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# Supersize It! Developing a "Supercytoplast" Through Platelet Fusion

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**Abstract**

Stem cell research opens a wide range of possibilities from lab-grown tissues for medical purposes to cloned animals for livestock production. The issue with one of the most popular forms of cell recombination into a stem cell, known as somatic cell nuclear transfer (SCNT), is that it requires an oocyte. The requirement of an oocyte is often the host of public scrutiny for its economic and ethical issues. Platelets may offer a less expensive and more accessible alternative to oocytes as sources of naturally enucleated cells. Thus, the objective of this explorative research project is to isolate and fuse platelets to form a large body of cytoplasm, which could be used to receive a donated somatic nucleus for recombination. Blood samples were collected first from a horse and then from cattle. The whole blood was centrifuged to create a density gradient, and then the platelets were isolated using a commercially available protocol (*Abcam*, Cambridge, MA) for the isolation of human platelets. Once isolated, the platelets were diluted using an RPMI solution to four dilutions using the volumetric ratio of μl of platelet-rich plasma (PRP) to μl of RPMI solution. The dilutions were 1:10, 1:100, 1:1000, and 1:10,000. Two samples of each dilution were then treated with either a 25%, 50%, or 100% concentration of polyethylene glycol (PEG). Fusion was found in the 1:100 dilution sample treated with the 100% PEG concentration. The rest of the samples appeared to have been destroyed. Two further replications were inconclusive as the platelets did not survive the isolation process. Any future attempts were canceled due to the shutdowns related to the COVID-19 pandemic. Despite inconclusive results, the fusion found in the first trial is encouraging that future research might find a method to consistently obtain platelet fusion.

*Keywords*: Platelets, isolation, fusion, recombination

## Introduction

In 1954, the medical community was revolutionized when a kidney became the first human organ to be successfully transplanted (Linden, 2009). Sixty-six years later, a new world exists where transplants are commonplace. Yet, transplant recipients still battle against their body's immune defenses that perpetually work to remove the foreign organ. Recipients are often prescribed a lifetime of immunosuppressive drugs to prevent rejection of the organ (Halloran, 2004). At the very least, these medications put transplant recipients at a higher risk of infection, while in the worst cases, the medications fail, and the recipient's body rejects the organ completely (Halloran, 2004).

There are, of course, some transplant patients who do not necessarily face the same concerns as others. Identical twins, by definition, have organs that are completely genetically similar to each other, meaning each twin's immune system can accept the other's organ as familiar (Linden, 2009). Even so, this advantage is not without its limitations. One identical twin might be able to give their sibling one of their two kidneys and survive, but this is not the case with most vital organs, and so they remain about as vulnerable as any other transplant patient. Still, identical twins point us toward the ideal world of transplantation and raise a compelling question: what if everyone could be a donor unto themselves?

Stem cells open the door to that idyllic world. As stem cell research has advanced, it seems inevitable that it will provide a viable alternative to traditionally donated organs. What is more, the possibilities go beyond transplantation (Ringe et al., 2002; Yokoo et al., 2005). Stem cell research may hold the secret to treating various conditions from traumatic brain injuries to Alzheimer's disease to diabetes (Ringe et al., 2002; Assady et al., 2001).

Originally, stem cell research used naturally occurring embryonic stem cells (ESCs) and somatic/adult stem cells (Ringe et al., 2002). These methods, particularly ESC research, have received heavy criticism from many members of the public (Lo & Parham, 2009). However, in recent years, a technique called somatic cell nuclear transfer (SCNT) has been developed, which allows the nucleus of a somatic cell to be removed and injected into the cytoplasm of an enucleated oocyte. Inside the oocyte, the somatic cell's nucleus is reprogrammed to an undifferentiated state (Young et al., 2006; Gurdon & Wilmut, 2011; Pasque et al., 2011).

As with ESC research, there has been some controversy surrounding SCNT due to its high cost and the invasive techniques required when harvesting oocytes (Ibtisham et al., 2016; Mertes & Pennings, 2007). In animal research, the donor is typically euthanized before harvesting the oocytes, or a surgery is performed to remove them. It is argued that the cost of harvesting oocytes makes SCNT difficult to pursue, and that the pain that a living donor must endure while recovering is inhumane (Ibtisham et al., 2016). In human medicine, the requirement of an oocyte from relatively young women raises the ethical issue of female exploitation. There is a higher tendency for donors to be from a poor socioeconomic background, which encourages targeting of these groups for oocyte donation. The donation in these cases might be made out of necessity and coercion, rather than enthusiasm (Mertes & Pennings, 2007). Additionally, informed consent is a major concern in oocyte donation. Donors are supposed to be given information about what they can expect from the harvesting procedure and the purposes for which their oocytes will be used. However, further investigation reveals that in many cases the donor does not fully understand the information being given (Lo & Parham, 2009).

As a solution to these issues, this study explores the possibility of developing a cytoplast body from fused platelets, similar in size to that of an oocyte, to be used as an alternative to

oocytes in recombination. Thus, the objective of this research is to isolate and fuse platelets to form a large body of cytoplasm, similar in size to that of an oocyte, which may be attached to a somatic cell. To achieve this, a method of cell fusion will be used wherein cells are treated with polyethylene glycol to initiate fusing.

Platelets are an abundant and easily obtainable resource had by every mammal on the planet. Platelets contain many growth factors to aid in the repairing of damaged tissues. To name only a few functions of these factors, they support angiogenesis, differentiation, and inhibit apoptosis. Particularly interesting is the finding that they have the ability to directly affect the DNA structure of other cells (Xu et al., 2018). The ability to interact with other cells’ genetic material means that there is some hope that platelets could function to rewrite other cells’ genetic code.

The development of a “super cytoplast” from the fusion of platelet cells could have significant benefits to the fields of animal and human research. In animal research and production, fields critical to the protection and improvement of the health and well-being of the planet, the accessibility of platelets could mean a more humane and cost-effective alternative in transgenic and cloned animal research than harvesting oocytes, and therefore, a greater opportunity for advances in the efficiency and environmental impact of our food supply. The use of platelets also means moving away from relying upon female donors to provide oocytes. This is especially important in human medicine as it could limit the exploitation of women and bolster the ethical credibility of somatic cell nuclear transfer research. Virtually any subject could provide the necessary platelets to serve as a supercytoplast. This method would provide a door to a widely available, patient-specific alternative to oocytes in the development of products like lab-grown organs, tissues, and insulin.

## Literature Review

Patel & Yang (2010) describe four of the most promising and successful methods for reprogramming somatic cells to the stem cell state. These methods include cell fusion, culture induced reprograming, direct reprogramming, and somatic cell nuclear transfer (SCNT). Cell fusion involves the fusion of a somatic cell and an embryonic stem cell. Factors in the embryonic stem cell promote the reprogramming of the somatic cell genetic materials to more closely resemble the pluripotent nature of the embryonic stem cell. This process has shown promise for in vitro tissue development; however, Patel & Yang (2010) point out that there is no way to remove the genetic material of the embryonic stem cells. The reason this is an issue, is that it limits the ability for a recipient to accept the products of this process. Another reprogramming technique is culture induced reprogramming. This is the process of extracting cell materials from embryonic stem cells and injecting the extracts into a somatic cell. This process has shown positive results in both lab studies of human cells for reprogramming the somatic cell and directing it to develop into a variety of tissue types. However, studies were unable to produce cells with complete pluripotency or cells capable of differentiation. Direct reprogramming is a unique method in that it does not require the nucleus of the somatic cell to be reprogramed to a pluripotent state. Instead, the genetic material of the somatic cell is directed to perform the desired function. Currently, there has been little success using direct reprogramming in vivo, and virtually no success in humans. Finally, the process of SCNT is the process on which this research focuses due to the interest it has garnered in both the medical and animal science fields. Somatic cell nuclear transfer is the process of placing the nucleus of a somatic cell into the enucleated body of an oocyte. Somatic cell nuclear transfer is most famous for successfully producing the cloned sheep, Dolly. Patel & Yang (2010) report that one of the

largest obstacles to overcome is the relatively low availability of oocytes as compared to other cell types. This is particularly pertinent to human medicine as the low rate of voluntarily available oocytes makes research and development of SCNT for medical purposes incredibly slow and expensive.

Lo and Parham (2009) outline the issue of using embryonic stem cells (ESCs) for recombination of human cells in stem cell research. ESC research has been fraught with controversy. The moral conclusions that result from being for or against ESC research are intrinsically tied to those of another divisive debate, abortion. Many members of the public believe the harvesting of ESCs is immoral and unethical as it requires destroying a human embryo. The argument against using ESCs in scientific research is often based in religious beliefs and political ideologies that claims an embryo should be granted full human rights from the moment of conception. Those who argue for the use of ESCs point to the very real progress that ESC research offers the medical community. Many patients with debilitating illnesses could find relief from stem cell therapies that are dependent upon the use of ESCs. This debate points to a need to find a method of recombination that allows patients to find the healing they need, while honoring the moral beliefs of a large portion of the population.

Nurden and coworkers (2008) detail platelets and their functions within the body. Platelets are clear, naturally enucleate, cell fragments that are the key to healing damaged tissue structures. They are known to aggregate during clot formation and to contain growth factors that promote tissue growth and angiogenesis. More recently, it has been discovered that they are involved in the recruitment of somatic cells and initiate morphological changes to cells to support healing (Reddy et al., 2018). Due to the many growth factors platelets contain, they are

excellent at repairing and building tissues, while preventing apoptosis (Sánchez-González et al., 2012).

So valuable are the healing abilities of platelets, that a type of therapy called platelet-rich-plasma (PRP) therapy has been developed to aid in the regeneration of tissues related to a variety of injuries and diseases. Reddy and coworkers discuss the benefits of platelets, and specifically the process of platelet-rich-plasma (PRP) therapy. Because the blood used in PRP therapy is harvested from the same individual who will be receiving the therapy, there is very little chance of causing an immune reaction. In fact, morbidity related to PRP-therapy is incredibly rare (Reddy et al., 2018).

Along with their ability to repair, platelets are also capable of damaging the DNA structure of other cells. Xu and coauthors explore the effect platelets have on the bacteria *Staphylococcus aureus*. Platelets are capable of preventing the growth of *S. aureus* by disrupting the bacteria's DNA structure. The research concludes that platelets are capable of antimicrobial actions, similar to those of immune cells (Xu et al., 2018). This is compelling for the present study, as this points to platelets ability to act on another cell's DNA structure. Although there is no current evidence that platelets can recombine a somatic nucleus to a pluripotent state, their ability to interact with DNA as enucleate cells implies that there is a possibility that they could be used in recombination.

There has been little research into the fusion of platelets. The most obvious function of platelets is clotting. The clotting process is achieved by platelets' propensity to aggregate. One of the few studies that exist on platelet fusion looked specifically at the mechanism of aggregation and clotting. In some instances, the platelets were observed to fuse after aggregating. The process of platelets fusing unassisted is slow and seemingly. Therefore, using aggregation of

untreated platelets to achieve platelet fusion is not practical for lab purposes. However, the ability of platelets to fuse is an important finding as it shows platelets are capable of doing so and may be able to do so in shorter amounts of time if under the right conditions (Woronowicz et al., 2007).

One of the most widely used chemicals in facilitating cell fusion is polyethylene glycol (PEG). It can be used for many types of processes requiring cell fusion, including nuclear transfer. Although the exact mechanism that allows PEG to cause cell fusion is unknown, it is believed that it causes a morphological disruption of the lipid bilayer of the cell membranes. This disruption allows the membranes of two cells to fuse where they are in contact with each other. According to Yang and Shen (2006), PEG is most effective in concentrations between 40% to 50% (Wojcieszyn et al., 1983).

## Materials and Methods

### Platelets

The platelets for Trials 1 and 2 were obtained from equine blood samples. The equine blood samples were collected by Dr. Paul Turchi at his privately-owned clinic, Norwest Arkansas Equine Services. The platelets for Trial 3 were obtained from blood samples collected from seven 15-week old heifers under the veterinary direction of Dr. Jeremy Powell.

### Trial 1

The first experimental trial for this research involved two replications and was held during the week of July 8, 2019. Six 10 mL blood samples were taken from a single horse by Dr. Paul Turchi at his private veterinary clinic. The blood samples were stored on ice for the 20-minute drive from the veterinary clinic to the laboratory at the University of Arkansas. The

samples were placed on a rocker to restore normal blood consistency. Three of the six blood samples were used during Trial 1.

***Platelet Isolation***

The first step of the experiment was isolating the platelets from the whole blood. A commercial protocol (*Abcam*, Cambridge, MA) for platelet isolation of human platelets was followed. Two of the three centrifuge speeds were converted from units of gravitational force to rotations per minute as required by the centrifuge available. Instead of Tyrode's buffer, RPMI solution was used as it is what was available and has been used for platelet isolation. A 10 ml RPMI solution was made using 11 mM of glucose, 10 mM of ethanesulfonic acid (HEPES), and 5 mg/mL of bovine serum albumin (BSA).

The whole blood samples were spun at 200 x g for 20 minutes with no brake applied to create a density gradient. The platelet-rich plasma (PRP) was observed at the top of the gradient. Approximately 2/3 of the PRP was removed and transferred to a separate plastic tube. Only 2/3 of the PRP was removed so as to avoid contamination of the PRP by the buffy layer. HEP buffer was added to the tubes containing each sample of PRP in a 1:1 volumetric ratio and mixed gently by hand. The samples were placed on ice when not being handled to prevent platelet activation. The samples were then centrifuged at 100 x g for 20 minutes with no brake applied to pellet any contaminating red or white blood cells. Then, 50 µl of the supernatant containing the PRP were removed and transferred to 1 mL plastic tubes and centrifuged at 800 x g for 20 minutes with no brake applied to pellet the platelets. The supernatant was removed and discarded. The remaining pellets were gently rinsed three times using phosphate buffered saline (PBS) without resuspending the platelets. Finally, the platelets were resuspended through the addition of 50 µl of RPMI 1640. From this point on, no ice was used in order to promote platelet aggregation.

### *Dilutions*

More RPMI was added to 10 µl samples of the PRP solution so that four different dilutions were formed based on volumetric ratios of µl PRP solution to µl of RPMI added. The final dilutions were 1:10, 1:100, 1:1000, and 1:10,000 µl of PRP solution to µl of total volume.

### *PEG Treatment and Fusion*

Three different concentrations of polyethylene glycol (PEG) 6000 were made. The concentrations were 25%, 50%, and 100%. The PEG concentrations were based on the volumetric ratio of PEG to water and subsequently recorded as percentages. The ratios were as follows: 1-part PEG to 4 parts water, 1-part PEG to 2 parts water, and 1-part PEG to 1-part water (25%, 50%, and 100% PEG, respectively). Six 10 µl samples of each PEG concentration were added to a 96-well plate. One µl of each PRP dilution was then added to two samples of each PEG concentration in the well plate. After 30 seconds, the samples were observed using a microscope. The only samples that showed evidence of cell fusion were the two samples containing the 1:100 PRP to RPMI ratio treated with 100% PEG. The fusion of the cells in Trial 1 was indicated by the gradual disappearance of the membranes between multiple platelets, as shown in Figures 1-3. The rest of the samples lacked any evidence of fusion, and furthermore revealed that most of the platelets had been destroyed.

## Trial 2

The second trial took place 12 hours later using the remaining three blood samples collected for the first trial. During this trial, the intention was to replicate the fusion seen in the first trial. To achieve this, a 1:100 ratio of PRP to RMPI treated with 100% PEG was planned to be used. The trial could not be completed because no platelet pellet was produced during

isolation. Observation using a microscope showed that most platelets were destroyed during isolation due to an unknown variable.

**Trial 3**

The final attempt to replicate the results took place on March 13, 2020. Several materials from the first two trials had to be replaced. All materials were purchase on the VWR website and are as follows, including their product number on the website.

500 ml RPMI 1640: 76045-320

100 ml PEG 1500 50%: 101443-456

30 ml Giemsa stain kit: 76265-784

25 ml Percoll: IC0219536925

100 ml HEPES: 97064-360

The Giemsa stain kit and the Percoll were never used, and therefore not mentioned in the following description of methods. A 100 ml HEP buffer was made using 140 mM NaCl, 2.7 mM KCl, 3.8 m//m HEPES, and 5 mM ethylene glycol tetraacetic acid (EGTA).

Seven blood samples were collected from 15-week old heifers owned by the University of Arkansas. One sample was collected from seven different heifers under the veterinary direction of Dr. Jeremy Powell. These samples were placed on ice for the 10-minute drive to the laboratory. They were then refrigerated to prevent platelet activation. The same protocol for platelet isolation was followed. As in the second replication of the first trial, no platelet pellet was formed and observation using a microscope confirmed that the platelets had been destroyed. The intention was to attempt to understand what was causing the platelets to be destroyed during isolation, and to finally replicate the fusion from the first trial. However, future trials were rendered impossible by the campus-wide shutdown related to the COVID-19 pandemic.

## Results and Conclusion

For the first trial, observation through a microscope showed that most samples showed platelet lysis. However, both samples containing the 1:100 PRP to RPMI ratio treated with the 100% concentration of PEG appeared to have successfully fused. The successful fusion of these platelets cannot be definitively confirmed based solely on this trial, but several observations indicated fusion took place. Referring to Figures 1 and 2, the platelets are seen aggregating, but the cell membranes are present and easily viewable. The presence of the cell membranes indicate that fusion had not occurred at the time the images were taken. In Figure 3, the cell membranes dividing the platelets are not visible. If, in fact, the membranes separating each cell were no longer intact, it is reasonable to conclude that the fusion was successful.

These results illustrate that fusion is more likely to be successful using the 1:100 ratio sample of PRP treated with the 100% PEG concentration. The intention from this point forward was to attempt to replicate those results using the same dilutions and PEG concentrations. Unfortunately, during Trial 2 and the second isolation of the platelets from whole blood, no platelet pellet was formed, indicating the platelets may have been lysed or contaminated. Observation of the samples confirmed that the platelets had lysed as there were only small cell fragments visible. Similarly, the final attempt in Trial 3 to replicate the fusion seen in the first trial yielded no platelet pellets during the isolation process, indicating that the platelets had been destroyed. Observation under a microscope confirmed that the platelets had been lysed.

In future research, it would be advisable to use a more realistic volumetric ratio of PRP dilution to PEG concentration. The amount of PRP dilution added to each PEG concentration was too small to be used efficiently on a large scale. The potential for applying this research to a

commercial setting would greatly depend on finding a viable and more efficient ratio of materials.

Additionally, future research would benefit from the use of a stain kit. Using a stain would definitively confirm or disprove any possible fusion. Although the observation through the microscope and examination of the images taken during Trial 1 strongly suggest fusion took place, without using a stain, fusion cannot be confirmed with complete certainty.

Although the results of this research are definitively inconclusive, a number of considerations might be made. Firstly, fusion was likely achieved during the first of the three trials. Although it cannot offer an answer to the overarching question of this project, that achieving a “supercytoplast” is possible using platelets, the observed fusion may be treated as the rough proof-of-concept for future investigation. Importantly, the results of the first trial demonstrate that using PEG could be a viable method for fusing platelets. Interestingly and in contradiction with Yang and Shen (2006), the 50% concentration of PEG did not result in fusion. Instead, the 100% PEG concentration resulted in the fusion.

Additionally, future research on this topic need not be hindered by cost. The fact remains that platelets are far more accessible at a lower cost than oocytes. The high expenses associated with using oocytes for cell recombination is motivating to find a less expensive alternative. Platelets are plentiful and cheap which would justify the allowance of more trials focused on polishing and continuing the methods of this project.

It is necessary that the process of isolating the platelets be adjusted to consistently achieve platelet pellets before attempting anything further. Several factors could have caused the platelets to lyse. Among these unforeseen factors could be damage to the blood samples during transport or storage, mechanical stress caused by pipetting the PRP into different tubes, or

incompatible amounts of a stabilizing chemical in the RPMI solution called bovine serum albumin (BSA).

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## Figures

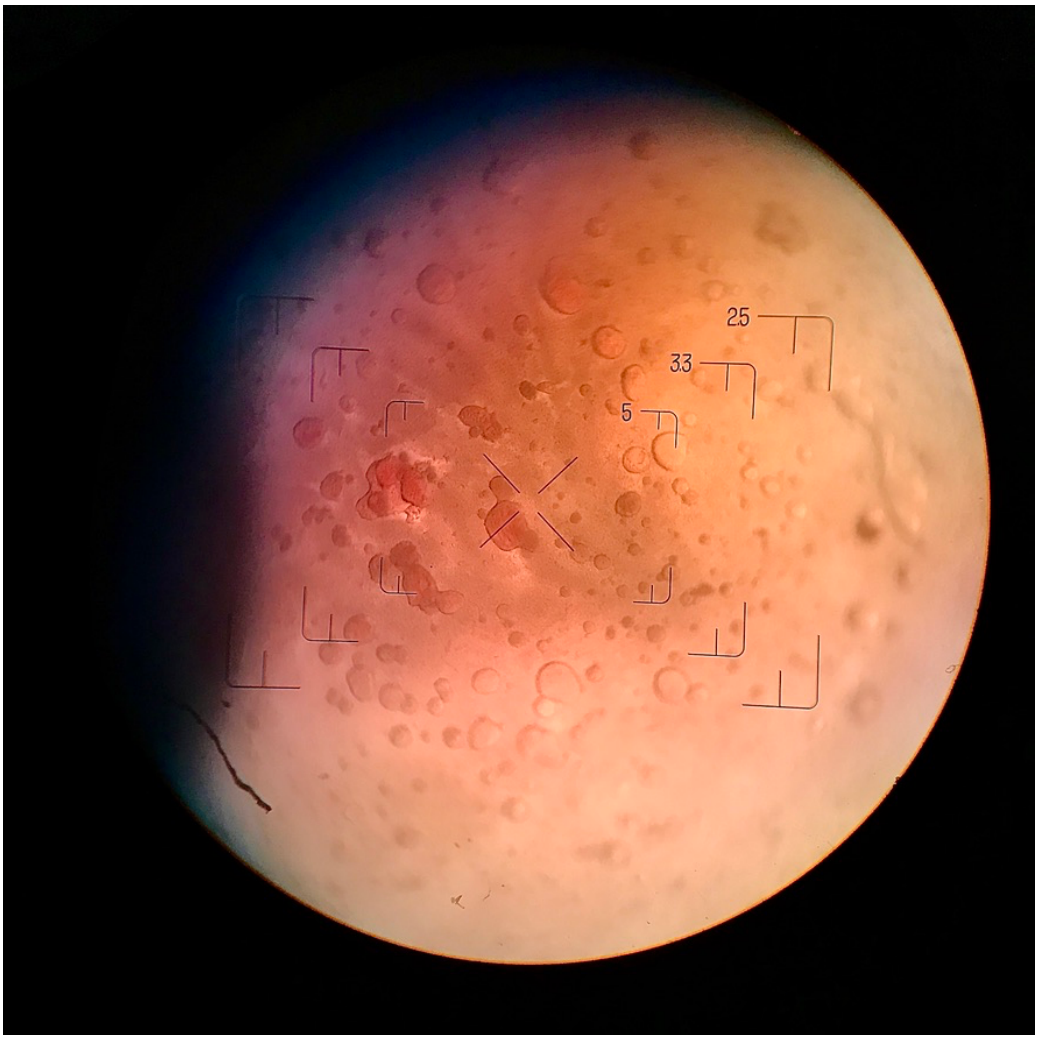


Figure 1. Several platelets have aggregated and begin to fuse approximately 1 minute after treatment with PEG.

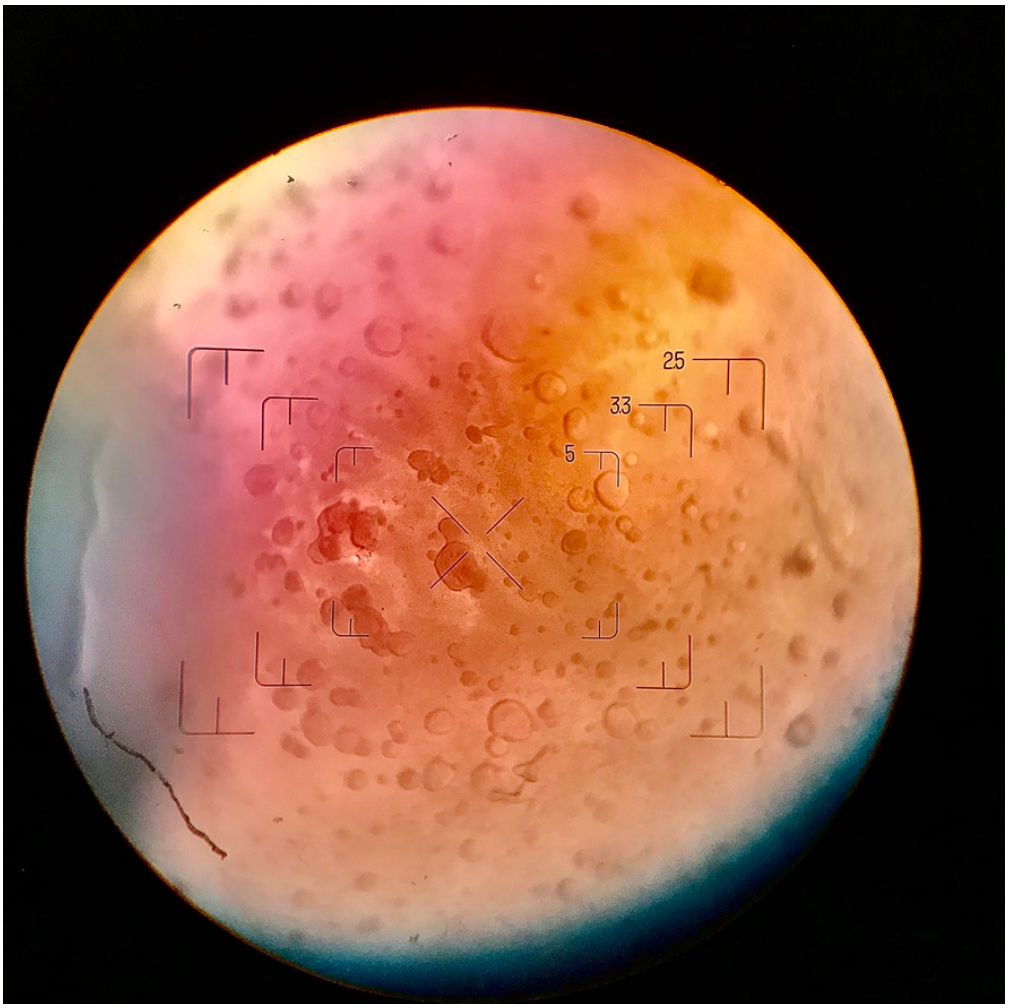


Figure 2. This image was taken approximately 1 minute and 10 seconds after treatment. No fusion progress is visible since the image in Figure 1 was taken.

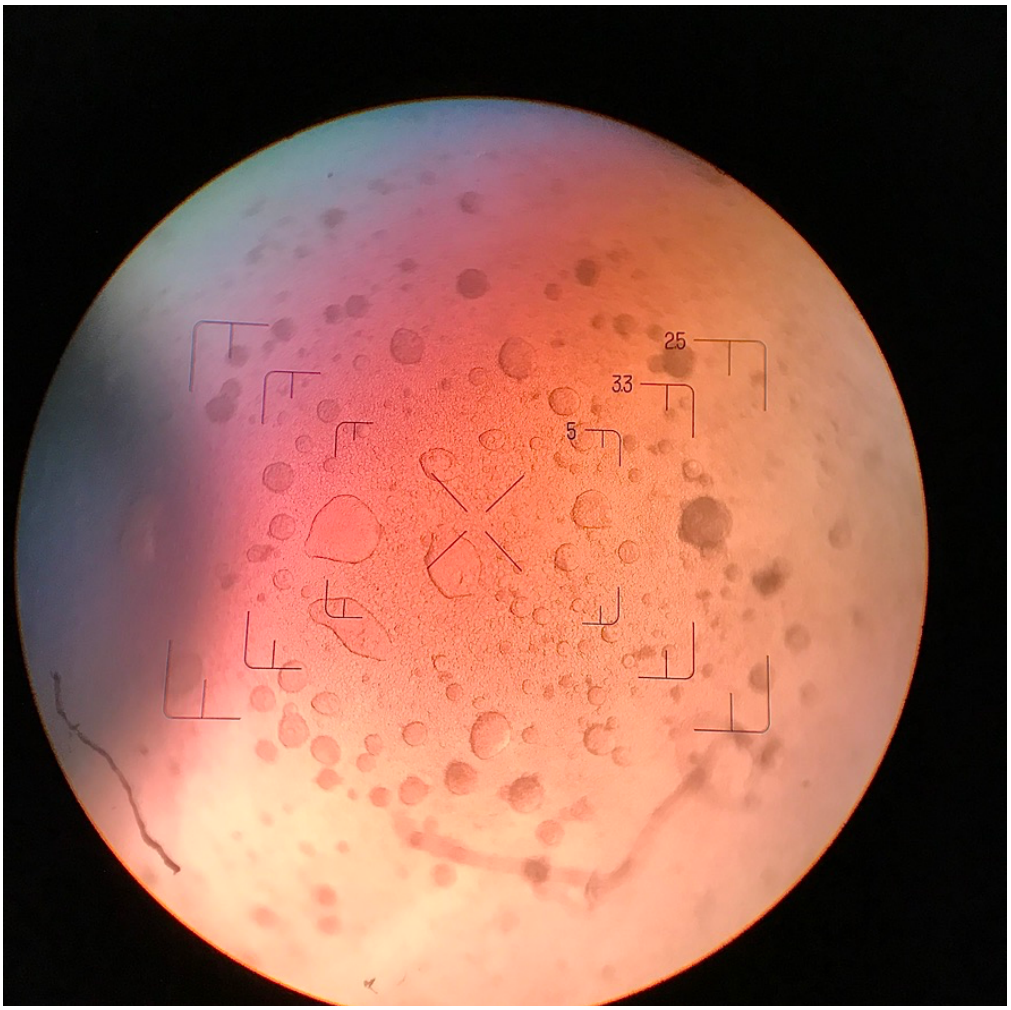


Figure 3. This image was taken approximately 3 minutes after treatment. The membranes separating the aggregated platelets have disappeared. Virtually all the platelet clumps have fused.