

## Authors' Response

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See Comment *here*

Editor,

The Commentary by A. Keten makes the case for considering evaporation and coagulation in research and applications of the forensic discipline of bloodstain pattern analysis (BPA). The author also mentions two important facts: first, blood stains are often present in crime scenes, and second, they can provide significant information towards crime scene reconstruction.

Evaporation and coagulation are two distinct and sometimes coupled mechanisms that we describe shortly before commenting on their relevance to bloodstain pattern analysis.

Evaporation occurs by transport of mass between a fluid and its environment, across the interface between the fluid and its environment. Evaporation is typically coupled with a change of phase (the liquid becoming vapor) and heat transfer (heat being needed for the phase change). As mentioned in the Commentary, external factors also influence evaporation, such as the thermodynamic conditions of the atmosphere, in terms of humidity and temperature, the aerodynamic conditions around the liquid, the characteristics of the stained material such as temperature, wettability, and wickability. For sessile drops and liquid films, evaporation is greatly enhanced at the contact lines—the location common to the fluid, the atmosphere, and the solid material on which the liquid rests. This explains why blood stains that have started to dry can be wiped off a surface except for a ring-shaped trace at their periphery. The physics of evaporation is well understood (1) and is relevant to multiple technical areas such as the design of combustion engines and climate modeling, which have been studied in more depth and breadth than BPA (2).

Coagulation is started by external or internal trauma and occurs via several multi-step phenomena, such as aggregation of blood platelets, and the production of a clotting protein called fibrin. Coagulation times can be measured with devices such as a prothrombin tester. Coagulation times vary between individuals and are complex to predict theoretically (3). Fluid dynamic stresses also influence coagulation (4): They can switch on and off the function of clotting polymers, and they play a role in the aggregation of platelets. Coagulation associated with violent events such as those associated with BPA has not been studied specifically, but several experimental studies show that coagulation of sessile microliter-volume blood drops (references [5–7] of the Letter by A. Keten) and of milliliter-volume liquid pools (8) occurs within minutes to hours.

So, what is the importance of evaporation and coagulation in our BPA study (9), which is cited in the first paragraph of the Letter? Our study is about the development and application of general artificial intelligence methods to automatically classify blood spatter patterns in terms of their cause: a gunshot or a blunt impact. Typical velocities and diameters of the drops of interest in these situations have been measured (10–12) between 10 to 100 m/s and 100  $\mu\text{m}$  to 1 mm, respectively. In study (9), the distance between the area of origin and spatter pattern is typically one meter or less. Thus, the overall typical time between spatter generation and stain formation on nonabsorbing surfaces

such as those on cardstock used in (9) is less than one second. Based on the present body of knowledge, it is unlikely that coagulation, which has been measured to occur on time scales orders of magnitude longer, would modify results of spatter experiments such as those in (9). Coagulation might however affect BPA of impact spatter or gunshot spatter patterns in at least two situations. The first situation is when a BPA experiment is performed using blood without anticoagulants (or nondefibrinated), and when coagulation starts between the drawing of the blood and the generation of the spatter. In that situation, the physical properties of the blood (its rheology and surface tension) may be affected by coagulation, and the experiment will not adequately describe what happens in, for example, gunshot spatters during actual crimes, where the pattern is produced immediately after the bullet generates blood drops. The second situation is the realistic situation where a surface covered with blood that has started to coagulate is then impacted, generating a spatter made of blood with physical properties affected by the coagulation. Such situation could occur during a struggle between two people involving a sequence of impacts over several minutes.

Regarding the effects (if any) of evaporation on the results of BPA study (9), evaporation during the drop flight or during the staining process is already considered, because the impact and gunshot spatter patterns were obtained experimentally. Evaporation during the observed staining process is not expected to have a significant effect on stain size, because the stain size is determined during the spreading time that follows the impact, and this time is much smaller than the evaporation time. Regarding evaporation in flight, classical mass transport correlations suggest that only the smallest spattered drops, with diameters of 100  $\mu\text{m}$  or smaller, will experience significant reduction in their diameter. These tiny drops are also mostly subjected to deflection by aerodynamic drag forces and might not even travel as far as the location where the spatter pattern is collected. It is thus likely that the effects of evaporation are not significant in gunshot spatter experiments such as the ones performed in (9).

Certainly, evaporation and coagulation can significantly affect slower aspects of BPA such as the drying of pools of blood (8), the evaporation of sessile drops with microliter or larger volume, the formation of drip trails, and wicking in fabrics. These situations can exhibit time scales on the order of minutes as well as enhanced evaporation at contact line and can thus be influenced by evaporation or coagulation.


On a side note, the Commentary suggests (based on study [13]) that horse blood would be more similar to human blood than pig's blood. We believe that this suggestion is not fully supported by (13). Reference (13) merely recommends that defibrinated horse blood could be used as a substitute for human blood, based on a small set of BPA experiments such as drop impact and reconstruction of the area or origin. Since human blood is a potential carrier of diseases, with a limited availability and shelf life, it makes sense to investigate the use of safer substitutes, artificial (14) or natural. This is no easy quest, because blood is a fluid with great structural and biological complexity, that varies significantly between individuals and even more between mammal species (15). Based on extensive

measurements of the physical properties of pig's blood (16), in terms of surface tension and rheology (shear and elongational viscosity), we decided to use pig's blood in our laboratory experiments as a safer substitute for human blood. Specifically, we measured that the relaxation time of pig's blood (a rheological parameter relevant to the formation of droplets upon impact) was in agreement (16) with that of human blood (17). Like human blood, blood from pigs, dogs, cats, or horses exhibits the formation of aggregate of red blood cells (called rouleaux) at low shear rates, unlike the blood of cattle, sheep, rabbits, and mice (15). The formation of rouleaux contributes to the decrease in shear viscosity with increasing shear rates, a behavior called shear thinning, which is also relevant to bloodstain pattern analysis. Ultimately, the choice of a blood substitute depends on several factors such as the availability of blood of a given species (there are many pigs in Iowa, where our laboratory is currently located), and the specific topic of bloodstain pattern analysis the research is focused on.

Finally, the Commentary recommends that experts from different scientific fields collaborate to perform research in BPA. We concur. The fluid dynamics phenomena associated with BPA involve capillary flow in complex geometries (18) and/or very fast deformation during impact or gunshots (19), interactions between traveling drops and muzzle gases (20), which are currently poorly understood. The physical properties of blood and its coagulation under conditions relevant to BPA have not yet been fully characterized. BPA events involve other phenomena such as the biomechanics of body tissues (21), human physiology, and the complex motion of the people involved in the bloodshed. Engineers, physicists, and physicians thus have to collaborate with crime scene experts to produce physically sound methods to reconstruct events relevant to BPA. Last but not least, a statistical framework is needed to identify and quantify experimental uncertainties relevant to the application of specific reconstruction methods to specific crime scene events (22). We believe that the combination of statistical methods and physics-based reconstruction models will help estimate the error rates of reconstruction expertise and expert opinion in legal proceedings.

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Daniel Attinger,<sup>1</sup> Ph.D.; Yu Liu <sup>2</sup> Ph.D.; and Kris De Brabanter,<sup>3,4</sup> Ph.D.

<sup>1</sup>Department of Mechanical Engineering, Iowa State University, 2036 Black Engineering, 2529 Union Drive, Ames, IA 50011

<sup>2</sup>Department of Computer Science, Iowa State University, Atanasoff Hall, 2434 Osborn Drive, Ames, IA 50011

<sup>3</sup>Department of Statistics, Iowa State University, 2419 Snedecor Hall, 2438 Osborn Drive, Ames, IA 50011

<sup>4</sup>Department of Industrial Manufacturing & Systems Engineering, Iowa State University, 3033 Black Engineering, 2529 Union Dr, Ames, IA 50011,

E-mail: liuyu0jlu@gmail.com