



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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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 to 75 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.

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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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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 $25\text{ }^{\circ}\text{C} \pm 0.05\text{ }^{\circ}\text{C}$ between shear rates 1 to $12,000\text{ s}^{-1}$ 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 $75\text{ }\mu\text{L}$ and impact velocities 1.4 to 6.0 ms^{-1} . White cardstock ($65\text{ lb}/176\text{ gsm}$) 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 $25\text{ }^{\circ}\text{C}$) compared to whole blood at physiological temperature ($37\text{ }^{\circ}\text{C}$).

| Fluid | Surface tension $\times 10^{-3}$ (Nm^{-1}) | Density (kg m^{-3}) | High shear viscosity ($>100\text{ s}^{-1}$) $\times 10^{-3}$ (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] |

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