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FINAL SUMMARY

PROJECT TITLE: “Transfer of Bloodstains from Textile Surfaces: A Fundamental Analysis”

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PURPOSE

The purpose of this study is to provide a fundamental understanding of the complex interactions that lead to transfer of a bloodstain from one textile material to another.

To achieve this goal, work was undertaken to achieve the following objectives:

OBJECTIVES:

To develop an in-depth understanding of the development of transfer bloodstain patterns on fabric surfaces incorporating:

a) textile engineering variables in common fabric structures (plain woven and knit),

b) time-pressure profiles leading to blood transfer between fabrics.

To achieve these tasks, a secondary objective was to create a new synthetic blood for forensic science that closely mimics human blood’s properties including surface tension, non-Newtonian viscosity, and red blood cells and that creates stains on textiles which faithfully reproduce real bloodstains.

PROJECT DESIGN AND METHODS

In this project, there are two main tasks: (1) to measure the transfer of blood from a bloodstained fabric to one that is not stained and to determine the underlying physics and chemistry involved; and (2) to develop a new artificial blood that contains particles of a similar size and shape as red blood cells, at a similar concentration (hematocrit), with the same viscosity and surface tension as porcine or human blood. To achieve these goals, we first need to determine how real blood interacts with fabrics and to determine the critical features of blood that results in the stains observed.
In the summary below, transfer of single 30µL drops of porcine blood from one simple fabric to another was studied as a function of the time between the application of the drop and the time that a second fabric contacted the stain on the first fabric and for different contact pressures and duration. In addition, the results of our attempts to create a new artificial blood substitute containing particles similar to red blood cells are reported.

MATERIALS
Cotton woven and knit fabrics were obtained from TestFabrics, and laundered according to the standard laboratory practice for home laundering fabrics by AATCC Monograph M7. Anticoagulated porcine blood was obtained from Lee Biosolutions and stored at 4°C. Prior to use, it was warmed to ambient temperature while being gently rolled on a jar roller. For synthesizing the artificial blood substitute, styrene (St, ReagentPlus®, ≥99%), di(ethylene glycol)diacrylate (OEDA, 75%), polyvinylpyrrolidone (PVP, M=40,000) and 2,2'-azobis(2-methyl-propionitrile) (AIBN, 98%) were purchased from Sigma Aldrich; ethyl alcohol (190 proof) was purchased from EMD Millipore Corporation; n-heptane (99%) was purchased from Fisher Chemical; Millipore deionized water is available within the laboratory.

METHODS
TRANSFER
Before beginning the experiments, the maximum force applied during "innocent" transfer was estimated. For this study "innocent" transfer was defined as the pressure required to lift a 55kg person using only the forearms with the intention of rendering assistance. Based on the shape of the forearms, this resulted in an estimate of 10kPa for the maximum. Due to equipment limitations, experiments were limited to 6kPa.
To perform transfer studies, one laundered fabric was placed flat on a glass plate. A 30µL drop of porcine blood was placed directly on the fabric and was allowed to soak into the fabric for a period referred to as the “wait time”. Then a second, identical fabric was placed onto the first, bloodied fabric and a weight was immediately placed on top and left for a time referred to as the “transfer time”. The weight and fabric were then removed and both fabrics photographed to determine the amount of transfer. Each experiment was repeated at least three times. ImageJ was used to determine the stain areas and both sides of both fabrics were examined to observe differences.

ARTIFICIAL BLOOD SUBSTITUTE

After several initial trials, a “particle aggregation polymerization” was carried out in a mixture of ethanol and heptane. 10g styrene, 0.2g OEDA, 0.3g AIBN and 1.0g PVP were dissolved into 45/15mL ethanol/n-heptane medium in a 250mL 3-neck round bottom flask under nitrogen atmosphere and stirred at constant rate (150 RPM) using magnetic stirrer. The flask was submerged into water bath with controlled temperature of 52°C. Reaction was continued for 3 hours to form St-OEDA seeds. After 3 hours, the rest of 45/15mL ethanol/n-heptane medium dissolved with the rest of chemicals (10g styrene, 0.2g OEDA, and 0.3g AIBN) were filled into syringes. A syringe pump was used to slowly add chemicals into flask at a constant rate during a period of 5 hours, after which, the reaction was continued for a total of 12 hours. The mixture was then centrifuged and the solvent removed, deionized water was added and the particles redispersed at a concentration of 44 volume % to simulate red blood cell concentrations. Finally, Acrysol 8306 was added to adjust the viscosity to match that of porcine blood.
Both porcine blood and the artificial blood substitute were dripped onto cardboard from 20, 60 and 100cm with impact angles of 10°, 30°, 60° and 90°. The stains were allowed to dry for 24 hrs and photographed for analysis.

**DATA ANALYSIS**

**TRANSFER**

All transfer stains were photographed along with a ruler. The image was brought into ImageJ and calibrated using the ruler image. Next, the images were converted to black-and-white using the threshold command. Finally, the area was determined using the Area function. (Figures A1-A2.) The stain areas were then graphed as functions of transfer pressure, transfer time, and wait time.

**ARTIFICIAL BLOOD SUBSTITUTE**

Particle sizes were measured by imaging using an SEM and measuring the particle sizes in ImageJ. Next, the size distribution was determined by counting the number of particles whose diameters < 1.5µm, between 1.5 and 2.5µm, between 2.5 and 3.5µm and so forth. The stain areas were measured in the same manner as for the transfer stains. The number of spines and scallops were counted manually for each stain. In addition, the major and minor axes of elliptical stains were measured manually using ImageJ.

**PROJECT FINDINGS**

**TRANSFER**

It was found that after a thickened red liquid was applied to knit fabric, it began to wick into the fabric. After only a few seconds (30s), most of the liquid had wicked into the yarns within the fabric. Once it entered into the yarns, it was very difficult to transfer this liquid from one knit fabric to another knit fabric. After 40s, it was difficult to transfer the liquid at pressures less than
4kPa. At 6kPa, transfer decreased with increasing wait time, extrapolating to zero transfer in under two minutes. Even with extended transfer times, little liquid was transferred. (It was observed that nearly the same amount of liquid was transferred for all transfer times tested beyond 40s.)

Porcine blood behaved very differently. Large areas of transferred porcine blood were observed and the area of the transferred stain increased with pressure and transfer time. It decreased only slowly with wait times. As wait time increased to 60s, the transfer area at 6kPa was 65% of the area after a wait time of 5s, while for the red liquid, the transferred area was only 10% under these conditions.

For woven fabrics, the red liquid wicked into the fabric more slowly than on knit fabrics. In addition, the stain areas were much larger, approximately 3x larger. At 6kPa and a wait time of 60s, the transfer stain area was 50% of that after a wait time of 5s, while on the knits, it was only 10%. Surprisingly, the porcine blood and the red liquid behaved similarly on the woven fabric, contrary to what was observed on the knit fabric. See Figures A3-A4.

It was also observed that for knit fabrics, the transferred blood was only observed on the side that had been in direct contact with the bloodied fabric. For woven fabrics, the transferred stain was observed on both sides of the fabric to which the blood was transferred. These results indicate that transfer of blood from one fabric to another depends sensitively on the structure of the fabric which changed as pressure increased. See Figure A5.
Aggregation polymerization is capable of producing a narrow distribution of particle sizes which are similar in size to red blood cells. (Figure A6) When these particles are dispersed in water at a concentration that matches the hematocrit of porcine blood, the viscosity of the artificial blood substitute is much lower than porcine blood. In addition, the stain areas and the number of spines and scallops on cardboard are markedly different than those of porcine blood. After adjusting the viscosity of the dispersing liquid, the viscosity of the dispersion closely matches that of porcine blood. In addition, the stain area and the number of spines and scallops closely match the stains observed for porcine blood. In short, this artificial blood substitute behaves the same on cardboard as porcine blood.

![Graph showing viscosity vs shear rate]

**IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE IN THE UNITED STATES**

Bloodstains on a witness’s clothing or the clothing of someone rendering assistance can occur
from airborne drops or transfer from a victim’s clothing. It has proven difficult to distinguish between these two types of bloodstains on fabrics. This study has shown that, even for two 100% cotton fabrics of the simplest fabric structures, and for the simplest blood stain, the transfer stains have very different characteristics.

It was also observed that transfer only occurs while liquid blood remains on the fabric surface. Once it enters the yarn structure, it becomes very difficult for the blood to exit the yarn under pressure, and thus no transfer occurs.

In addition, by incorporating particles of a similar size as red blood cells, at a concentration the same as for the hematocrit and where the liquid dispersing liquid has a similar viscosity to serum the viscosity profile of the artificial blood substitute closely matches that of porcine blood. Stains created by this artificial blood substitute closely resemble those of porcine blood. This artificial blood substitute is storage stable at room temperature and should be a safer alternative for use in BPA training and scene recreation.
Figure A1. Black colored L-shaped forensic calibration scale placed on a stained knit fabric. The black doubleheaded arrow representing the wale direction for knit fabric and warp direction for woven fabric.

Figure A2. Filtered image to analyze particles, to get circularity, and total area of stain.
Figure A3. Area of transfer stain on knit fabric (top graph) and woven fabric (bottom graph) decreases rapidly as the wait time increases.
Figure A4. Transfer stain area on knits shown for increasing contact pressure at a wait time of 20s.
Figure A5. Fabric porosities at contact pressures.

Figure A6. Polystyrene aggregated particles in an ethanol/heptane solution (left) compared to red blood cells (right). Scale bars are 50µm long.