Synthetic platelets: can bioengineering realize in few years what evolution made in over 200 million years?

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Platelets are essential in the maintenance of hemostasis and vascular integrity, and thus platelet transfusions have been used for more than a century to treat thrombocytopenia and to interrupt or prevent bleeding.[1] Currently, more than 2 million platelet transfusions are administered yearly in the USA,[2] often as emergency treatment for uncontrolled bleeding in subjects with a normal platelet count.

Despite progress in processing and storing platelet components, there are still significant limitations and risks associated with platelet transfusions, including immunogenicity, possible bacterial or viral contamination, febrile non-hemolytic transfusion reactions, transfusion-associated circulatory overload, and transfusion-related acute lung injury.[3] Moreover, due to donor dependency and limited shelf life, there is often a shortage of platelet concentrates for blood transfusion, as occurred critically during the recent COVID-19 pandemic.[4]

For all these reasons, an intensive search for alternative sources of platelets for transfusion purposes, either through methods of *in vitro* production of platelet concentrates or by the manufacturing of artificial platelet-mimetic materials, has been undertaken over the last few decades.

While the first approach has seen significant progress, with the first in-human clinical trial of *in vitro*-generated, induced pluripotent stem cell-derived platelets,[5] there are still major barriers to its generalized clinical use for transfusion purposes. These barriers include long production times, high costs, and uncertainties about pro-hemostatic efficacy and circulatory capacity.[6,7] Therefore, the production of artificial platelets may represent a promising alternative, especially for complex clinical settings such as in traumatology or remote locations, like war theaters, because they may be compatible with all blood groups, preservable for long periods, and have production costs lower than those required for *in vitro* platelet generation.

A recent study by Nellembach and co-workers reports on the development, safety, and efficacy of ultra-soft platelet-like particles as pro-hemostatic agents in various preclinical models of acute bleeding.[8] The basic principle is that platelet-like nanoparticles (PLPs), formed by highly deformable microscale colloidal hydrogels about 600 nm in diameter cross-linked to fibrin-specific antibody fragments, may recapitulate important platelet functions, like the ability to induce clot retraction.

The authors optimized the production of biomimetic biomaterials composed of ultrasoft poly(N- isopropylacrylamide) microgels coupled to an antibody fragment with high specificity for activated fibrin and little or no affinity for circulating fibrinogen. The fibrin-binding antibody facilitates targeting to sites of tissue injury. The microgel body of the microparticles tends to collapse from a spread to a more energetically favorable spherical conformation, allowing them, when bound to the fibrin fibers of a forming clot, to exert strain, thus generating clot retraction. In this way, they enhance clot stability and favor wound healing.

The authors showed that the incorporation of these PLPs into clots formed *in vitro* from purified fibrinogen and thrombin enhanced clot density. They then intravenously infused these PLPs into normal mice, showing that they were cleared from the circulation within one hour, primarily through the kidneys, while in mice with induced liver laceration injuries, PLPs accumulated mostly in the wound tissue with no signs of off-target clot formation. They then tested the pro-hemostatic efficacy of PLPs in a severe murine liver laceration model, showing a significant decrease in blood loss, an effect also observed in animals with dilutional coagulopathy induced before liver injury. Interestingly, in a less severe liver injury model, PLPs were compared with an equal number of transfused platelets and were found to reduce blood loss more effectively. Moreover, seven days after injury, wound areas were smaller in PLP-infused animals compared with controls, suggesting an improved healing response. Finally, PLPs were infused into pigs after a liver injury was inflicted, to mimic the potential real setting of clinical use, and it was shown that they reduced blood loss compared with both saline-infused and platelet-transfused animals with apparently no side effects.

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While these results seem very promising, several points deserve further investigation. These include the safety of repeated administrations, as would likely be used in a clinical setting, a thorough comparison with platelet transfusions at conventional therapeutic dosages, and safety and efficacy in conjunction with currently used pro-hemostatic interventions. It is also important to assess their ability to target wound tissue in conditions of severe coagulopathy, such as in severe hemophilia or disseminated intravascular coagulation patients.

Moreover, although remarkably able to reproduce some specific activities of blood platelets, such as wound targeting and clot retraction, PLPs lack the myriads of specialized actions that platelets display in primary and secondary hemostasis. These actions include the trophic function on the endothelium,[9] the ability to migrate within the clot and exert microbicidal activity against infiltrating pathogens,[10] the capacity to release cytokines and cooperate in the immunological response,[11] the ability to release growth and angiogenic factors favoring wound healing,[12] and the capability to elicit blood clotting activation,[13] among many others.

These multifaceted and highly specialized functions of mammalian platelets in host defense have developed over several hundred million years,[13] and it is unlikely that even the most sophisticated biotechnologies will be able to produce artificial platelets endowed with all the crucial properties of real platelets.

An interesting alternative approach being explored is the engineering of circulating blood platelets by loading them with molecules that will be delivered to the site where platelets get activated. This approach is actively investigated for cancer therapy, given the high affinity that platelets display for several types of tumors,[14] but has also been exploited to enhance the natural pro-hemostatic function of transfused platelets through the loading with liposomal thrombin. The modified platelets became more responsive to stimulation and improved clotting even under conditions that cause platelet dysfunction or impaired coagulation.[15]

In conclusion, while it is unlikely that to all effects platelets will be artificially synthesized in the foreseeable future, it is conceivable that man-made platelet-like particles or engineered whole platelets will become useful as pro-hemostatic agents as an adjunct to platelet transfusions and other anti-hemorrhagic therapeutics.

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