

**NANOTECHNOLOGY: THE FUTURE
OF FRONTLINE TRAUMA CARE?**

BY

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PASS WITH MERIT



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ABSTRACT

Blood loss is the leading cause of preventable death in combat casualties and the haemorrhage being the leading cause of death in civilian trauma cases.¹ Research in both medicine and bioengineering has focussed on developing chemical and mechanical agents to safely induce haemostasis, and prevent death due to excessive blood loss.

This paper will look at how recent advancements in nanotechnology have led to the development of synthetic platelets and clottocytes, two designs aimed to stop clotting without causing further damage to the tissue. It will also examine whether either of these designs could be a viable alternative for treatment in both civilian and armed forces trauma cases.

INTRODUCTION

Haemostasis is the process of stopping bleeding when there is damage to the endothelium of the blood vessel. There are three main stages: the vasoconstriction of the blood vessel to limit the flow of blood to the affected area therefore the volume of blood lost via the injury; the blocking of the open wall of the vessel by a platelet plug (FIG 1); finally the thrombosis (formation of a blood clot) which keeps the wound closed until it fully heals. The aim with nanomedicine's application to haemostasis is to speed up the second and third phase to form a temporary repair to the vessel to stop the loss of blood, without causing harm to the vessel or any other tissue around it.

Platelets can be 'activated' by colliding with exposed collagen from beneath the endothelium in damaged vessels, collision with the von Willebrand factor (vWF) protein secreted by the endothelial cells, or the presence of thrombin or negatively charged particles.²

Although a clot can begin to form in 15-20 seconds in severe trauma³, the whole process resulting in a fully formed clot can take 2-5 minutes, during which time enough blood can have been lost to cause shock and severe enough blood loss to be fatal. In battlefield trauma in particular, it is important that a clot is formed in the least possible amount of time and as safely as possible, because practical limitations such as treatment in the field does not allow for careful reparations of the blood vessels. Instead, the aim is to reduce or stop the bleeding moments after injury in order to improve chances of survival. Nanotechnology has proven that it has the capabilities to improve clotting time, but the technology currently in place such as aluminosilicate and chitosan particles have faults. Technology currently in development has the potential to drastically improve clotting time and reduce blood loss within moments of injury safely, therefore saving the lives of countless trauma patients.

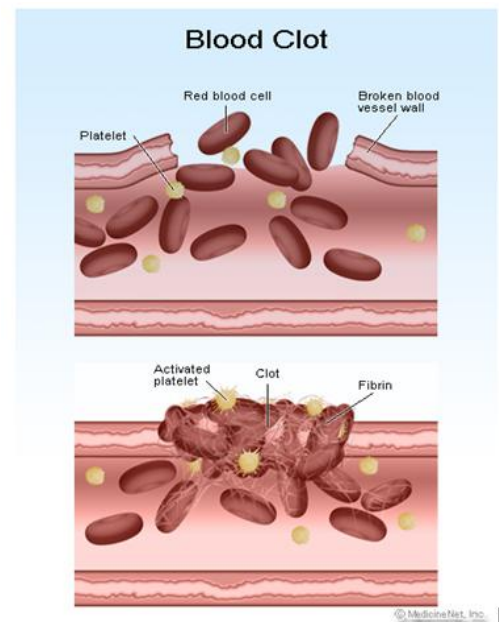


FIG 1

One of the current haemostatic agents used in military and civilian trauma is the QuikClot Combat Gauze bandage, developed by Z-Medica in order to control life threatening haemorrhages⁴. QuikClot uses aluminosilicate particles to absorb water and increase clotting factors and platelets at the site of injury.⁵ It is the number one recommended haemostatic agent by the COTCCC (Committee on Tactical Combat Casualty Care Committee)⁶ and carried by all branches of the US Military, as well as being distributed to some European countries. However, QuikClot has several major flaws, the first of which is a direct result of the science which makes it effective. Although Combat Gauze is less affected than the loose granules, the reaction which occurs in order to start the localised coagulation of the blood to form the clot is exothermic, and can therefore cause mild to severe burns. The second is its possible ineffectiveness when dealing with high pressure bleeds. These are issues I will examine in more detail further in the paper.

There are two new developments in nanotechnology that may provide alternatives to current haemostatic agents, and quickly reduce the number of deaths caused by excessive blood loss due to trauma. Synthetic platelets, as developed by Erin Lavik et al (2009) were synthesised by binding the polypeptide *poly(lactic-co-glycolic acid)-poly-L-lysine* to polyethylene glycol to form a nanoparticle approximately 170nm in core diameter.⁷ This synthesis uses peptides already in use within a medical context as well as being biodegradable, and therefore is thought to produce safer particles than Zeolite granules such as used in QuikClot.

The synthetic platelets are administered intravenously, and work by adhering to activated platelets, increasing the number of platelets at the site of injury. In rat models, they have been shown to reduce the clotting time by 23% when administered 20 seconds after injury.⁸ The polyester core of the nanoparticles induce clots by activating the coagulation cascade⁹ (the series of reactions which activate platelets) but the presence of PEG (FIG 2) in the nanoparticle reduces the reaction, allowing the synthetic platelet to reach the site of injury before forming a clot.¹⁰ It is thought that the surface charge of the particles and their aggregation in water is the mechanism by which they activate platelets and induce thrombi, caused by the PLGA-PLL core. (FIG 2)

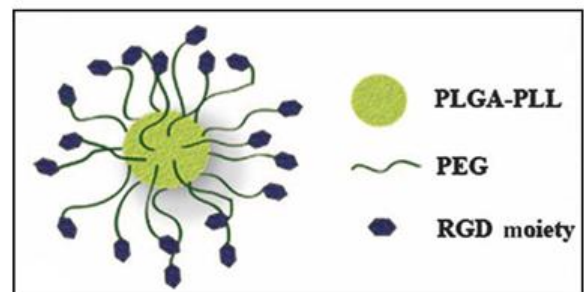


FIG 2

The developments in nanotechnology and polymer engineering have allowed synthetic platelets to be developed. The nanoparticles are smaller than a normal platelet which is due to their components' size, and as the components have already been approved by the FDA in other medical and bioengineering applications. This makes the likelihood of synthetic platelets being approved as safe for use in the human body high. It also prevents the clots from being unmanageably large, therefore becoming harmful to the casualty; this is

supported by the recommended dosage for the rat model. 10 mg/ml had no effect, but 40 mg/ml had cardiovascular and respiratory complications such as increased pulse rate and gasping. As a larger dose of the synthetic platelets has shown to be dangerous to the casualty, this would support the artificial platelets operating on a nanoscale because the larger particle size may have the same effect as the larger dose.

Clottocytes are a theoretical design by Robert A. Freitas Jr which are artificial, mechanical nanobots to simulate platelets, able to achieve complete haemostasis in one second. The nanobots – powered by serum oxyglucose – would contain a fibre mesh able to unfold near to the site of injury and adhere to the blood vessel. The clottocytes would be approximately 2 microns in diameter, roughly the same size as the body’s own platelets. The mesh itself would be biodegradable, and upon release, a soluble film coating the appropriate parts of the mesh would dissolve in contact with the plasma to expose sticky mesh (FIG 3). This ‘stickiness’ would be blood-group specific, in order to trap blood cells by binding to the antigens on the blood cells’ (specifically red blood cells) antigens (FIG 4). Each mesh would overlap with a neighbouring mesh and attract enough blood cells to immediately stop bleeding, requiring no additional or increased concentration of cells.

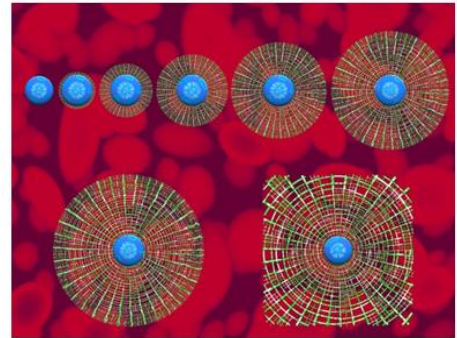


FIG 3

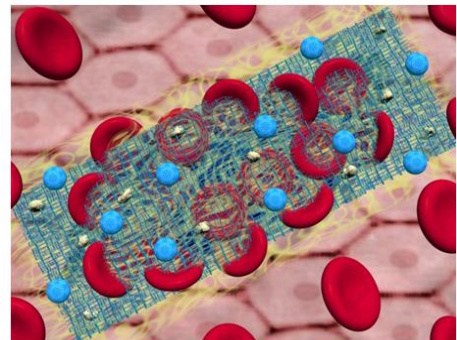


FIG 4

DISCUSSION

When trauma is the leading cause of death in people under the age of 45 in the US¹¹, it stands to reason that resources and ideas must be pooled to research new treatment and lifesaving options. Developments such as synthetic platelets and clottocytes have the potential to vastly improve response to trauma, and limit the number of deaths caused by excessive blood loss. Although the method of administration is most likely to be intravenous for both, if paramedics/Army Medics are trained to administer them safely, they could increase the rate of haemostasis enough to allow the casualty the chance to receive professional care in a sterile or at least safer environment.

Long-standing treatments of battlefield trauma are tourniquets and direct pressure. However, problems arise when traumatic injuries are suffered in places inaccessible by a tourniquet. Also, a tourniquet is not complex enough to discriminate between blood vessels; its primary aim is to prevent blood from reaching the damaged vessel, but the collateral damage is the constriction of other vessels in the limb. 'Tourniquet Injuries' are usually compression injuries to the limb such as to the skin, muscle, nerves and blood vessels¹². Research shows that if tourniquets are released by someone other than a medical professional, they can release blood clots or toxins into the main bloodstream¹³. In any case, the application of a tourniquet should be considered a last resort due to possible injuries and chance of loss of limb below the tourniquet, and only applied by physicians and paramedics. However, it is clear the tourniquets, although commonly used in the military are not always practical, and safer alternatives need to be considered. Nanotechnology offers the potential to stop clotting without the risk of damaging surrounding tissue as is the case with tourniquets.

QuikClot and HemCon are two haemostatic agents currently used by Armed Forces and civilian medics in traumatic injuries. However, both have associated risks and limitations which could make them less effective in trauma situations.

HemCon is a haemostatic bandage which works as the polysaccharide chitosan particles infused in the bandage have a positive charge, which attracts the red blood cells. The cells are drawn into the bandage and a tight seal is formed around the wound, preventing more blood from being lost. The chitosan also has antibacterial properties, making it an effective bandage for use in trauma. The bandage can be left in place for up to 48 hours, making it useful for battlefield trauma, if the wound needs to remain sealed until it can be operated on. However, the bandage takes 2-5 minutes to stop bleeding and natural haemostasis can occur in 2-5 minutes or up to 9-10 minutes if aspirin is present¹⁴, so the bandage does not drastically improve clotting time. Also, due to chitosan being derived from shrimp shells, it is not considered kosher. On the other hand, the bandage does not interfere with the body's natural clotting cascade, so the wound can heal naturally once the bandage is removed.

QuikClot has more significant associated risks. The reaction in basic QuikClot granules had an exothermic reaction, causing mild to severe chemical burns (FIG 5) in some casualties. It has been established that QuikClot is ineffective in high pressure bleeds, such as those to arteries. Given the severity of traumatic injuries and the likelihood that they will sever an artery, especially in battlefield trauma, the effectiveness of QuikClot should be questioned. An Iraqi woman was shot near the brachial artery, and the QuikClot dried and flaked off, and the corpsman treating her found standard pressure to be more effective. Another case is of a woman shot in the femoral artery and QuikClot was unable to be effectively applied due to the high pressure of the bleed¹⁵. The previous QuikClot granules in its powder form was documented to have caused embolisms in some patients due to its free granular form, increasing already unstable patients' risk of a transient ischemic attack (or "mini-stroke").



FIG 5

A major problem with both haemostatic bandages is that their applications are limited to external use, and to areas which can be treated with direct pressure. For wounds where this is difficult, HemCon and QuikClot are no more effective than a standard gauze bandage.

Robert A. Freitas Jr's artificial mechanical platelet design may be considered to be part of the future of induced haemostasis. Their size relative to natural platelets means they can operate on a cellular level with other blood components and move safely around the vascular system without blocking any vessels. As explained earlier in the paper, it is speculated that clottocytes could induce complete haemostasis in approximately 1 second as this is the amount of time it has been estimated the net would take to unfurl and adhere to the damaged vessel wall¹⁴. Due to the predicted density of the clottocytes, each nanobot would be no more than 370 microns away from each other¹⁴, so if the vessel wall is severely damaged, enough devices could work together, each net overlaying with its neighbours' to ensure that the bleeding was stopped. In trauma situations, this would allow for the injury to be haemostatically stabilised in order for the casualty to be transported to a hospital. Although current design limitations mean the nanobots would be limited to use for penetrating injuries and would not currently work with internal bleeding, their presence and speed could reduce the time taken to treat the patient on-scene, providing more time for the transportation and treatment at a hospital or by surgeons during the first 'golden hour' (promoted by *Dr. R Adams Cowley* at the University of Maryland Shock Trauma Center).

Clottocytes would need to ensure that the onboard mesh was not released in the wrong place or at the wrong time. There are several possible ways to manage this; for one, the partial pressures of oxygen and carbon dioxide are significantly different inside the bloodstream and outside the body¹⁴. If the onboard computer system could manage this, it could manage when to deploy the net of fibres.

The risk of the mechanical platelets operating at a much quicker rate than the body's natural platelets is that the two could interfere so it has been proposed that the clottocytes would have to release certain chemicals to signal to the natural platelets to continue at a normal or increased speed, which would include controlling the platelets throughout the stages of haemostasis¹⁴.

The other main associated risk of the clottocytes is that the additional activity of the mechanical platelets triggers DIC (disseminated intravascular coagulation) which is essentially an increased level of thrombin being released into the body's circulation other than the site of injury, which overrides the body's natural control and results in multiple microthrombi. The microthrombi can cause platelet and fibrin activation and can eventually lead to haemorrhage. The proposed solution to this is for the onboard computer to monitor decreases in fibrinogen or increased levels of thrombin, both of which have been documented to suggest a diagnosis of DIC¹⁴. If increased levels of thrombin or other DIC conditions are found, the clottocytes could ideally metabolise the excess thrombin, or releasing inhibitors to suppress the process.

However, there is another significant issue more relevant to the proposition of clottocytes being used in cases of trauma. The immediate haemostasis would mean sealing the vessel, which although is the aim when preventing death due to excessive blood loss, the mechanism by which it does so also seals in any ballistics/foreign objects. These may circulate around the body, either causing other trauma to vessels or organs, or causing an infection due to bacteria on the object. Although Robert A. Freitas Jr suggests that clottocytes are merely one part "*the complete haematological upgrade package*" and other artificial components could eventually be manufactured to clean and dispose of these objects¹⁴, other than artificial red blood cells (respirocytes) they have yet to be designed even theoretically. This raises concerns as to whether clottocytes would be safe to use on their own, which also depends on the question; which is more dangerous to a patient with traumatic injuries, the immediate problem of blood loss, or the possibility of further damage and infection?

Although critics may argue that it is a waste of resources to pay presumably large amounts for the clottocytes then to have them cause additional problems which require further funding to rectify, it may be raised that this is still the case with some haemostatic agents in use. These products are championed by Armed Forces medics regardless of critics, such as QuikClot and HemCon and their associated problems discussed earlier. Also, although clottocytes may not be suitable for civilian casualties where the risk of primary infection is at the forefront of most medics when treating trauma, the same cannot always be said for injuries sustained in battle. It has been repeatedly said that the most important thing in battlefield medicine is to stop the bleeding as quickly as possible, as if the bleeding is stopped long enough for the casualty to receive professional attention, they have a greater chance of survival. If attention is given within the first 'golden hour' then their chances of survival are increased dramatically, and they can be transported quicker once the bleeding is under some control. So in battlefield

trauma, should the potential of infection caused by foreign objects which can be removed in a more sterile and less urgent situation be more important than immediate haemostasis and stabilising a casualty long enough to gain further medical assistance?

It has yet to be decided when clottocytes would need to be administered. Practically, they would be required to be transfused intravenously, but would need to be in some form of solution in order to be transfused, such as saline. But to be effective, the clottocytes would need to be administered prior to injury, so until the 'complete haematological upgrade', it would be impractical and expensive to administer the clottocytes to every citizen, which raises ethical issues. Who is more likely to suffer traumatic injuries leading to blood loss, and are they more deserving than others? Soldiers in the Armed Forces would be highly likely to suffer traumatic injuries if in an active warzone, and clottocytes could provide them the time to be evacuated and receive life-saving medical attention. I have already proposed that clottocytes may be more suitable in battlefield trauma than civilian due to the associated risks, but should the transfusion be limited to those on the frontline? Also, there are civilians who are likely to suffer traumatic injury such as those working with industrial machinery, law enforcement officers, and contact sport players. If trauma is the leading cause of death for persons under the age of 45 in the US, why would only some people receive pre-emptive treatment? There are many factors to consider – both practical and ethical – before clottocytes can be determined to be part of the future of induced haemostasis, but if these issues can be researched and reviewed, clottocytes have been designed to possess the properties required of a good haemostatic agent.

Synthetic platelets are composed of the same polymer used in dissolvable sutures, surrounded by a water soluble polymer used in food and drug products. The molecules surrounding the polymers help the synthetic platelets only bond to the platelets involved in the clotting, to avoid uncontrollable/unwanted clots which could lead to embolisms¹⁶, making them theoretically safe for use in the human body.

Though all nanoparticles injected into the body pose some risk of nanotoxicity, the developers have taken some measures to reduce the risk of this as much as possible. The synthetic platelets are surrounded by a shield of water to prevent them binding to each other and forming clots which could lead to embolisms, and tests have shown that excess synthetic platelets have been flushed from the body within 24 hours in rat models¹⁷. None of the artificial platelets were found to be present after 7 days¹⁸. The synthetic platelets do not activate natural platelets under normal circumstances, limiting the risk of the casualty developing a thrombosis¹⁵. Despite the central polyester nanoparticle's proven ability to induce thrombosis, these effects have been limited by the surrounding PEG 'shield' which prevents it

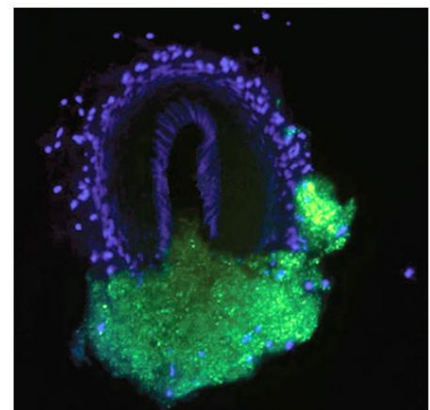


FIG 6
(green shows clot consisting of artificial platelets, blue shows lumen of vessel)

from altering normal clotting behaviour, and allows the artificial platelets to travel to the site of injury before beginning to bind to the body's own platelets¹⁵.

Natural platelets have a short shelf life and must be kept refrigerated, as well as requiring administration in a hospital, making them impractical for battlefield trauma. Synthetic platelets on the other hand have been shown to have a shelf life of approximately two weeks, and can be stored at room temperature¹⁹. They can be used as a free-standing treatment to treat acute bleeding, or in conjunction with a natural platelet transfusion to decrease the amount required, and increase platelet concentration.

Synthetic platelets also have one thing in common with clottocytes which make them significantly more useful than existing haemostatic agents; they can be used to treat internal injuries, and can therefore be used in blunt force trauma injuries as well as sharp force trauma. They can be administered to injuries which make application of pressure difficult, and therefore can make a difference in the more difficult to treat injuries using existing methods.

The artificial platelets are required to be administered intravenously post-trauma to function well, but the lack of requirement for continuous transfusion and storage instructions means that they are an ideal candidate for haemostatic agents in battlefield. Proven to be stable in normal conditions both in the body and in storage, their lack of platelet activation when not required, their quick clearance from the body and their unique ability to treat internal injuries suggests that they would be a useful and less harmful addition to the battlefield and civilian trauma kit to make a significant impact on prevention of death due to excessive blood loss.

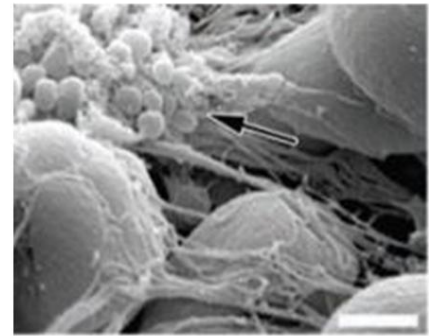


FIG 7
(arrow shows synthetic platelets integrated into clot)

CONCLUSION

As I have thoroughly discussed above, it is clear that although nanotechnology's use in trauma care is still in its early stages, it has a huge amount of potential to improve the treatment of casualties. If clottocytes and synthetic platelets can be developed and integrated into trauma kits of civilian responders and Armed Forces field medics, they have proven themselves to have the capabilities to vastly decrease the time taken for complete haemostasis. Though they would be limited to trained personnel due to the administration method, their ability to work without causing damage to the vessels makes them an innovative idea in the field of trauma care.

Clottocytes are still in the theoretical stages, which means that they would not be available for use for many years, as they have yet to be manufactured and safety tested. So although theoretically they have the potential, it has yet to be seen as to whether they would be practical in real situations, and whether being administered post-trauma would still improve clotting time.

Synthetic platelets are said to be more than ten years away from commercial use, but since the developers have been awarded a \$2.3 million grant as part of the Innovator award in September 2010²⁰ it is clear that their development is crucial, and could make a revolutionary change to trauma care on both the home front and the battlefield.

Both designs have practical issues to be considered and resolved, but they are hindered by funding and demand. Their use and promise once manufactured depends on contract negotiations, and how much governments are prepared to pay in order for the chance to improve trauma care, and whether their benefit outweighs the expense.

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FIG 1: MedicineNet Inc

FIG 3: Tim Fonseca 2003

FIG 4: Tim Fonseca 2003