

# Procenta Binds and Immobilizes Human Platelets for Concise Delivery to Soft Tissue Injuries

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## Introduction:

The fields of biologics, which spans an array of fields including orthopedics, wound care and surgery had three principal focuses: scaffolds, progenitor cells, and soluble factors<sup>1-4</sup>. Depending on the clinical need, physicians select their therapeutic route based on which of these conditions are already satisfied. Often, the delivery of platelets to the treatment site is used as a method to deliver autologous growth factors in a time-release fashion to stimulate a native or co-delivery cell population (e.g. chondrocytes verse bone-marrow aspirate concentrate)<sup>5-7</sup>. However, these platelet preparations, known as platelet-rich plasma, are in a fluid form and readily disperse/diffuse from the delivery site. As a result, clinicians seek a scaffold which will readily bind platelets and retains them at the delivery site<sup>8</sup>. Here, we examine the ability of Procenta, a conformable soft-tissue allograft to bind platelets for delivery.

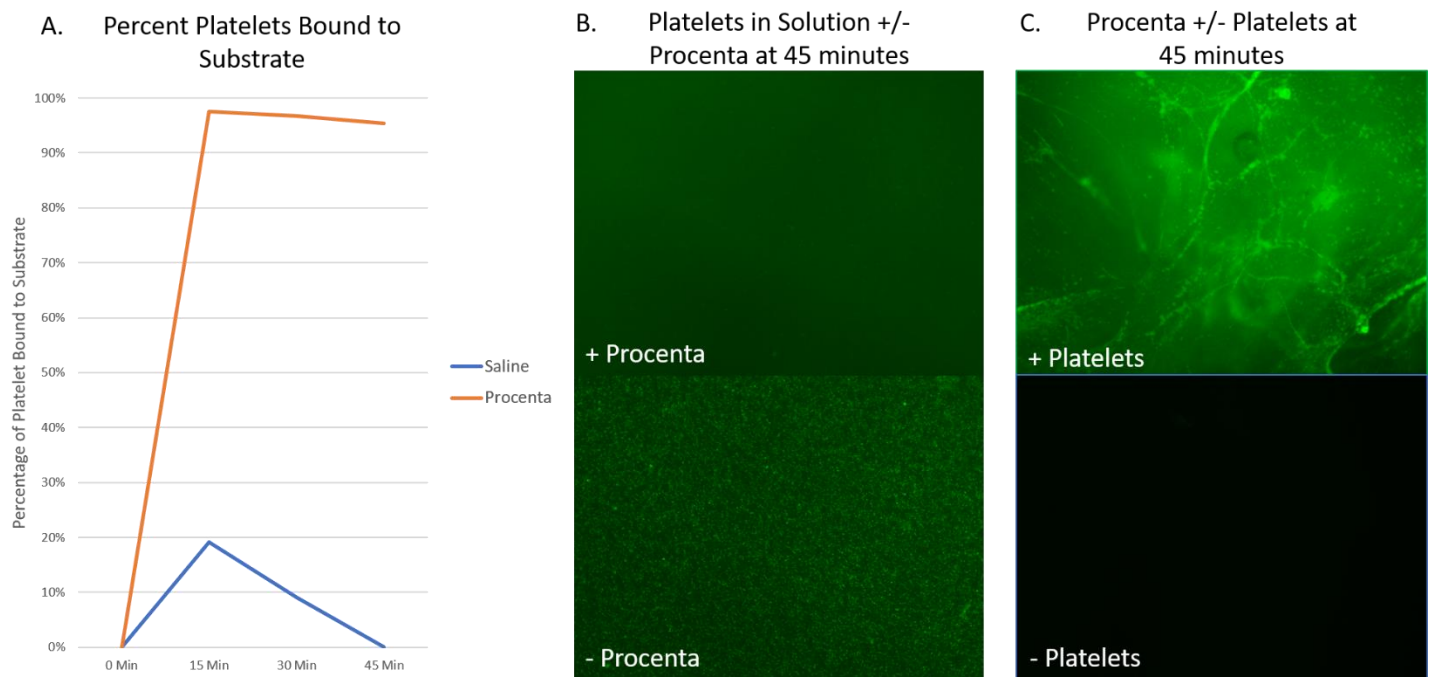
## Method:

A total of 38 mL of whole blood was collected into acid citrate solution diluted to a final concentration of 15% by volume. The specimen was centrifuged at 1,150 xg for 5 minutes and the plasma fraction (approximately 20mLs) was collected. The plasma was split into two 10mL aliquots and further centrifuged at 2,200 xg for 5 minutes. The resulting platelet pellets were resuspended in 1mL phosphate-buffered saline (PBS). A unit of Procenta matrix (approximately 250mg) was added to one of the tubes containing platelets suspended in 1mL PBS. A total of 0.25 mL PBS was added to the second tube containing 1mL PBS and platelets to account for volume added by Procenta in the comparative condition. Both tubes were incubated at 37°C in a shaking water bath for 45 minutes and 10µl of the platelet suspension was collected at time points: 0 minutes, 15 minutes, 30 minutes and 45 minutes. The sample was incubated with APC-conjugated CD61 (diluted 1:20) and Calcein AM (2.5µM) for 45 minutes, diluted 1:100, then analyzed via flow cytometry. Following the 45-minute time point and sample collection as described above, Procenta was removed from and placed in a 12-well plate. The Procenta tissue and residual platelet suspensions from each tube along with unexposed Procenta (to account for autofluorescence) were imaged using an AMG EVOS FL microscope and GFP light cube (Ex. 470/22 Em. 510/42).

## Results:

Platelet counts in solution declined rapidly within the first 15 minutes when exposed to Procenta. This phenomenon was not observed in the control platelet suspension lacking scaffold (Figure 1A). There were no significant changes in platelet counts in suspension at the 30 and 45FF minutes time points compared to the respective counts at 15 minutes for each condition. Imaging at 45 minutes showed a robust signal from free, unbound platelets in the control solution and few from the solution exposed to Procenta (Figure 1B). Further, imaging of Procenta following incubation for 45 minutes in the platelet solution showed robust Calcein signal, indicating platelet attachment to the scaffold (Figure 1C). Procenta from a sample not exposed to platelets showed no autofluorescence.

Figure 1:



#### Discussion:

Based on the parameters of this experiment, platelets appear to rapidly attach to the matrix components of Procenta. Here, we show via flow cytometry that platelets in suspension are reduced by >95% within 15 minutes and remains unchanged over the examined time course. This indicated platelet interaction with the available scaffold, where 95% were bound to Procenta, which was further verified by fluorescent microscopy. Once bound to matrices, such as collagen and hyaluronan, platelets are known to become activated and begin secreting soluble factors from their intracellular granules<sup>9-13</sup>.

#### Significance:

While further investigation is necessary, this data suggests the potential of allogeneic tissue scaffolds for the concise delivery of platelets to sites of clinical interest.

#### References:

- 1 Tada, S., Kitajima, T. & Ito, Y. Design and synthesis of binding growth factors. *Int J Mol Sci* **13**, 6053-6072, doi: 10.3390/ijms13056053 (2012).
- 2 Tollemer, V. *et al.* Stem cells, growth factors and scaffolds in craniofacial regenerative medicine. *Genes Dis* **3**, 56-71, doi: 10.1016/j.gendis.2015.09.004 (2016).
- 3 De Witte, T. M., Fratila-Apachitei, L. E., Zadpoor, A. A. & Peppas, N. A. Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. *Regen Biomater* **5**, 197-211, doi: 10.1093/rb/rby013 (2018).
- 4 Dzobo, K. *et al.* Advances in Regenerative Medicine and Tissue Engineering: Innovation and Transformation of Medicine. *Stem Cells Int* **2018**, 2495848, doi: 10.1155/2018/2495848 (2018).
- 5 Sakata, R. & Reddi, A. H. Platelet-Rich Plasma Modulates Actions on Articular Cartilage Lubrication and Regeneration. *Tissue Eng Part B Rev* **22**, 408-419, doi: 10.1089/ten.TEB.2015.0534 (2016).
- 6 Kim, S. J., Song, D. H., Park, J. W., Park, S. & Kim, S. J. Effect of Bone Marrow Aspirate Concentrate-Platelet-Rich Plasma on Tendon-Derived Stem Cells and Rotator Cuff Tendon Tear. *Cell Transplant* **26**, 867-878, doi: 10.3727/096368917X694705 (2017).
- 7 Dhillon, M. S., Behera, P., Patel, S. & Shetty, V. Orthobiologics and platelet rich plasma. *Indian J Orthop* **48**, 1-9, doi: 10.4103/0019-5413.125477 (2014).
- 8 Solovieva, A. *et al.* Immobilization of Platelet-Rich Plasma onto COOH Plasma-Coated PCL Nanofibers Boost Viability and Proliferation of Human Mesenchymal Stem Cells. *Polymers (Basel)* **9**, doi: 10.3390/polym9120736 (2017).
- 9 Harrison, S. *et al.* Platelet activation by collagen provides sustained release of anabolic cytokines. *Am J Sports Med* **39**, 729-734, doi: 10.1177/0363546511401576 (2011).
- 10 Roberts, D. E., McNicol, A. & Bose, R. Mechanism of collagen activation in human platelets. *J Biol Chem* **279**, 19421-19430, doi: 10.1074/jbc.M308864200 (2004).
- 11 Toyoda, T. *et al.* Direct activation of platelets by addition of CaCl<sub>2</sub> leads coagulation of platelet-rich plasma. *Int J Implant Dent* **4**, 23, doi: 10.1186/s40729-018-0134-6 (2018).
- 12 Cavallo, C. *et al.* Platelet-Rich Plasma: The Choice of Activation Method Affects the Release of Bioactive Molecules. *Biomed Res Int* **2016**, 6591717, doi: 10.1155/2016/6591717 (2016).
- 13 Iio, K. *et al.* Hyaluronic acid induces the release of growth factors from platelet-rich plasma. *Asia Pac J Sports Med Arthrosc Rehabil Technol* **4**, 27-32, doi: 10.1016/j.asmart.2016.01.001 (2016).

Disclosures: The author is the Chief Scientific Officer of Lucina BioSciences.