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Visualization of Plasma and Serum Pattern Formation in Drying Drops of Blood

Kelly P. Kearse*

Knoxville Catholic High School, 9245 Fox Lonas Road, Knoxville Tennessee 37923

Corresponding Author:** ***Kelly P. Kearse, *Knoxville Catholic High School, 9245 Fox Lonas Road, Knoxville Tennessee 37923*

Abstract: *Whole blood is a colloidal suspension consisting of ~55% liquid plasma and ~ 45% cellular components. A thorough understanding of drying blood drop pattern formation is important in forensic applications involving the blood of a murder victim or suspect. Previous studies on drying blood drops have focused on the movement of the blood cellular portion, or on plasma that has been physically separated from whole blood; however, no analyses have been performed to evaluate the movement of plasma within whole blood during this process. The current study utilizes ultraviolet light 365 (UV 365) to monitor the migration of plasma in drying blood on both glass and skin. These data show that a distinct sequence of events occurs in the movement of plasma within drying whole blood and provide the first specific visualization of this process.*

Key Words: *blood plasma, serum, ultraviolet light, red blood cells, halo*

Abbreviations: *ultraviolet light 365 (UV 365), visible light (VL)*

1. Introduction

Whole blood is comprised of multiple cellular components, including red blood cells, white blood cells, and platelets; and liquid solution termed plasma. As drying blood begins to solidify, serum is the fluid that remains from the plasma, devoid of clotting factors [1]. Glass is regularly the model substrate for blood drying studies [2-4], and to this end, Brutin et al. has identified five distinct stages based on red blood cell movement and blood drop appearance [4]. Initially, the drop becomes pinned on the substrate at the first stage, establishing a perimeter at the edge. In stage 2, fluid in the central part of the drop flows outward to replace liquid lost at the edge due to evaporation; concurrently, red blood cells move from the center to the edge of the drop. Stage 3 is defined by rapid desiccation, indicated by the central part of the drop becoming a lighter color of red, and the appearance of crack formation at the edges. In stages 4 and 5, the central part of the drop and the corona (outer rim) begin to desiccate in the final steps of drying [4].

The movement of plasma in whole blood during blood drying remains to be evaluated, as its presence is typically veiled by red blood cells in the mixture. UV 365 has recently been shown to be an effective alternative light source in the detection of diminutive amounts of dried blood plasma and serum [5,6]. Transfer of clotting blood to assorted materials have shown that fluorescent serum halos are imprinted in a time-dependent manner, which were not observed when blood was absorbed directly by the cloth [5]. Here, ultraviolet light 365 (UV 365) was used to specifically monitor the flow of plasma during blood drop drying and the formation of serum halos during this process. Studies were performed on both glass and skin substrates. These data provide the first specific visualization of plasma movement in drying whole blood and show that a distinct sequence of events occurs during this process.

2. Materials and Methods

2.1. Blood, Bilirubin Preparation, and Mice.

Human blood was obtained from healthy volunteers by the finger stick method using a Health Lancing device (CVS pharmacy, USA) fitted with a micro lancet (CVS Pharmacy, USA). Blood was dropped onto Parafilm® M Laboratory Film (Bemis Company, Inc., Oshkosh, WI) and transferred to various substances using a 1-10 or 10-100 microliter (ml) Eppendorf® micro pipettor (Hamburg,

Germany. Bilirubin (Lee Biosciences, Maryland Heights, MO) was added at 10 mg/dL to whole blood containing an endogenous bilirubin level of 0.6 mg/dL total. Newborn mice approximately 1-2 weeks of age were obtained from Rodent Pro LLC (Inglefield, IN).

2.2. Ultraviolet Light Source and Photography

A LED UV flashlight, 365 nm, LED-UV301-365 nm (Shenzhen Lightfe Light Limited, Shenzhen, China), positioned at a ~ 45^{o} angle from the subject was used as the UV source. All photographs were taken using a Sony 6500 digital camera fitted to a Unitron ZST stereomicroscope.

3. Results and Discussion

Whole blood was added to untreated glass slides and photographed at three-minute intervals using either visible light or UV 365. Analysis under UV 365 showed that within the first few minutes a distinct "plasma blister" had formed in the center, and a small rim of plasma was present at the edge (Figure 1A and Figure 1C, white and black arrows, respectively). As time progressed, the opacity of the plasma blister decreased with an accompanying thickening of plasma/serum at the edge, peaking at approximately twenty to twenty five minutes (Figure 1A). After this time, there was an area of dim fluorescence in the center, corresponding with crystallization of serum proteins (Figures 1B and 1D). No further change in appearance of the fluorescent serum halo was observed after approximately 30 minutes under either VL or UV 365 (Figure 1B). This pattern formation of plasma movement was not observed when whole blood that had been frozen and rethawed was used (Figure 2), suggesting that intact red blood cells are required for this process.

As a further means of verifying that the fluorescent halo in dried blood drops in these experiments was indeed due to serum, exogenous bilirubin was added as a tracer using levels similar to certain physiological conditions seen with impaired liver function. Bilirubin was chosen as it is a serum protein which should mix with endogenous bilirubin in whole blood and is easily distinguished by its greenish/yellow fluorescence under UV 365 [7]. As shown in Figure 3, dried blood drops containing exogenously added bilirubin shared a similar morphology as control groups under visible light but displayed a unique corona with a distinct greenish/yellow tint under UV 365 (Figure 3). Additionally, crystallization of serum proteins in the center portion of blood globules was effectively highlighted by the greenish hue in groups receiving exogenous bilirubin (Figure 3).

Similar blood drying experiments were performed on skin, using newborn mice as the model system. Mouse skin is similar to human skin in that it consists of epidermal and dermal layers but is much thinner with four total layers relative to ten in humans [8]. In newborn mice, the skin is moderately transparent, with numerous, darker internal organs being visible, providing a suitable background for photography under uv. The upper chest region was chosen for blood deposition as it is a relatively flat, smooth area. As shown in Figure 4, several minutes after blood addition a central "plasma blister" had developed (Figure 4A, Figure 4C, white arrow), similar to what was seen with blood drying on glass. Relatedly, plasma was visible at the edge (Figure 4A), which thickened with time (Figure 4B, Figure 4D, black arrow). Interestingly, unlike what was observed using glass, the fluorescent serum ring was internal to the outer contact edges on skin (Figures 4D), which may result from the comparatively increased elasticity of skin relative to glass.

Previous studies have established that blood behaves as a non-Newtonian fluid with evaporation directed by Marangoni flow [2-4 ,9]. The current study importantly extends prior work by providing the first specific visualization of the movement of plasma within drying whole blood on both glass and skin substrates. When blood was subjected to several rounds of freeze-thawing prior to deposition, formation of the serum ring was disrupted, suggesting that intact red blood cells are necessary for efficient phase separation and movement of cellular and liquid components during drying.

As blood begins to dry, the liquid in the central portion flows outward to replace liquid lost at the edge due to evaporation [1-4]. The results in the current study provide a further visualization of this process by specifically showing the formation of a "plasma blister" in the center, which lessens with time, and is accompanied by the thickening of the plasma ring at the periphery. Indeed, plasma flowed relatively quickly to the outer edge of the

blood globule on both glass and skin as evidenced by a fluorescent rim/edge that was apparent within the first few minutes after blood addition. With glass, the final serum halo remained at the periphery, unlike skin, where the serum ring was interior to the outer edge. As the drying proceeded on skin, clotting blood at the edge appeared to draw the skin inwards (Figures 4A and 4B), orienting the plasma (and eventual serum ring) more towards the center. Skin is a relatively pliant surface compared to glass, which may account for this difference. Previous studies have detailed the transfer of clotting blood to various materials, particularly textiles, and imprinting of fluorescent serum halos [5], which was not observed when blood was absorbed directly by the cloth. The results in the present report support those findings, showing that migration of plasma is an active process in the initial stages of blood drying.

In conclusion, the current study has evaluated the movement of plasma in whole blood drops drying on glass and skin substrates. The data importantly extend previous studies on blood drop drying by providing the first visualization of plasma migration and serum halo formation during this process.

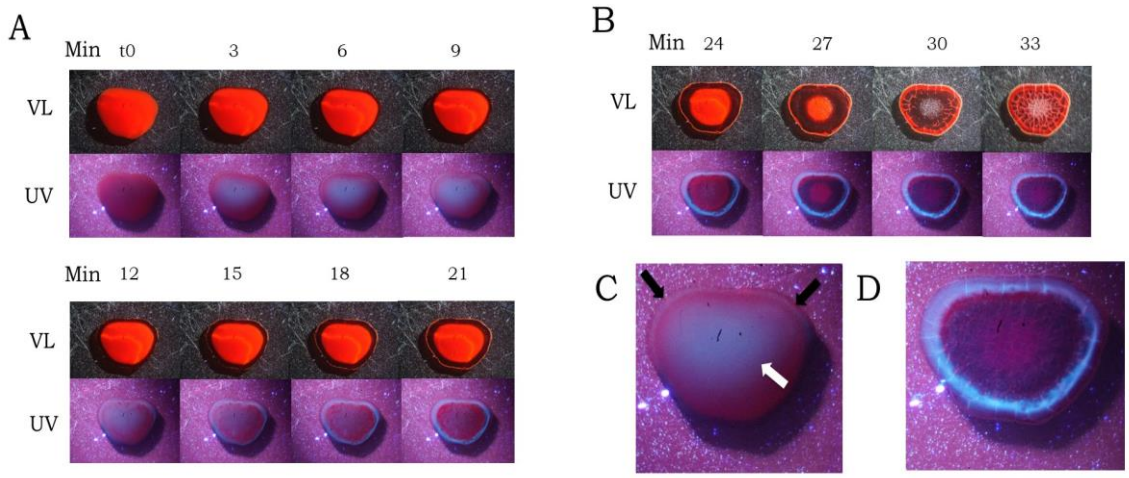

Figure1. *Whole blood was added to glass slides and photographed under visible light (VL) or ultraviolet 365 (UV) at the time indicated (A & B). In (C) an enlargement of the 6-minute time point is shown, with the plasma blister and plasma/serum edge highlighted by white and black arrows, respectively. An enlargement of the 33-minute time points is show in (D). Note the fluorescent halo around the corona (see text for details).*

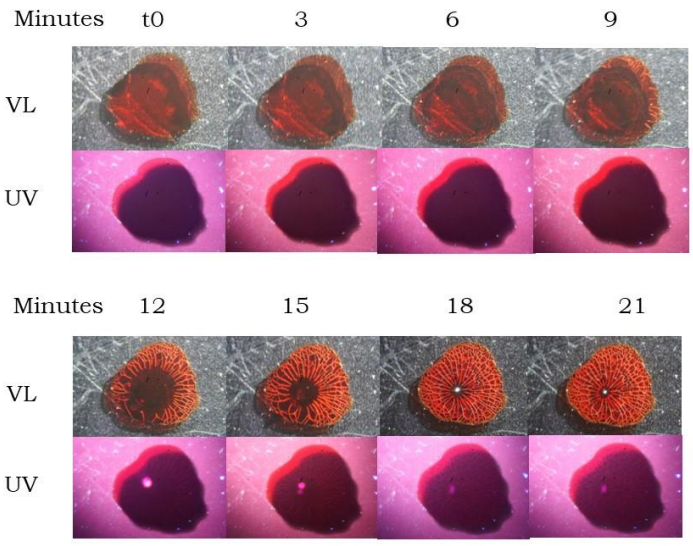

Figure2. *Whole blood that had been freeze-thawed for several cycles was added to glass slides and photographed under visible light (VL) or ultraviolet 365 (UV) at the time indicated (see Materials and Methods for details).*

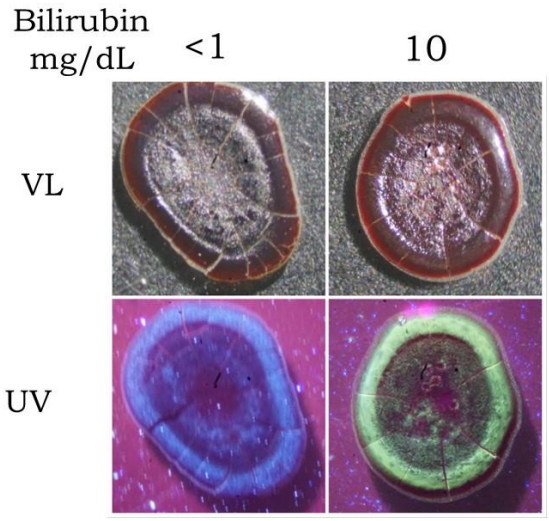

Figure3. *Appearance of dried whole blood containing elevated levels of bilirubin. Samples containing endogenous levels of bilirubin (<1 mg/dL total) or exogenous added bilirubin (10 mg/dL) were added to glass slides and photographed under visible light (VL) or ultraviolet 365 (UV) after 35 minutes of drying. Note the yellow/greenish fluorescence in groups receiving high levels of exogenous bilirubin.*

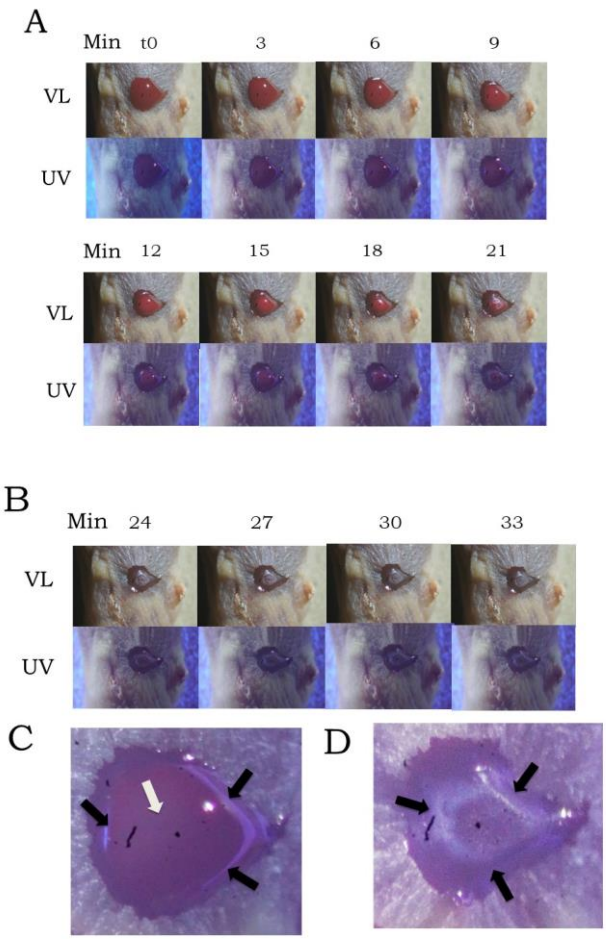

Figure4. *Whole blood was added to the upper chest of newborn mice and photographed under visible light (VL) or ultraviolet 365 (UV) at the time indicated (A & B). In (C) an enlargement of the 6-minute time points is shown, with the plasma blister and plasma/serum edge highlighted by white and black arrows, respectively. An enlargement of the 33-minute time points is show in (D). Note the fluorescent halo interior to the periphery (see text for details).*

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