Lyophilized iPS-derived platelets as a hemostatic agent for uncontrolled bleeding

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Introduction

Uncontrolled hemorrhage caused by warfare, trauma, surgery, or thrombocytopenia is a major contributor to morbidity and mortality, especially in military, prehospital, and resource-limited settings. In these environments, conventional liquid-stored platelets are frequently unavailable because of their short shelf life of only 5–7 days and the strict requirements for continuous refrigeration and agitation. Lyophilized (freeze-dried) platelets address these limitations by permitting room-temperature storage for more than one year, enabling rapid reconstitution, and providing immediate procoagulant activity as a ready-to-use hemostatic agent [1,2]. In addition, the generation of platelets from stem cells—independent of donor—offers a potential solution to further constraints on platelet supply for hemostatic and resuscitation applications. The present study therefore aims to develop an effective freeze-drying method for platelets while developing a commercially viable and cost-effective method for producing platelets from induced pluripotent stem (iPS) cells.

Materials & Methods

In order to generate platelet from human undifferentiated iPS, the cells were genetically modified to conditionally overexpress a combination of genes including SCF etc using Tetracycline-On (Tet-On) system. The modified iPS cells were normally maintained on Matrigel and induced to differentiation by adding doxycycline (DOX) following activation by cytokine BMP4, FGF2 and GSK3 inhibitor. Platelets were collected during 10 to 14 days. For lyophilization, both the iPS derived and donor platelets were incubated in a trehalose-containing solution (30-300mM) for 2 hours at room temperature, followed resuspended in a protecting medium containing 8% Dextran 40 and 2% BSA, then dehydrated on a freeze drier at -40 ^{o}C chamber temperature, 0.5mBar for 36 hours, followed by gradual increase of temperature to 25 ^{o}C at a rate of 0.25 ^{o}C /min and maintained for 15 hours. Platelets samples were analyzed for surface antigens CD41a,CD42b and CD62p by flow cytometry, aggregation and thrombin generation functions. NOD-SCID mouse model was used to evaluate the lyophilized dried platelets for hemostatic property.

Results

Following our protocol iPS can generate functional platelet after 10 days in the differentiation culture (Fig.1). The platelet morphology appeared normal with diameter 2.9±0.8 um , and >90% of the collected platelets showed double positive for surface makers CD41a and CD42b. Upon activation by ADP and collagen, the platelets aggregated at levels higher than the fresh adult donor platelets. Thrombin generation is comparable to the fresh control.

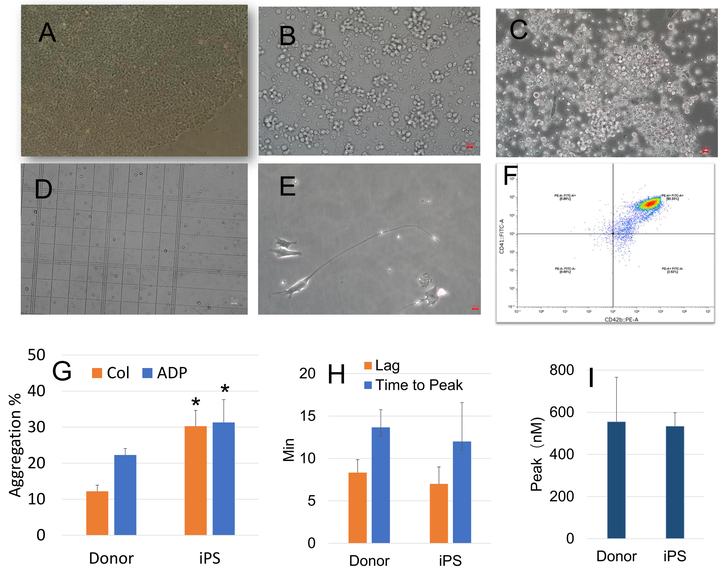

Fig.1 Platelets generated from iPS cells. Morphological change during the 10-day culture (A-C). Platelets show normal particular shape(D). Proplatelet attaching on the culture plate (E). Flow cytometric analysis showed >90% double positive for CD41a and CD41b. Thrombin generation is comparable to donor platelets (G-I). *: p<0.05, comparing to donor control.

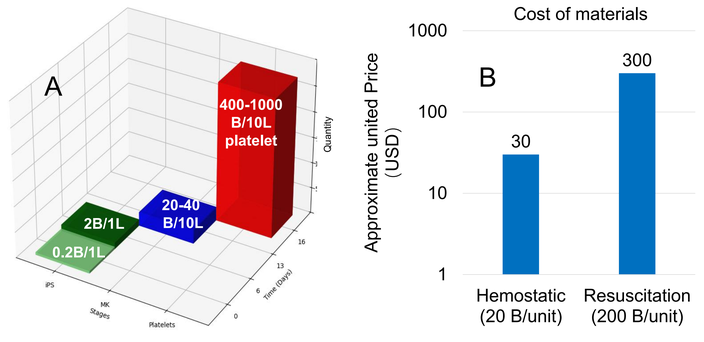

Fig.2 illustration of platelet production process in current laboratory setting (A) and estimated materials cost based on the best available non-GMP grade raw materials (B).

Comparing to conventional approaches, the bioengineered iPS cell line enables dramatically reduction of material cost (>50x) and labor cost (>2.5x). Commercial scale bioreactor processes may further improve productivity and cost effectiveness.

Both donor and iPS derived platelets were lyophilized following our proprietary method (Fig.3 A-F). The lyophilized platelets retained membrane integrity and membrane glycoprotein expression, but high CD62p expression. In vitro, they displayed unchanged aggregation upon activation by arachidonic acid, adenosine diphosphate, collagen and epinephrine (p>0.05), and increased thrombin generation in donor platelet. In vivo, administration of the lyophilized platelets significantly reduced 80% blood loss and 50% bleeding time compared to control (p <0.05) in the rodent models (Fig 3. G-I).

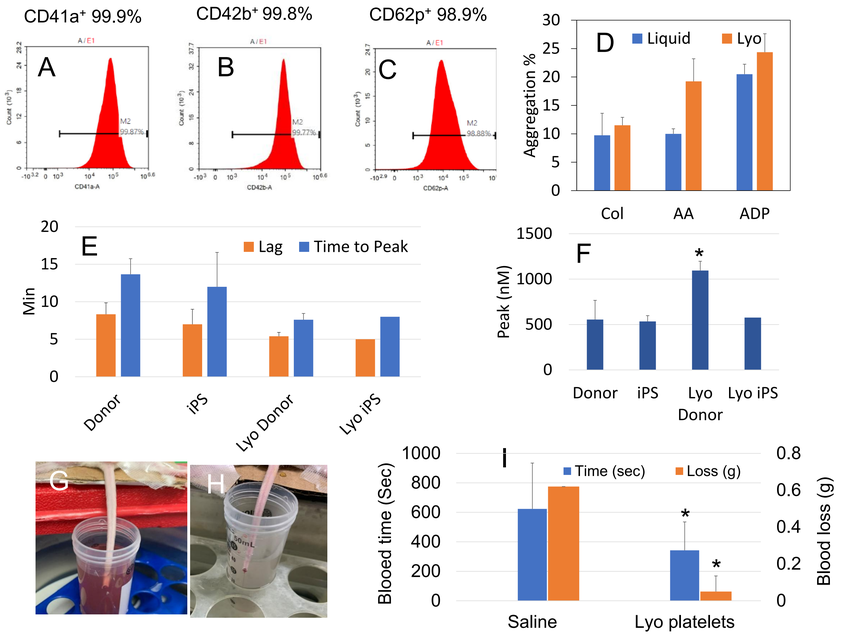

Fig.3 Lyophilized platelets displayed normal levels of membrane glycoproteins CD41a and CD42b and high CD62p. Aggregation was not changed (p>0.05). Thrombin generation was comparable before and after lyophilization for both donor and iPS platelets except for the lyophilized donor platelet showed higher peak value (E-F).*: p<0.05, comparing to donor control. Mouse tail cut model shows bleeding (G,H). Bleeding and blood loss were dramatically reduced after administration of lyophilized platelet (I) .

Conclusion

Lyophilized platelets derived from donor or iPS stem cells represents as a potential transformative option for warfare and traumatic hemorrhage in acute and resource-limited settings. The high efficiency and cost effectiveness of generating functional platelet from iPS cells indicates the potential for clinical and commercial development.

References

[1] Fitzpatrick GM, et al. Transfusion. 2013;53(Suppl 1):100S–106S.
[2] Wolkers WF, et al. Cryobiology. 2001;42(2):79–87.