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The role of CXORF21 in systemic lupus erythe Porcine blood in forensic bloodstain pattern analysis: Hemorheological issues

104 – Biomedizin Innovativ – patientInnenfokussierte, anwendungs-orientierte sowie interdisziplinäre Forschung am Puls der Zeit

Abstract

Keywords:

Bloodstain pattern analysis, dynamic shear viscosity, forensic science, hemorheology, storability

Introduction: Bloodstain Pattern Analysis

Definition and objectives

Bloodstain pattern analysis (BPA) is a discipline of forensic sciences dedicated to the detection, categorization, analysis and interpretation of blood spatter with the aim of reconstructing a course of criminologically relevant events. In an interdisciplinary manner BPA experts help to distinguish between natural death, accident, suicide and homicide, try to solve the question of fault for an accused person and assist to determine the intensity of a criminal action (Peschel et al. 2011). The International Association of Bloodstain Pattern Analysts serves as an umbrella organisation for analysts and offers professional networking and educational programs around the world (International Association of Bloodstain Paterrn Analysts 2016).

BPA practice

BPA casework follows a specific scheme. If possible, it starts with a detailed examination and photographical documentation of the crime scene. Next, analysts will review reports, photographs and notes from officers, emergency medical technicians, hospital staff and autopsy personnel. Hypotheses

will be formulated and need to be tested. Testing might include a review of past cases or there might be a demand for actual experimentation (James et al. 2005)

In cases where bloody fingerprints are not accessible for analysis or where blood has been washed off by an offender, chemical enhancement is used. The most prominent technique employs luminol and is based on the presence of the metalloprotein hemoglobin that can be found in red blood cells (RBCs) (James et al. 2005).

To enable communication between experts, bloodstains are divided into several categories and subgroups depending on their formation mechanism. The shape, distribution and size of spatter allows to determine the velocity and type of weapon, as well as the location of the victim (blood source) and the offender. It might even clarify, whether an assailant war left or right handed (Brodbeck 2012).

The necessity of simulations

It must be clear that although very much dependent on experience, a correct understanding of traces of blood in a criminal case can sometimes only be achieved through experimental setups. Furthermore, the training of qualified BPA professionals also requires practical simulations. Using different types of apparatuses, trainees are able to create patterns similar to the ones present at actual crime scenes. To demonstrate back and forward spatter occurring after gunshots for example, shooting through blood-soaked polyurethane sponges is a very common procedure. Besides, re-enactment experiments might also be used as demonstrative evidence to support expert testimony at a trial (James et al. 2005).

The physical properties of blood

Even though blood is connective tissue, it's physical properties are those of a liquid. Earlier studies indicate that whole blood viscosity is influenced by hematocrit (HCT) and temperature. Blood is a shear thinning fluid and so viscosity significantly varies with the shear rate. Low shear rates are accompanied with RBC sedimentation and aggregation (rouleaux formation), as well as the formation of a clear plasma phase, causing blood to act like a non-Newtonian liquid. At high shear rates, blood behaves like a homogenous liquid and becomes Newtonian (Laan 2016).

Previous findings on suitable samples

Due to blood-transmitted infections and a limited availability even for transfusion therapy, human blood can often not be used for BPA purposes. Therefore, in some countries anticoagulated animal whole blood - especially from pig - is a commonly used alternative for simulations. In 1996, a study was made, comparing human and porcine samples, both fresh and after refrigerated storage. The authors postulated that it would be valid to use EDTA pig blood instead of human samples for BPA issues, even if stored at 4 °C for two weeks (Raymond et al. 1996). In another project, it was also shown that samples from slaughtered pigs are less physiologic than samples obtained via venipuncture (Serp 2015).

Project fundamentals

Academic void

As previous work only provided viscosity measurements for pig blood at a singular shear rate and did not address the influence of HCT and temperature on viscosity to the extent required, further research was needed for a profound characterization of the substance. Moreover, the suspension stability of porcine blood - given by the number of structural elements as well as the forces between those structures and the surrounding plasma - had not been analysed before and had to be assessed with

rheological tests in the oscillating shear field. Additionally, there was a demand for improved and prolonged storability of BPA samples.

Research questions

1. Rheological characterization of porcine blood

   - What impact do the factors HCT and temperature on pig whole blood viscosity and suspension stability have?

   - What conclusions regarding the applicability of porcine whole blood for BPA re-enactment experiments can be made considering these effects?

2. Hemorheological analysis of pig blood storability

   - What hemorheological changes does pig blood show during storage at 4 °C?

   - What steps can be taken in order to prolong the storability of pig blood samples intended for BPA?

   - To what extent can the storability of porcine blood intended for BPA be prolonged in comparison to present conservation methods with EDTA?

3. Pilot study on bloodstain pattern simulation with aged samples

   - In what way are bloodstain patterns created with aged pig blood different to patterns originating from simulations with fresh samples?

Materials and Methods

Rheological characterization of porcine blood

To study the influence of HCT and temperature on viscosity and suspension stability, ten EDTA blood samples were drawn from conscious pigs via venipuncture of Vena cava cranialis. HCT dilutions (30, 40, 50, 60 %) from each sample were analysed with rotational measurements at 7, 12, 17, 22, 27, 32, 37 and 42 °C with shear rates of 1 - 1000 s-1. In addition, small amplitude oscillation measurements were performed at 7, 22 and 37 °C with frequencies of 0.1 - 3.16 s-1 and constant shear stress of 10 mPa.

Analysis was conducted with a rheometer featuring a double gap cylinder system and a Peltier element (Fig.1). Once brought to movement in rotational analysis, the sample tends to move the measurement body around. A torsional moment is recorded and is used to determine the shear stress. The software then calculates dynamic shear viscosity η as a quotient of shear rate and shear stress.

During small amplitude oscillation, reversible material deformation is used to determine storage module (G´) and loss module (G´´). G´ represents the energy being stored in the sample during shearing. G´´ depicts the deformation energy that is being released to the surrounding environment. If G´>G´´, the sample exhibits gel character. If G´´>G´, the sample shows characteristics of a fluid. Loss factor tanδ (G´´/G´) describes both elements of a viscoelastic material (Mezger 2012).

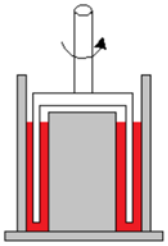

Fig. 1: Illustration of the rheometer. The measurement body (white) performs rotations or oscillations. The measurement cup (grey) is immobilized.

Hemorheological analysis of pig blood storability

To study and improve storability of pig whole blood for BPA simulations, blood samples from eleven pigs were anticoagulated with Citrate Phosphate Dextrose Adenine (CPDA-1) solution. They were stored at 4 °C for 32 days and analysed every second day. Testing included rotational and oscillation measurements at 22 and 37 °C only, as well as standard hemograms and analysis of free hemoglobin (fHb). Additionally, bacteriologic screening was performed at the beginning and at the end of storage.

Pilot study on bloodstain pattern simulation with aged samples

To study the effect of sample ageing on bloodstain patterns in experimental setups, four pig blood samples with CPDA-1 were used for a simple standardized simulation immediately after withdrawal and again following 32 days of storage at 4 °C. A template with concentric bands was used to check whether the resultant patterns were different after storage.

Results

Rheological characterization of porcine blood

During rotational measurements, dynamic shear viscosity of pig whole blood increased with HCT increments and decreased with temperature increments. Contour plots were used to show the HCT-temperature-dependency at different shear rates (Fig.2).

During frequency sweep tests, shear moduli G´ and G´´ increased with HCT enlargement and decreased with rise in temperature. Boxplots were used to show both shear moduli at different HCT-temperature-combinations at distinct frequencies (Fig.3). At 7 °C in all dilutions, as well as at 22 °C with 60 % HCT, blood featured characteristics of a viscoelastic solid (jelly-like material).

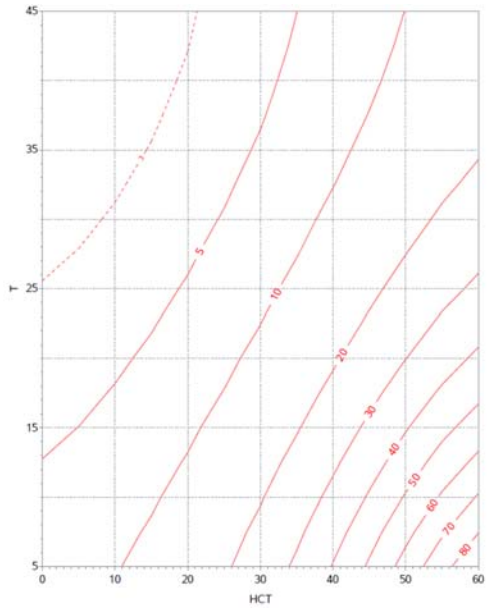

Fig. 2: Contour plot example. Red lines show pig whole blood viscosity [mPa*s] at different HCT-temperature-combinations at a shear rate of 10 s-1.

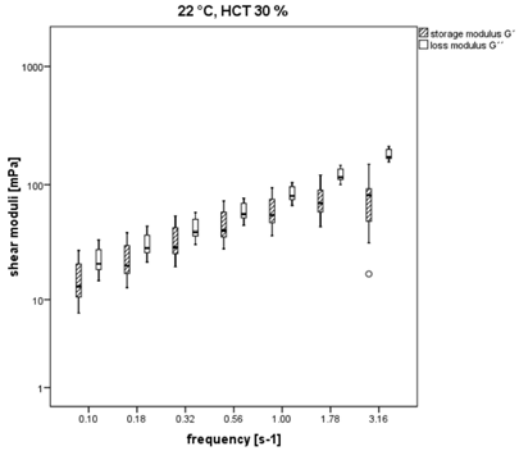

Fig. 3: Frequency sweep test example. Shear moduli reveal characteristics of a viscoelastic liquid for pig blood at 22 °C with 30 % HCT.

Hemorheological analysis of pig blood storability

Samples were germ-free prior to storage, but were contaminated with Staphylococcus xylosus and Pantoea agglomerans afterwards. Antibiotic susceptibility was within normal limits.

Upon ageing, RBC count fell constantly. HCT increased until day 4, remained the same until day 12 and decreased afterwards. Mean corpuscular volume (MCV) of RBCs ascended until day 12, then decreased again. Mean corpuscular hemoglobin (MCH) featured an almost constant upward movement. Mean corpuscular hemoglobin concentration (MCHC) decreased until day 4, then displayed a constant rise following day 12. Analysis of fHb revealed a steady increase over storage time. Following day 26, a remarkable leap of fHb was observed.

Whereas at low shear rate (1 s^{-1}) dynamic shear viscosity showed a biphasic course, at high shear rate (1000 s^{-1}) a constant increase was noted. Oscillation analysis revealed a progressivity of tanδ at 1 s^{-1}. At 22 °C, the ascent started between day 20 and 22 (Fig.4). At 37 °C, it started earlier, but was pronounced following day 22.

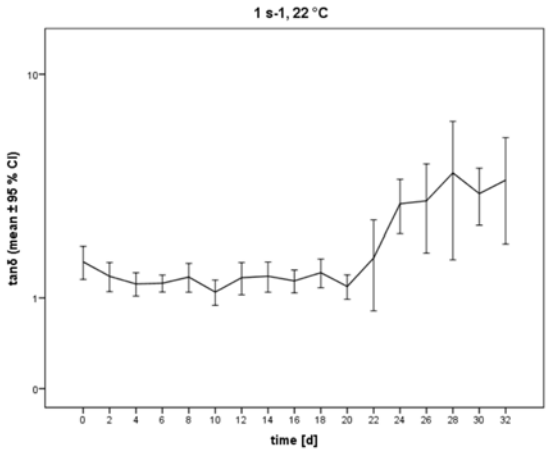

Fig. 4: The course of loss factor tanδ at 22 °C and 1 s-1.

Pilot study on bloodstain pattern simulation with aged samples

The comparison of bloodstain patterns revealed that parent stains were smaller after storage (Fig.5). Also, there was an increase of satellite spatter in proximity to the point of impact. In some of the comparisons, a decrease of satellite spatter size was observed.

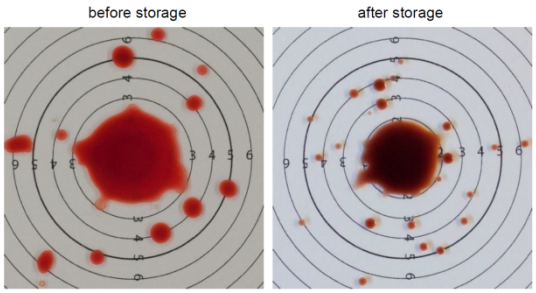

Fig. 5: Example of a simulated bloodstain pattern before and after sample ageing.

Discussion

Rheological characterization of porcine blood

What impact do the factors HCT and temperature on pig whole blood viscosity and suspension stability have?

Data obtained in this study indicate that dynamic shear viscosity of pig whole blood increases with HCT increments, decreases with temperature ascent and reduces with shear rate enlargement. G´ and G´´ also increase with HCT increments and decrease with rise in temperature. Loss factor tanδ acts contrariwise.

What conclusions regarding the applicability of porcine whole blood for BPA re-enactment experiments can be made considering these effects?

In respect of the far-reaching consequences for offenders and victims, which might result from insufficient simulations, it seems inconceivably important to ensure maximum quality and accurateness for BPA experiments. Samples should therefore be warmed up to body temperature prior to usage. If available, HCT should be customized according to medical records of the victim. Leastwise, HCT should be adjusted to gender and age. Further research is recommended in order to appreciate possible changes in physical behaviour due to anticoagulation additives.

Hemorheological analysis of pig blood storability

What hemorheological changes does pig blood show during storage at 4 °C?

Samples displayed a number of hematological changes due to ageing - see results section. Conceivably, osmotic Na+-influx leads to RBC swelling in the early phase of storage and cell shrinkage and the formation of echinocytes plays a role during later stages (D'Alessandro et al 2010). Presumably, the rise in fHb might in fact be linear and not exponential like observed in this study. Haptoglobin, an acute phase protein, binds fHb in order to avoid iron loss and prevent highly reactive heme groups from causing tissue damage. The haptoglobin-hemoglobin-complex is not recorded with the colorimetric method used to determine fHb (Alayash et al 2013).

The biphasic course of dynamic shear viscosity at low shear rate can be associated to the gain of RBC mass. The reasons for the increase of viscosity at high shear rate in spite of a decrease of HCT can only be speculated. Presumably, morphological changes of RBCs or a gain of cell debris hinder the gliding of cells during shear. Also, altered RBC surface charge might modify the interaction with surrounding molecules. Protein clustering in blood plasma might have an influence as well. In summary, a loss of structural strength over time is likely to occur. This assumption was confirmed by the increase of tanδ. Aged samples approach towards Newtonian behaviour, which is not common for blood.

Bacterial contamination in this study is considered to be non-hazardous and presumably originates from sample carryover. Still, it's effect on rheological behaviour remains unanswered.

What steps can be taken in order to prolong the storability of pig blood samples intended for BPA?

As CPDA-1 is well-established for whole blood preservation in blood donor services and improves RBC survival, it is suggested to use CDPA-1 instead of EDTA (Beutler, West 1979). To eliminate bacterial growth, antibiotics can be added to the samples. As it has been shown that the nature and quality of storage containers has an impact on whole blood storability, further research is recommended to compare different containers (Prowse et al. 2014).

To what extent can the storability of porcine blood intended for BPA be prolonged in comparison to current conservation methods with EDTA?

Raymond, Smith and Liesegang stated that it would be valid for BPA issues, to store porcine EDTA blood at 4 °C for 14 days (Raymond et al. 1996). Based on the course of loss factor tanδ in this study, a maximum storability of 20 days is postulated. This applies to pig whole blood with CPDA-1 stored at 4 °C. Further research including pattern simulations at regular intervals is recommended.

Pilot study on bloodstain pattern simulation with aged samples

In what way are bloodstain patterns created with aged pig blood different to patterns originating from simulations with fresh samples?

Analysis revealed a clear tendency towards a decrease of parent stain diameter and an increase of satellite spatter. Thus, hemorheological changes in the sample are not only reflected in measuring parameters, but also have a practical relevance. Samples for BPA should therefore not exceed a certain duration of storage or otherwise distorted patterns might occur.

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