Forensic Science International 275 (2017) 308–313

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Technical Note

The application of silicon sol–gel technology to forensic blood substitute development: Investigation of the spreading dynamics onto a paper surface

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ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 23 March 2017
Accepted 24 March 2017
Available online 1 April 2017

Keywords:
Bloodstain pattern analysis
Passive drip stain
Spreading
Forensic blood substitutes
Silicon sol–gels
Thin films

ABSTRACT

This work investigates the spreading dynamics of three candidate sol–gel solutions, of ranging viscosities, surface tensions and densities, and compares them with water and two commercial blood substitute products. Droplets were created with different sizes (10 to75 μL) and impact velocities (1.4 to 6.0 m/s) to strike 176 gsm cardstock. Over 2200 droplets were created using the six different fluids and their final dried stain diameter was measured. Droplet spread was plotted using the Scheller and Bousfield correlation and uses effective viscosity as a parameter for non-Newtonian fluids. Comparing the results to an expected whole human blood range validated the spread of the candidate FBS sol–gel material in passive drip bloodstain pattern simulation. These findings complement the practical application of the material as a safe substitute for demonstrating droplet spread under controlled conditions on hard paper surfaces.

1. Introduction

The Organization of Scientific Areas Committee's (OSAC) bloodstain pattern analysis (BPA) subcommittee has recently reported a call to determine the differences between fresh, whole human blood and various blood substitutes for use in research, training and case experimentation [1]. The subcommittee suggests that further research can help with understanding the relationship between the physical characteristics of blood and other fluids and the mechanisms that cause pattern formation [1]. This work complements this idea by exploring the contributions of surface tension, density and viscosity to droplet spread and passive drip stain formation of various forensic blood substitutes (FBS).

Previously, our sol–gel formulation has demonstrated validity in passive dripping simulation [2]. We can further evaluate the utility of our materials by investigating the fluid dynamics of spread. We extend our previous data set [2] by creating more droplets of various size and impact velocities. This helps to provide a more comprehensive understanding of droplet spread in practical crime scene reconstruction. We can then compare the results of the sol–gel materials to the expected whole human blood range in passive drip bloodstain pattern simulation. Validation of a forensic blood substitute is an important component of its implementation into research and training exercises. This is particularly true with commonly encountered [3], well researched [4] and extensively taught [5] bloodstains patterns associated with bloodletting events.

Droplet impact and spreading dynamics have been heavily explored in the literature [6]. Traditionally, spread has been modeled in bloodstain pattern analysis, and other areas of fluid dynamics, using either the droplet's Weber number and/or Reynolds number [4,7]. A recent study suggests that the accuracy of the model can be enhanced by blending terms from both the capillary and viscous regimes of the fluid [8]. Our work supports this idea, and we use the Scheller and Bousefield relation [9] to linearize the spreading ratios of six different fluids. This work extends previous work that uses the correlation as a platform to test candidate FBS materials [10] as it can be used to express both Newtonian and non-Newtonian spreading by using a fluid's high shear, or effective viscosity [11]. In this paper we investigate the spread of six fluids, water, two commercial blood substitutes, and

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http://dx.doi.org/10.1016/j.forsciint.2017.03.020
0379-0738/

three sol–gel materials used to develop what we describe as our candidate FBS. This candidate FBS has been tested against whole blood and validated using another passive dripping test [10]. The focus of this work is to demonstrate the spreading dynamics of the validated material in comparison to those observed for other materials.

2. Materials and methods

2.1. Test liquids

Six different fluids were characterized in this experiment: water, three sol–gel materials and two commercial products. The sol–gels were created using an established protocol [2]. These included the base formulation (GT), the base formulation that undergoes a process for alcohol removal (LAGT), and the LAGT gel with fillers, which is our candidate FBS. The GT and LAGT formulations had <1% weight by volume (w/v) food coloring, to enhance visualization of the dried stains. Millipore water was used with <1% w/v food coloring. Two commercial products were also tested; one intended for use by forensic practitioners [12] and the other for use in educational settings [13].

2.2. Physical property measurements

The surface tension of each fluid was measured using the Pendant_Drop plugin for ImageJ [14]. A 1 cc glass syringe was cleaned with water and dried with acetone prior to droplet formation. Scaled images of the fully developed droplets were captured using a Nikon D3400 under ambient conditions prior to droplet release. Fifty measurements were obtained per fluid and averaged.

Density was determined by measuring the mass of known volumes of each material drawn in a 1 cc glass syringe with an analytical balance. Ten measurements were obtained per fluid and averaged.

The viscosity of each fluid was measured in triplicate at 25 °C ± 0.05 °C between shear rates 1 to 12,000 s^{-1} using a TA AR 2000 rheometer. Viscosity was measured in ambient conditions as each of the materials are considered stable and ready-to-use at room temperature. Measurements were taken and averaged over 60 s for each of the shear rates analyzed. The high shear viscosity was determined by averaging the fluid's viscosity at shear rates greater than 100 s^{-1}.

Statistical test measures were applied to the density, surface tension and viscosity measurements obtained for each of the six tested fluids. It is important to note that the whole human blood values were not included in the testing, as the values were obtained from the literature using different sources, where means of the physical properties are reported rather than the raw data, where variance in sample measurements are used and required for statistical testing. A one-way ANOVA test was used to determine whether there were any statistically significant differences between the means of the different fluids' densities, surface tensions and high shear viscosities. A Tukey honestly significant different test was then used for post hoc testing for pairwise analysis of each of the physical properties of the fluids. Statistically significant differences were also confirmed using a pairwise test with a Bonferroni correction factor for multiple testing.

2.3. Stain deposition

Droplet volume was controlled using different syringe tips with various diameters. Single droplets were formed at specific dripping heights between 20–120 cm and allowed to fall perpendicular to the target paper. Droplet volumes ranged from 10 to 75 μL and impact velocities 1.4 to 6.0 ms^{-1}. White cardstock (65 lb/176 gsm) was the target surface for stain deposition. Each piece had a circle printed on it as an area that stains could be dropped within to avoid any interference by the paper's edges. 10 stains per dripping height were created and allowed to dry prior to measurement. The diameter of each stain was measured from scaled images of the stains using ImageJ. Over 2200 stains were created and measured. The droplet's spread, D_S/D_0, was determined as the quotient between the measured stain diameter, D_S, and original droplet volume D_0.

3. Results and discussion

3.1. Physical property measurements of test fluids

Table 1 reports the average measured properties of the fluids, and includes an estimated whole human blood range that is taken from a survey of the scientific literature on the scientific literature. Combined, they form a wide range of testable properties to model droplet spread, and therefore assess the capabilities of the fluids to act as forensic blood substitutes in dripping simulation. Interestingly, the commercial substitutes have significantly different high shear viscosities and surface tensions compared to whole human blood, and are in fact, more similar to water. The LAGT and FBS have similar properties to whole human blood, with a slightly lower surface tension and higher density than required.

The one-way ANOVA testing determined that statistically significant differences exist between the means of each of the tested fluid's densities, surface tensions and high shear viscosities ($p < 2^{-16}$ for each test). Using the post-hoc testing, only three similarities in means were determined and were: the surface tension between the LAGT and FBS ($p = 0.250$), the density between water and Commercial 1 ($p = 0.951$) and the high shear viscosities between water and Commercial 1 ($p = 0.685$). These results support the idea that the sol–gels have physical properties that are much different than water and the other commercial blood substitutes, as well as demonstrate how the physical properties of Commercial 1 are similar to water. The rest of the pairwise comparisons were determined to have statistically significant differences between

Table 1
Surface tension, density and high shear viscosity of the test fluids at ambient temperatures (22 to 25 °C) compared to whole blood at physiological temperature (37 °C).

Fluid	Surface tension × 10^{-3} (Nm^{-1})	Density ($kg\,m^{-3}$)	High shear viscosity (>100 s^{-1}) × 10^{-3} (Pa.s)
Water	71.4 ± 2.8	1028.7 ± 0.0044	1.04 ± 0.09
Commercial 1	57.1 ± 5.9	1126.1 ± 0.0065	1.26 ± 0.14
Commercial 2	65.9 ± 4.1	1026.5 ± 0.0044	2.15 ± 0.11
GT	36.6 ± 1.4	1083.0 ± 0.0071	6.46 ± 0.97
LAGT	48.0 ± 2.0	1170.3 ± 0.0070	4.59 ± 0.35
FBS	49.5 ± 2.3	1149.3 ± 0.0023	4.99 ± 0.54
Whole human blood	54 ± 4.2 [15,16]	1053 ± 0.0113 [16–21]	4.63 ± 1.94 [22–25]

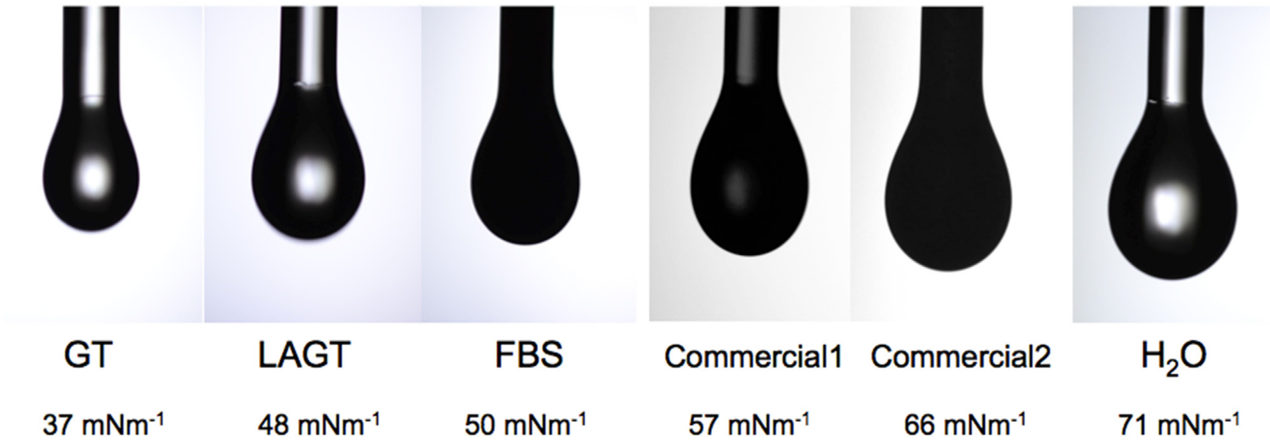

Fig. 1. Droplets that have formed prior to release from the tip of a 1 cc glass syringe for surface tension measurement. The average surface tension values for each of the fluids are given. The differences in opacity of the FBS and commercial products from the GT, LAGT and water are a result of the solid content of each fluid.

the means of the pairs ($p \leq 3.1^{-10}$). The results of this find primarily suggest that each of these fluids must be plotted independently when mapping droplet spread. It also supports the idea of using descriptors for fluids being more "water-like" and "blood-like".

Fig. 1 shows an example of a fully developed droplet of each fluid using the same 1 cc glass syringe. There is a visual difference in droplet size and contour when they are fully developed at the syringe tip. Since droplet size influences stain size, it is important, then that consistency in droplet size is maintained when testing candidate materials in passive dripping simulation.

The alcohol removal step when converting GT to LAGT is key to increasing the surface tension of the base sol–gel material, while not significantly affecting its viscosity and density. The increase in density from LAGT to FBS is a result of filler addition.

3.2. Dried stain characteristics

Fig. 2 highlights the observed difference in the average spreading ratio of the dried stains of each fluid. This figures shows the average spreading ratio of the stains created from droplets of the same approximate size (36.9 μL ± 2.0 μL). Plotting the spreading ratio, D_s/D_0 accounts for minor volume differences between the fluids As expected, the water stains are much larger than the commercial and sol–gel products, GT, LAGT and FBS.

3.3. Droplet spread

Using the Weber and Reynolds numbers individually to plot final stain diameter is not an effective way to understand spreading in this case. Fig. 3 shows that most of the data falls below the theoretically and experimentally depicted capillary and viscous regimes commonly used to model spread [7,26–28]. This has also been reported in a previous study of forensic relevance [8].

If anything, these models are best suited for water, rather than fluids with a higher viscosity, like the commercial and candidate fluids. It is fitting, then, that the fluid spread is more appropriately modeled using a combination of dimensionless numbers.

The Scheller and Bousefield Correlation [9] using the An & Lee's concept of effective viscosity [11] shown in Fig. 4 is one example of a method that linearizes the data ($R^2 = 0.71$) and shows a clear

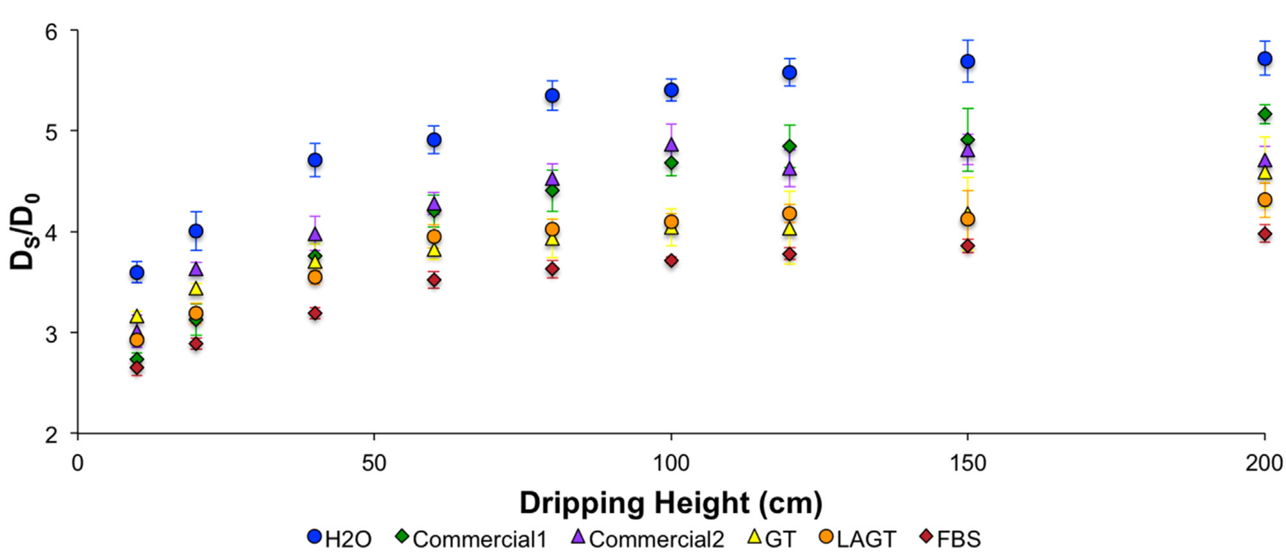

Fig. 2. Average droplet spread as a function of dripping height for water (blue circles), Commercial1 (green diamonds), Commercial2 (purple triangles), GT (yellow triangles), LAGT (orange circles), and FBS (red diamonds). Error bars represent standard deviation of n = 10 samples per dripping height. The average droplet volume for the fluids is 36.9 ± 2.0 μL. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

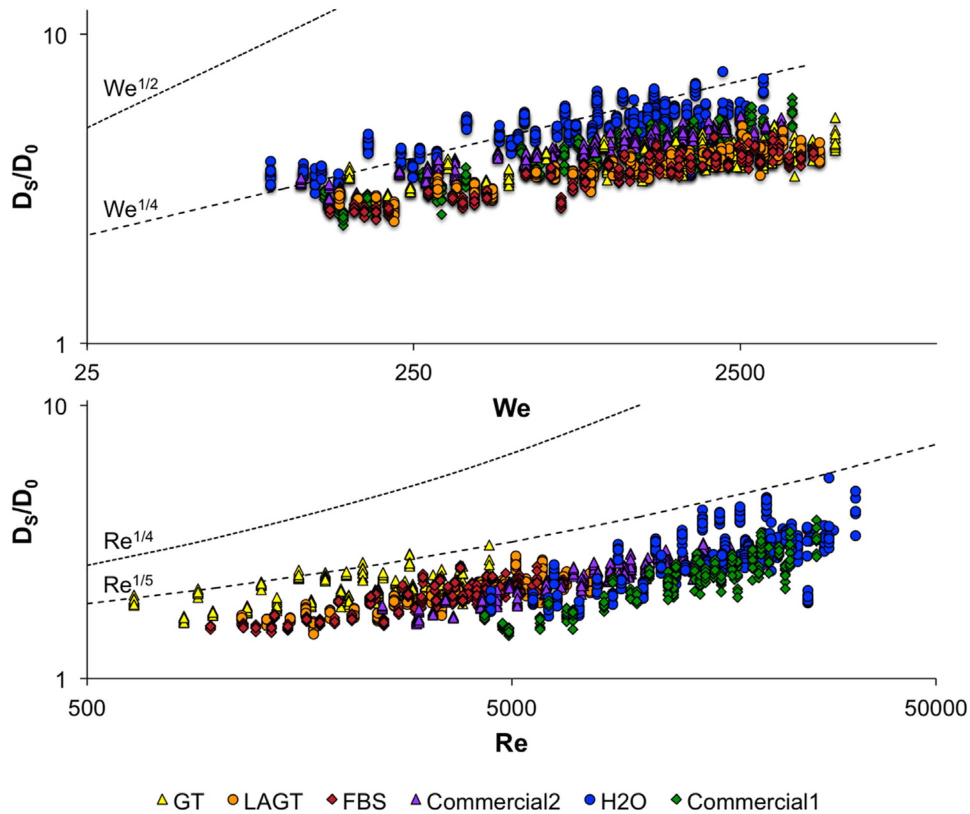

Fig. 3. Spread of the fluids as a function of their droplet's Weber, We, (above) and Reynolds, Re, (below) numbers. The tested fluids are water (blue circles), Commercial1 (green diamonds), Commercial2 (purple triangles), GT (yellow triangles), LAGT (orange circles), and FBS (red diamonds). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

difference in scalability of passive drip stains created by all of the fluids. The correlation uses a combination of a fluid droplet's density, surface tension, viscosity, volume and impact velocity to map its spread. The scaling factor (slope of the line of best fit), 0.55, is different than the 0.61 reported [11]; however, this is most likely a result of surface constraint as stains were created on two slightly different hard, porous surfaces [10]. Paper surfaces have complex interactions at the fluid-substrate interface, where absorbency can conceivably limit spread.

Scalability is highlighted by the overlap in stain data between fluids. For example, a small water droplet can be made to look like a larger stain created from larger droplets made from sol–gel and vice versa. This speaks to the versatility in creating a wide variety of formulations for FBS; however, practically this is ill-suited for

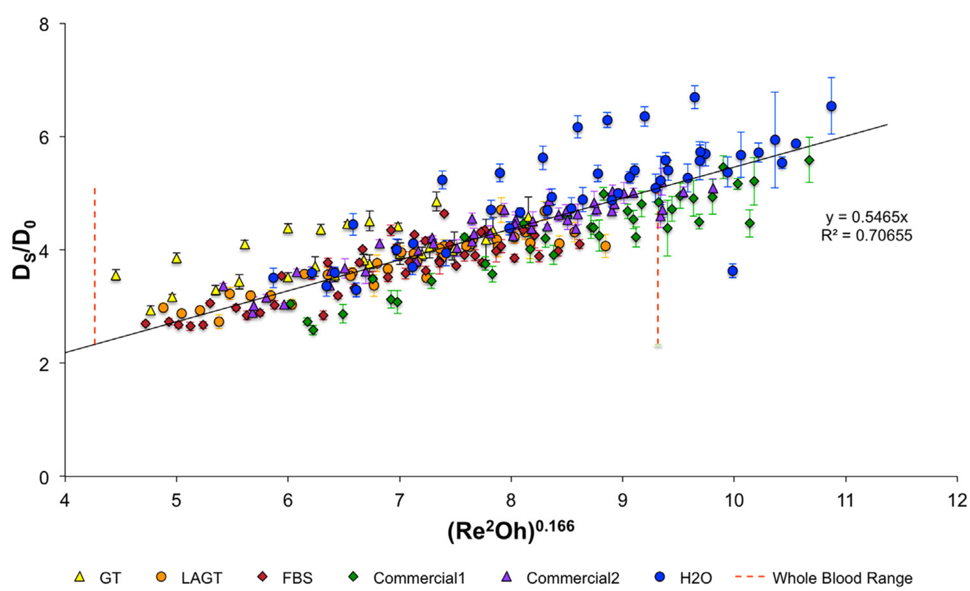

Fig. 4. Droplet spread of the fluids as a function of Scheller and Bousefield correlation. The tested fluids are water (blue circles), Commercial1 (green diamonds), Commercial2 (purple triangles), GT (yellow triangles), LAGT (orange circles), and FBS (red diamonds). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

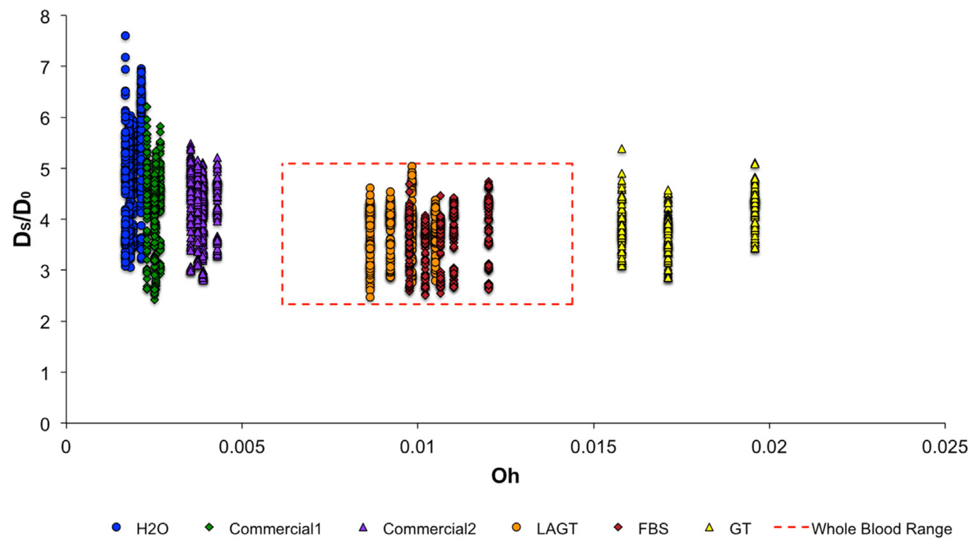

Fig. 5. Spread of the fluids as a function of their droplet's Ohnesorge number, Oh. The tested fluids are water (blue circles), Commercial1 (green diamonds), Commercial2 (purple triangles), GT (yellow triangles), LAGT (orange circles), and FBS (red diamonds). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

implementation into forensic training and reconstruction as discussion of the scaling factors would require further and unnecessary follow-up/instruction.

There is a clear difference between the LAGT and FBS solutions compared to the other fluids. In fact, the commercial products share similar spreading characteristics to water. This is primarily due to the products' low viscosity. When applying the range of experimental conditions (10 to 75 μL droplet volumes and impact velocities 1.4 to 6.0 ms^{-1}) to the reported whole human blood range, a theoretical range of spread is determined. This range, shown in Fig. 4, was determined using an accommodating approach, one that exploits the entire range of physical property values reported in the literature. Again, some stains made by water and the commercial standards fall outside of this range, while the sol–gel solutions, particularly the LAGT and FBS, do not. This is best demonstrated by Fig. 5, which uses the fluids' Ohnesorge numbers in relation to droplet spread. The vertical spread in data at a specific Ohnesorge number is dictated by the droplet's impact velocity. Again, this plot demonstrates how the LAGT and FBS solutions spread in a manner similar to whole human blood range. Caution is recommended when using and applying the commercial fluids in dripping experiments under the tested conditions.

The obvious extension of this work is to investigate splashing; particularly in spine and scallop formation as well as the generation of satellite stains from the parent stain.

3.4. Importance for validation prior to implementation in training

Establishing validation protocols are important for implementing an artificial fluid into bloodstain training and research programs [29]. Using fluids that can accurately represent the mechanisms of bloodshed is important for training, particularly where whole blood is not used. This holds true for both quantitative and qualitative stain and pattern characteristics. It is important to consider how well a fluid mimics a well-researched bloodstain pattern type. This is because there is little user-benefit in using a material with physical properties that are significantly different than whole blood as it could potentially provide a false visualization of bloodstain pattern formation. This is particularly important in training, as providing an accurate representation of a scientific concept is important for a learner to understand the theoretical and experimental core principles of pattern formation [30]. This is why investigating the fundamental dynamics of candidate FBS materials during pattern simulation is so important for implementation into the industry.

4. Conclusion

This work extends the validation of the silicon sol–gel material as a candidate FBS for simulated passive dripping under controlled conditions on a paper substrate. Passive drip stains of six different fluids were created and their spread analyzed. Spread was modeled using the Scheller and Bousfield correlation, with an applied effective high-shear viscosity for non-Newtonian fluids. A theoretical range for whole human blood was determined and applied to the data. This was used to show that droplet spread from the LAGT and FBS solutions were more similar to blood, and the two commercial products more similar to water. This work emphasizes the need to characterize and thoroughly test FBS materials for use in crime scene research and training.

Conflict of interest

The authors declare no conflict of interest from the work of this paper.

Acknowledgements

We would like to thank Dr. Suresh Narine of the Trent University Center for Biomaterials Research for access to equipment as well as Dr. Laziz Bouzidi and Peter Andreas for their technical advice with the rheometer. The author TS was supported by the Vanier Scholarship from the Natural Sciences and Engineering Research Council of Canada.

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