.405	0.003
0	30	2.019	0.008	0.384	0.002
0	20	1.305	0.096	0.359	0.006
0	10	0.675	0.009	0.322	0.005
10	50	3.281	0.022	0.820	0.007
10	40	2.608	0.011	0.810	0.005
10	30	1.916	0.029	0.790	0.009
10	20	1.243	0.012	0.775	0.005
20	50	3.303	0.018	1.526	0.008
20	40	2.642	0.021	1.503	0.004
20	30	2.008	0.009	1.486	0.005
30	50	3.168	0.019	2.008	0.010
30	40	2.442	0.065	1.934	0.052
40	50	3.012	0.025	2.479	0.020



Vs, systolic peak velocity; Vm, mean velocity; Vd, end diastolic velocity

Figure 6. Linear regression analysis for the flow velocities measured by the Doppler ultrasound device: **(A)** peak; **(B)** mean; and **(C)** minimal flow velocity



Vs, systolic peak velocity; Vm, mean velocity; Vd, end diastolic velocity

Figure 7. Bland-Altman plots comparing the measured velocities from the Doppler ultrasound device with the velocities of the flow phantom: **(A)** peak; **(B)** mean; and **(C)** minimal flow velocity

IV. DISCUSSION

1. Flow phantom for a tooth and pulp

Phantoms used in ultrasound studies must possess acoustic properties similar to the tissue of interest. This include critical acoustic properties such as the compressional speed of sound, acoustic impedance, attenuation, backscattering coefficient, and nonlinearity parameter (International Commission on Radiation Units Measurements 1998). In the present study, to mimic a tooth, a microfluidic chip was fabricated with PDMS and a glass slide. In relation to the acoustic properties of TMMs for dental hard tissues (Table 1), the 1.2 mm-thickness glass slide could properly emulate the enamel layer of a tooth. A suitable material for dentin substitute should be considered in further investigations for appropriately mimicking human teeth. The width and depth of the channel on the microfluidic chip was 200 μm, which is larger than the arterioles found in the pulp of human teeth (Cheng and Provenza 1959; Provenza 1958). In addition, as the shape of the channel was rectangular, the fluid flow in the phantom could be different from the blood flow *in vivo*. Microfluidic chips containing channels, whose size and shape are more comparable to the real blood vessels, should be fabricated.

BMF is used to mimic blood, both acoustically and rheologically. Human blood itself has been used in previous studies (Shevkoplyas et al. 2003; Weskott 1997), however, the use of blood is limited due to its short lifespan and change in acoustic properties at room temperature (Oates 1991). A number of different recipes of BMF as well as their

modifications have been reported (Boote and Zagzebski 1988; Hoskins et al. 1990; Ramnarine et al. 1998; Rickey et al. 1995), and used for flow phantoms (Hoskins 2008; Kenwright et al. 2015; Meagher et al. 2007). In this study, the BMF containing nylon particles, which was developed as part of an European Commission project and found to have most similar characteristics to real human blood (Ramnarine et al. 1998), was used. Yoon et al. (2010) reported that the PBF velocity of vital teeth was 0.3-0.8 cm/s, and Cho and Park (2015) reported a mean PBF velocity of 0.56 cm/s in maxillary anterior teeth. Considering the high deviation of the measured values, the flow profiles simulated in this study were chosen to cover a wider velocity range (0.3 to 3.3 cm/s).

Within the limitations of this study, the microfluidic system was able to generate the flow profiles as intended, and the fluid flow could be easily monitored and controlled. With the support of microfabrication techniques, it was possible to fabricate a micrometer-sized channel. It is hoped that this study can offer various possibilities in future researches and educations involving Doppler ultrasound technique in dentistry.

2. Doppler ultrasound device for measurement of PBF velocity

The MM-D-K is the only Doppler ultrasound device that is currently available for dental use. It uses a high-frequency CW transducer to assess the dynamics of the blood flow in microvessels. In this study, we used the 20 MHz-CW transducer, which is recommended for detecting vessels at 0.1-8 mm depth, according to the manufacturer's

instructions. It has a sensor with a small diameter (1.5 mm) so that it is possible to position it in a tight area such as the tooth surface.

It was suggested that high-frequency Doppler ultrasound has the potential to play an important role in examining the PBF of teeth in both clinical and research settings (Berson et al. 1999). The spatial resolution of an ultrasound device increases proportionally with the center frequency of the system. Therefore, by using high-frequency transducers, dental structures should be resolved in higher detail.

CW transducers use continuous transmission and reception of ultrasound waves (Herwig and Schäberle 2010). Doppler signals are obtained from all vessels in the path of the ultrasound beam until it becomes sufficiently attenuated. Although CW Doppler ultrasound is a convenient technique, it is unable to determine the specific location of velocities and separate Doppler signals arising from different vessels along the transmitted ultrasound path. Further development and research of various types of transducers is necessary. Additionally, a technique of estimating the volume of moving blood in the pulp should be applied for more relevant information regarding the pulp physiology.

By using the flow phantom, we observed that the MM-D-K is able to detect and measure a blood flow velocity of about 0.3 to 3.3 cm/s in the 200×200 μm-sized channel. The peak, mean, and minimal velocities measured using the MM-D-K demonstrated excellent correlations with those of the flow phantom. However, the overall results show that the velocities are overestimated by the MM-D-K compared to those of the flow

phantom. This is in agreement with previous phantom studies (Camfferman et al. 2014; Schulten-Wijman et al. 2008; Teirlinck et al. 1998). Although Doppler ultrasound can be an effective diagnostic tool for the quantitative measurement of PBF velocity, the validation and calibration of the system, prior to clinical use, is essential.

3. Sources of error in Doppler ultrasound

There could be several factors that contribute to the overestimation of velocity. First, errors can occur during the raw data processing by the Doppler ultrasound device (Carol et al. 2011; Pozniak and Allan 2013). Most Doppler ultrasound devices consider the mean propagation speed in tissue to be 1540 cm/s and use this value to calculate velocity parameters. The ultrasound velocity in the glass is about 6000 cm/s (Singh et al. 2008), therefore the calculated velocity could be overestimated. All types of Doppler ultrasound devices employ filters to cut out the high amplitude, low-frequency signals resulting from tissue movement, such as vessel wall motion. Depending on this filter frequency, the measured flow velocities can be altered.

The Doppler angle could affect the estimated velocity. Steinman et al. (2001) investigated the sources of error in the estimation of maximum velocity and found that the Doppler angle was one of the chief sources of error. Even a little difference in the angle can make a significant change in the estimated velocity, especially at large angles. To minimize errors, the Doppler angle should be kept as small as possible. In the present study, the angle

was fixed at approximately 60º, which is considered as the maximal angle in clinical practice. Though we tried to minimize the effects of the Doppler angle, it is likely that it could cause some errors.

Lastly, the difference in acoustic impedance between the phantom and the coupling material can cause errors. Due to the mismatch of acoustic impedance between air and tissue, a coupling material is used to transfer ultrasound energy into the tissue. In clinical practice, the most common couplant is water-based gel, however, it is demonstrated that water is a poor couplant for teeth (Kossoff and Sharpe 1966). The difference in the acoustic impedance between water and dental hard tissue is so high that it leads to a reduction in the ultrasound amplitude when entering the enamel layer. Further investigations are necessary to find an appropriate coupling material for a tooth.

4. Clinical considerations and future perspectives

Doppler ultrasound offers obvious advantages for the estimation of PBF, as it directly measures the frequency changes in ultrasound reflected from RBCs. The velocity can be measured in standard units such as cm/s. However, there are still unsolved issues that prevent the clinical application for human teeth. The layers of enamel and dentin have natural variations among individuals, tooth types, and sites on particular teeth. The scatter, reflection, and attenuation of ultrasound will be different for each tooth. As the outer surface of a human tooth is uneven, it remains unclear as to how consistent application

angles can be obtained. Since the Doppler angle is unlikely to be known, the velocity may not be properly calibrated. Further research is required for the improvement in the clinical performance of Doppler ultrasound devices.

This study demonstrates one aspect of the collaboration between microfluidics and dental research. Considering that microfluidics is actively used in various research fields, it could certainly contribute to the advancement of dental research.

V. CONCLUSION

We developed a tooth-pulp flow phantom using a microfluidic system and BMF. With the soft lithography technique, it was possible to fabricate a micrometer-sized channel. The microfluidic system was able to generate the flow profiles as intended, and we could easily monitor and control them by the software program. It is considered that this phantom can offer various possibilities in future researches and educations involving the Doppler ultrasound technique.

By using the phantom, we evaluated the accuracy of a Doppler ultrasound device in making velocity estimations. Within the limitations of this study, the peak, mean, and minimal velocities measured using the Doppler ultrasound device demonstrated excellent linear correlations with those of the flow phantom. However, the overall results show that the velocities are overestimated by the Doppler ultrasound device. Although Doppler ultrasound can be an effective diagnostic tool for the quantitative measurement of PBF, validation and calibration of the device is needed prior to a clinical use.

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Abstract (Korean)

도플러 초음파 기기의 유효성 평가를 위한 미세유체공학 기반 치아-치수 혈류 팬텀의 개발

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I. 서론

도플러 초음파는 의학 영역에서 다양한 혈관의 혈류를 측정하기 위해 사용되고 있다. 최근에 도플러 초음파를 이용하여 치수의 혈류 속도를 측정한 연구 결과들이 보고되고 있으나, 치수 혈류 측정에 있어 도플러 초음파 기기의 사용에 대한 신뢰도는 아직 충분히 검증되지 않았다. 도플러 초음파 기기의 혈류 속도 측정을 평가하기 위해 “flow phantom” 이라는 모형이 사용되고 있다. 하지만 대부분의 팬텀은 연조직 내부를 지나가는 상대적으로 큰 직경의 혈관을 모사하고 있기 때문에, 치아 경조직과 치수를 재현하기에는 부적절하다. 미세유체공학은 마이크로 또는 나도 단위의 유체를 다루는 학문으로, 다양한 연구 분야에 응용되고

있다. 최근 미세유체공학을 이용하여 조직-혈관 네트워크를 재현하기 위한 시도들이 이루어지고 있다. 하지만, 도플러 초음파 기기의 평가 또는 치아 경조직 및 치수의 재현에 사용된 경우는 아직 보고된 바가 없다.

본 연구에서는, 미세유체 시스템을 이용하여 도플러 초음파 기기에 적용가능한 치아-치수 팬텀을 개발하고, 팬텀을 사용하여 도플러 초음파 기기의 혈류 속도 측정 능력을 평가하고자 하였다.

II. 재료 및 방법

미세유체 시스템을 사용하여 다양한 속도의 박동성 유체 흐름을 만들고, 이를 통해 혈류 모사 용액을 미세유체 칩 내부의 200×200 μm 크기의 직선형 유로 내부로 흘려보냈다. 20 MHz-continuous wave transducer 가 장착된 도플러 초음파 기기를 사용하여 용액의 유속을 측정하였다. 실제 팬텀에서 흐르는 용액의 최고, 평균, 최저 속도와 도플러 초음파 기기에서 측정된 최고, 평균, 최저 속도를 선형 회귀분석 및 Pearson 상관계수를 통해 분석하였으며, Bland-Altman analysis 를 통해 실제 속도와 측정된 속도 간의 차이를 평가하였다.

III. 결과

미세유체 시스템을 사용하여 원하는 형태와 속도의 유체 흐름을 생성할 수 있었으며, 컴퓨터 소프트웨어를 통해 실시간 모니터링 및 조절이 가능하였다. Soft lithography 기술을 사용하여, 마이크로미터 크기의 유로 제작이 가능하였다.

도플러 초음파 기기에서 측정된 최고, 평균, 최저 속도는 실제 팬텀 용액의 속도와 높은 상관관계를 나타내었다 (각각 $r = 0.94, 0.98, 0.996$, $p < 0.001$). 하지만, 도플러 초음파 기기에서 측정된 속도가 실제 팬텀에서 흘려보낸 속도와 비교하여 과측정되는 경향을 보였다 (최고, 평균, 최저 속도에서 각각 2.23, 2.00, 1.69 cm/s 과측정).

IV. 결론

본 연구를 통해 제작된 팬텀은 도플러 초음파를 활용한 연구 및 치수 혈류에 관한 연구에 도움을 줄 수 있을 것으로 기대된다. 도플러 초음파는 치수 혈류 속도를 정량적으로 측정할 수 있는 좋은 진단 도구가 될 수 있지만, 임상에 적용하기 위해서는 기기의 정확도와 유효성에 대한 평가 및 검정이 선행되어야 할 것이다.

핵심되는 말 : 치수, 혈류 속도, 도플러 초음파, 팬텀, 미세유체공학, 미세유체칩