

Characteristics of Platelet Lysate Compared to Autologous and Allogeneic Serum Eye Drops

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Purpose: Platelet lysate produced from platelet apheresis components has been proposed as an alternative to serum eye drops in the treatment of ocular surface disease. This study compared the effects of platelet lysate and serum on growth factor, cytokine and nanoparticle concentrations, and corneal epithelial cell proliferation.

Methods: The concentration of growth factors, cytokines, and nanoparticles in platelet lysates manufactured from either fresh or expired platelet apheresis concentrations collected with Trima or Haemonetics technology was characterized and compared with those of allogeneic, autologous, and fetal calf serum. The ability to promote corneal epithelial cell proliferation and wound healing was tested in vitro.

Results: Platelet lysate enriched the amount of transforming growth factor β 1, platelet-derived growth factor –AB and –BB, fibroblast growth factor, and epidermal growth factor compared with the two sera groups. The concentrations of insulin-like growth factor 1, hepatocyte growth factor, and fibronectin were significantly lower than in sera. There were no differences in nanoparticle concentrations. There was no significant difference in corneal epithelial cell proliferation. Platelet lysates were comparable to fetal calf serum in accelerating corneal epithelial wound healing in vitro.

Conclusions: Fresh and expired platelet lysates from the Trima and Haemonetics systems had higher growth factor concentrations than sera. The ability of platelet lysates to promote corneal epithelial cell proliferation and wound healing was equivalent to sera.

Translational Relevance: Platelet lysates may serve as an efficient and reliable source of human growth factors for the treatment of ocular surface diseases.

Introduction

Serum eye drops (SEDs) have been shown to be beneficial in the management of ocular surface diseases such as persistent epithelial defects, severe dry eye, Stevens-Johnson syndrome, neurotrophic keratopathy, ocular graft-versus-host disease, and limbal stem cell deficiency. This treatment accelerates corneal epithelial wound healing due to the greater concentration of growth factors in serum compared with normal tear composition.^{1–3} Although serum (which does not contain platelets and other clotting factors) contains growth factors, platelets are known to be great reser-

voirs of growth factors that aid wound healing, stored in α -granules.⁴ Seventy percent of the growth factors are released from platelets within 10 minutes of clotting and almost all of the remainder within 1 hour.⁴ High concentrations of growth factors that are stored in platelets are known to influence proliferation, differentiation, and migration of corneal epithelial cells.^{1,5} The α -granules are a storage organelle for mediators that promote the stages of wound healing and tissue repair, including PDGF-AB/BB, transforming growth factor- β isoform 1 (TGF- β 1), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF).⁶

EGF and fibronectin support proliferation and migration of epithelial cells.⁷ Platelet-derived growth factor (PDGF), HGF, and FGF stimulate cell proliferation, while in contrast, TGF- β 1 inhibits corneal epithelial cell proliferation and enhances apoptosis.⁷

Other autologous blood-derived products investigated for use in ophthalmology include platelet-rich plasma (PRP) and plasma rich in growth factors (PRGF). These are commonly used terms that cover more than 30 different protocols that produce autologous plasma with elevated platelet concentrations above the normal level.^{4,8} PRP and PRGF can be produced by single or double centrifugation of anticoagulated blood and be applied without clotting as eye drops or after clotting (aka activation, to induce growth factor release) as a matrix.^{4,9,10} For example, eye-PRP (E-PRP) eye drops were produced by single centrifugation of anticoagulated autologous blood and contained 1.6 to 2.5 times the normal platelet concentration in blood.⁹ They have shown success in the treatment of nonhealing corneal epithelial ulcers, moderate to severe dry eye syndrome, and ocular surface syndrome after laser in situ keratomileusis.⁹ Applied as eye drops, platelets released growth factors in a more prolonged time frame.^{4,9} Applied as a clotted E-PRP matrix, it was an effective adjuvant for ocular surface reconstruction.⁹

Autologous serum eye drops are contraindicated or not available to infants, the elderly, or patients who cannot donate enough blood due to underlying diseases, poor venous access, or low hemoglobin¹¹; therefore, allogeneic serum is also provided for these recipients by many blood services. Alternatives that are easier to collect and more straightforward to produce than serum are also being investigated. Another future alternative is platelet lysates prepared by repeated freeze-thawing of platelet components. Autologous or allogeneic platelet components are easily available and can be converted to platelet lysate via freeze-thawing.^{5,12} This process could serve as a source to obtain formulations containing higher concentrations or an alternative source of growth factors than current serum eye drop products.

Review of production methods for SEDs shows variation in methods of product manufacturing, such as collection volume, centrifugation speeds, platelet content, dilution factors, and serum diluents.^{11,13} Quality control of products is required to ensure consistency between batches in the quantification of growth factors and cytokine concentrations, as well as nanoparticle content.¹⁴

In an attempt to standardize the method for preparing platelet lysate for possible alternative eye drop formulations, our first aim was to assess the growth

factor, cytokine, and nanoparticle concentrations in platelet lysate produced from Trima and Haemonetics platelet apheresis components and to compare these levels to those derived from autologous and allogeneic serum. Our second aim was to test the effects of Trima and Haemonetics platelet lysates on corneal epithelial cell proliferation and wound healing compared with autologous and allogeneic serum.

Materials and Methods

Donor Selection and Platelet Units

All blood donations were from eligible, voluntary donors following the New Zealand Blood Service (NZBS) guidelines for collections. Donor consent was obtained for use of the platelets in this study. Leuko-reduced ($<1 \times 10^6/L$ white blood cells) apheresis platelet components were collected using two apheresis systems. In the Trima Accel system (Terumo BCT, Lakewood, CO), platelet components were stored in platelet additive solution (PAS), with a final composition of approximately 70% PAS-E and 30% plasma. In the Haemonetics system (MCS+; Haemonetics Corp., Braintree, MA), platelet components were stored in 100% plasma. The platelet components were collected as a double unit (equivalent to two transfusion doses) and then split into two components for storage at 22°C on a shaking incubator and routine usage.

Preparation of Platelet Lysate

Platelet lysate was prepared from single-donor apheresis platelet components either collected fresh (stored overnight until day 2, D2) or left to expire (D8). The platelets were transferred via docking onto Maco Biotech Freezing EVA bags GSR 8000AU capable of holding 140 to 280 mL (Macopharma, Mouvaux, France). The platelet component was transferred into a -80°C freezer (Revco; Thermo Electron Corp, Asheville, NC) for 24 hours. The EVA bag was removed from the freezer and warmed in a Lab Companion BW-10H water bath (Jeiotech, Daejeon, Korea) at 37°C for 30 minutes. The freeze-thaw process was performed twice to enhance PDGF release. After the second freeze-thaw, the platelet suspensions were transferred to 600-mL transfer packs (VSE 4001Q; Macopharma, Mouvaux, France). Platelets were sedimented by centrifugation at $3500 \times g$ for 30 minutes, acceleration 9, brake 9 using the Heraeus Cryofuge 6000i (Thermo Scientific, Waltham, Massachusetts). The centrifuged supernatant was transferred into a 600-mL plasmaflex bag 0MABSV6000XB (MacoPharma) passing through

a 0.65- μ M filter using a Fenwal plasma extractor (Baxter Healthcare, Zurich, Switzerland). The filtration unit was removed from the plasmafex bag using a tubing heat sealer, and an exchange coupler (Pfmmedical, Otzenhausen, Germany) was spiked into the bag. The cell-free supernatant, termed *platelet lysate*, was then filtered through a Millex GP 50-mm 0.22- μ M filter (Millipore, Molsheim, France) using a three-way stop cock ZMC7401 (Baxter Healthcare) and 50-mL syringe (Terumo Corp, Tokyo, Japan) within a class II laminar-flow cabinet (HERAsafe; Thermo Electron LED GmbH, Langensfeld, Germany) and stored in 10- to 30-mL aliquots in EVA Maco Freezing bags GNR 1000AU (Macopharma) at -80°C .

Preparation of Allogeneic and Autologous Serum

Whole blood was collected from patients requiring autologous serum eye drops ($n = 6$) and healthy allogeneic blood donors ($n = 6$). Units were left to clot at room temperature for 2 hours in dry collection bags (MRV5001LB; Macopharma). Serum was prepared by centrifugation at $4303 \times g$ for 10 minutes, acceleration 9, brake 5 using the Heraeus Cryofuge 6000i (Thermo Scientific). A Fenwal manual plasma expresser (Baxter Healthcare) was used to express the serum into an empty dry collection bag MRV5001LB (Macopharma), heat sealed, and centrifuged at $4303 \times g$ for 10 minutes as above. The resulting serum was expressed into an empty dry collection bag MRV5001LB (Macopharma) and stored at -30°C . Serum was *not* diluted to 25% with 0.9% sodium chloride as per usual SED manufacturing by the NZBS for patient use.

Growth Factor and Cytokine Analysis of Serum and Platelet Lysate

The concentrations of soluble proteins in platelet lysate and serum were analyzed using commercially available kits. Specifically, TGF- β 1, PDGF-AB/BB, IGF-1, EGF, VEGF, bFGF, HGF, and fibronectin were analyzed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN).

Detection of Nanoparticles by Dynamic Light Scattering Technology (ThromboLUX)

Dynamic light scattering (DLS) technology (ThromboLUX; LightIntegra Technologies, Vancouver, BC, Canada) was used to assess the number and

size of nanoparticles present in the serum (autologous, $n = 6$; allogeneic, $n = 6$) and platelet lysate components (Trima d2 [fresh], $n = 6$; Trima d8 [expired], $n = 6$; Haemonetics d2 [fresh], $n = 6$; Haemonetics d8 [expired], $n = 6$). The DLS score was generated using the manufacturer's settings (ThromboSight, Version 1.18; LightIntegra Technologies).

Human Epithelial Cell Culture

Donor corneal limbal rims were used to obtain primary cultures of human corneal epithelial cells, after the central corneas had been used for corneal transplantation. The rims were provided by the New Zealand National Eye Bank (Auckland, New Zealand), with approval from the Northern B Health and Disability Ethics Committee (NTX/06/19/CPD). The corneal epithelium was scraped from donor corneal rims, then cultured in Minimal essential medium (MEM) (Invitrogen, Waltham, Massachusetts) containing 10% fetal calf serum (Invitrogen), 1% antibiotic-antimycotic (Invitrogen), and 1% GlutaMax (Invitrogen). The culture medium was changed twice a week, and cells were passaged at 90% confluency using Tryple Express (Invitrogen).

CyQuant Proliferation Assay

Cells were plated into 96-well plates at 4000 cells per well in 200 μ L standard culture medium as above. The number of cells in each well was assessed the next day using the CyQuant assay, Thermo Scientific, Waltham, Massachusetts. Cell numbers on day 1 in each well were generated from a standard curve. Immediately afterward, the culture medium was changed to MEM with 1% antibiotic-antimycotic, 1% GlutaMax, 4 IU/mL heparin, and 10% of one of the following: Trima D2 (fresh) ($n = 6$), Trima D8 (expired) ($n = 6$), Haemonetics D8 (expired) ($n = 6$), autologous serum ($n = 6$), allogeneic serum ($n = 6$), and fetal calf serum ($n = 2$) as the experimental positive control. A series of wells containing 8000, 4000, 2000, 1000, 500, 250, and 0 cells was also included for generation of the standard curve. After 24 hours of incubation in different treatment groups, the CyQuant assay was repeated to obtain cell numbers on day 2. A percentage increase in cell numbers over the last 24 hours was calculated for each well. Each sample was replicated four times (i.e., in four wells), and the mean was calculated.

Wound-Healing Assay

Cells were plated into 96-well plates at 8000 cells per well in 200 μ L standard culture medium for 2 days

until cells reached 100% confluency. Culture medium was changed to MEM with 1% antibiotic-antimycotic, 1% GlutaMax, 4 IU/mL heparin, and 10% of one of the following: Trima d2 (fresh) ($n = 6$), Trima d8 (expired) ($n = 6$), Haemonetics d8 (expired) ($n = 6$), autologous serum, allogeneic serum ($n = 6$), and fetal calf serum ($n = 3$) as the experimental positive control. A scratch wound was created in the center of each well using a 200- μ L pipette tip. Images were captured at 4 \times magnification immediately after wounding and at 6, 12, and 24 hours at the same location. The area of wound was outlined and measured using ImageJ software (National Institutes of Health, Bethesda, MD). The area of wound healed from 0 to 6 hours, 6 to 12 hours, and 12 to 24 hours was calculated by subtracting the wound areas at different time points. The speed of wound healing from 0 to 6 hours, 6 to 12 hours, and 12 to 24 hours was calculated by dividing the area of wound healing by the number of hours taken. Each sample was replicated four times (i.e., in four wells), and the mean was calculated.

Statistical Analysis

The data are expressed as mean \pm standard deviation (SD). Data were analyzed using computer software (GraphPad Prism, Version 7.0; GraphPad, Inc., La Jolla, CA), by one-way analysis of variance (ANOVA). Holm-Sidak multiple comparison test was carried out post hoc, using allogeneic or autologous serum as the comparator. Corneal epithelial cell proliferation and wound-healing speed were analyzed using one-way ANOVA and Tukey's post hoc test for pairwise comparisons. A P value of less than 0.05 was considered significant.

Results

Platelet Lysate and Serum Eye Drop Growth Factor Concentrations

Growth factor concentrations differed between the serum and platelet lysate groups (Table). The platelet lysate derived from Trima liquid-stored platelets (70% PAS-E/30% plasma) showed a very similar overall growth factor and nanoparticle profile to the platelet lysate obtained from Haemonetics MCS+ platelets collected in 100% plasma. Both expired (day 8) and fresh (day 2) Trima and Haemonetics platelet lysates yielded significantly higher growth factor concentrations compared with autologous and allogeneic serum

with the exception of fibronectin, IGF-1, VEGF, and HGF. The pH of all apheresis platelet units prior to production of lysate via the freeze-thaw process remained within the range 6.4 to 7.4, as recommended by the European Directorate for the Quality of Medicines and HealthCare (EDQM).¹⁵ Importantly, all units were above the minimum requirements for platelet content of greater than 200×10^9 /unit, set by the EDQM.¹⁵

The concentration of EGF was significantly higher (two- to threefold) in expired and fresh Trima and Haemonetics platelet lysate formulations when compared with autologous and allogeneic serum ($P < 0.0001$). Similarly, the concentrations of PDGF-AB ($P = 0.0028$) and PDGF-BB ($P < 0.0001$) were significantly higher (two- to threefold) in the platelet lysate formulations when compared with serum. The concentrations of bFGF and TGF- β 1 were also significantly greater in platelet lysate formulations ($P < 0.0001$) (four- to ninefold and fourfold respectively) when compared with serum.

The concentrations of IGF-1, fibronectin, and HGF were substantially lower in the platelet lysate samples than autologous and allogeneic serum ($P = 0.0036$, $P < 0.0001$, and $P = 0.002$). The freeze-thaw procedure applied to all four platelet lysate groups did not enhance IGF-1, fibronectin, and HGF release from platelets. There was no significant difference in VEGF concentrations between any of the serum or platelet lysate formulations ($P = 0.7095$). Fresh and expired Haemonetics platelets collected in 100% plasma were most able to replicate the growth factors present in serum.

Nanoparticle Profile

The concentration of nanoparticles was extremely variable across both fresh and expired platelet lysate samples as well as autologous and allogeneic serum (Table). The freeze-thaw procedure did not contribute to an increase in the nanoparticle concentration. Rather, a small decrease was observed when compared with serum samples ($P = 0.2811$). There was also no statistically significant difference in nanoparticle radius.

Corneal Epithelial Cell Proliferation

Analysis of primary cultured human corneal epithelial cell proliferation (percentage increase in cell number over 24 hours of culture) revealed no significant differences between groups (one-way ANOVA, $P = 0.363$), suggesting that all groups were equally effective in promoting epithelial cell proliferation

Table. Characteristics of Serum Eye Drops and Platelet Lysates*

Growth Factor and Nanoparticles	Serum Autologous			Serum Allogeneic			Trima (70% PAS-E:30% Plasma)			Haemonetics LDP (100% Plasma)			P Value [†]
	D2 (Fresh)	D8 (Expired)	D2 (Fresh)	D8 (Expired)	D2 (Fresh)	D8 (Expired)	D2 (Fresh)	D8 (Expired)	D2 (Fresh)	D8 (Expired)	D2 (Fresh)	D8 (Expired)	
EGF (pg/mL)	656.3 ± 175.8	884.3 ± 303.6	1531 ± 334.3 [‡]	2373 ± 1017 ^{‡,§}	1206 ± 259	2451 ± 370.5 ^{‡,§}	<0.0001						
PDGF-AB (pg/mL)	12,432 ± 6398	11,236 ± 3207	22,404 ± 5518 [§]	17,532 ± 5868	25,590 ± 11,072 ^{‡,§}	26,403 ± 6540 ^{‡,§}	0.0028						
PDGF-BB (pg/mL)	2814 ± 610.8	2880 ± 1188	5209 ± 1147 ^{‡,§}	5719 ± 1599 ^{‡,§}	5819 ± 1141 ^{‡,§}	10,809 ± 1702 ^{‡,§}	<0.0001						
bFGF (pg/mL)	7.79 ± 5.233	4.911 ± 4.024	164.9 ± 62.06 ^{‡,§}	126.2 ± 53.94 ^{‡,§}	92.58 ± 58.61 ^{‡,§}	65.35 ± 48.03	<0.0001						
TGF-β1 (pg/mL)	27,802 ± 6538	32,719 ± 13,980	49,280 ± 7186	169,271 ± 51,345 ^{‡,§}	129,626 ± 73,952 ^{‡,§}	158,932 ± 63,517 ^{‡,§}	<0.0001						
IGF-1 (ng/mL)	55.01 ± 41.92	71.52 ± 31.71	23.68 ± 3.33 [§]	24.7 ± 11.87 [§]	70.23 ± 25.58	55.16 ± 7.66	0.0036						
Fibronectin (ng/mL)	261,147 ± 80,868	211,908 ± 45,066	24,498 ± 40,468 ^{‡,§}	14,468 ± 8028 ^{‡,§}	88,150 ± 61,267 ^{‡,§}	193,703 ± 145,800	<0.0001						
HGF (pg/mL)	1003 ± 198.3	922.5 ± 215.4	492.8 ± 225.9 ^{‡,§}	623.2 ± 240.1 ^{‡,§}	533.5 ± 131 ^{‡,§}	738.9 ± 83.65 [‡]	0.0002						
VEGF (pg/mL)	254.5 ± 115.8	304.3 ± 202.1	269.2 ± 140	404.4 ± 167	303.6 ± 228.2	304.4 ± 119.2	0.7095						
ThromboLUX nanoparticle radius (nm)	138 ± 31.06	186.3 ± 31.87	129 ± 39.94	199.3 ± 111.2	207.2 ± 50.78	186.7 ± 44.58	0.1262						
ThromboLUX nanoparticles × 10 ¹² /L	3.217 ± 3.998	2.317 ± 2.156	0.65 ± 0.1871	0.8333 ± 0.8017	1.8 ± 1.293	2.15 ± 1.522	0.2811						

*Values shown as mean ± SD; n = 6 for all groups.

[†]Obtained using one-way ANOVA indicating an overall difference between the serum eye drops and platelet lysate formulations.

[‡]P < 0.05 between serum autologous eye drops and platelet lysate, determined using one-way ANOVA and Holm-Sidak multiple comparison test.

[§]P < 0.05 between serum allogeneic eye drops and platelet lysate, determined using one-way ANOVA and Holm-Sidak multiple comparison test.

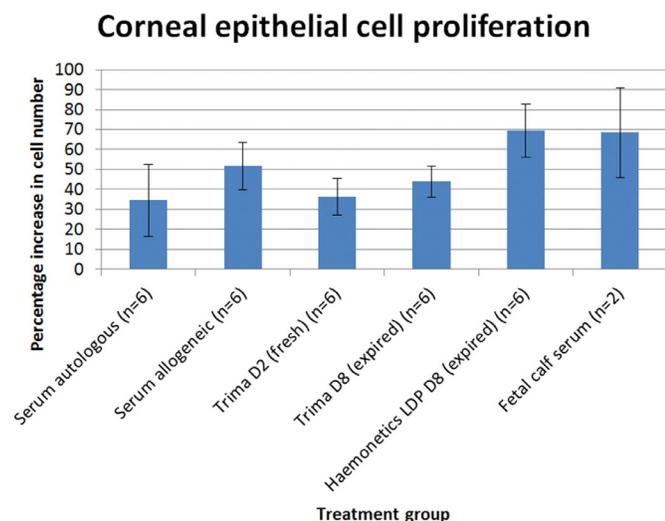


Figure 1. Proliferation of corneal epithelial cells supplemented with 10% of serum, platelet lysate, or fetal calf serum. There was no significant difference between groups (one-way ANOVA, $P = 0.363$). $n = 6, 6, 6, 6, 6, 2$.

in culture, to a similar extent as the experimental control fetal calf serum, normally included in standard medium to maintain optimal epithelial cell proliferation (Figure 1).

Corneal Epithelial Cell Wound Healing

One-way ANOVA of corneal epithelial cell wound-healing speed showed a significant difference between groups at 0 to 6 hours ($P = 0.001$). Tukey's post hoc test (pairwise comparisons) showed that the speed with which corneal epithelial cells migrated to close the wound was significantly lower in autologous serum, compared with standard medium containing fetal calf serum ($P = 0.001$) (Figures 2, 3). However, the speed was also significantly lower in allogeneic serum, compared with standard medium containing fetal calf serum ($P = 0.002$). The three platelet lysates were comparable to fetal calf serum control (no statistically significant difference in pairwise comparisons). Although there was no significant difference in other Tukey pairwise comparisons at 0 to 6 hours, including no significant difference between any of the platelet lysate groups and any of the serum groups, the results still suggest that platelet lysates were at least equivalent, if not better, than serum in initiating corneal epithelial cell migration to close a wound. There was no significant difference between any of the groups at 6 to 12 hours and at 12 to 24 hours, suggesting that all groups were comparable or as good as fetal calf serum in promoting corneal epithelial cell wound healing in the mid and late stages of healing.

Discussion

This study assessed growth factor and nanoparticle concentrations in two different types of platelet lysates, prepared fresh and after expiration of platelet concentrations, compared with autologous and allogeneic serum used for the preparation of eye drops. The results demonstrated significant differences in growth factor levels between platelet lysates and serum, regardless of whether the platelet lysates were fresh or expired formulations. The concentrations of growth factors EGF, PDGF-AB/BB, bFGF, and TGF- β 1 were significantly greater in platelet lysate formulations, whereas the concentrations of IGF-1, fibronectin, and HGF were significantly lower in platelet lysate formulations compared with serum. VEGF concentration was the same in all formulations.

The comparatively large differences found in TGF- β 1 levels between large serum and platelet lysates manufactured using Trima and Haemonetics apheresis platelet collection technologies assume that the different technologies used in the two cell separators (Haemonetics MCS+ and Terumo Trima Accel) will have the same effect on platelets during the collection processes, and their activation states during storage may differ. The additional platelet storage lesion effects when using fresher platelets (2 days of storage) when compared with outdated platelets (8 days of storage) are seen in the levels of TGF- β 1 found in the subsequent platelet lysate generated from these starting components. These differences have direct effects on the downstream manufacturing techniques used to produce the platelet lysate product. The increase in TGF- β 1 suggests that the TGF- β 1 levels obtained in 2-day stored platelets and in serum that has been prepared by clotting in blood collection bags may not cause maximal release of this growth factor.

Production of the platelet lysates used a simple freeze-thaw process, whereas the serum eye drops product achieved release of platelet granule contents by blood clotting, that is, it involved activation of platelets by thromboxane A2, ADP (Adenosine diphosphate) (both released endogenously by platelets during the activation process), and thrombin (which was produced by the coagulation cascade).

The observation of different levels of TGF- β 1 in the different products was not expected but is not a surprise. Since each growth factor tested within this study selectively regulates different cellular processes involved in cellular healing and repair, the high levels of TGF- β 1 in platelet lysate require further optimization, testing, and standardization. By ensuring the correct

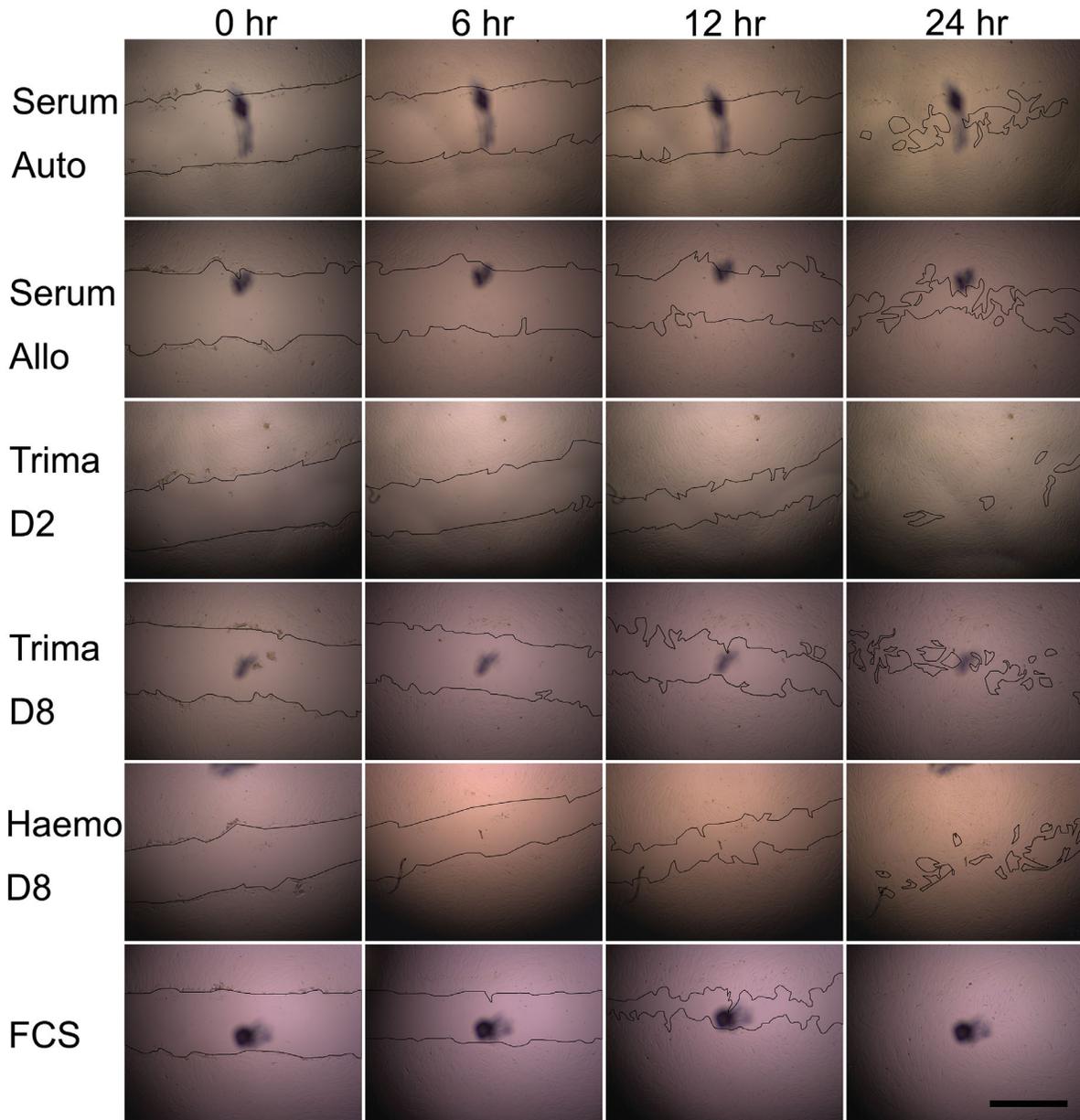


Figure 2. Representative images showing migration of corneal epithelial cells to close a scratch wound, taken at different time points. The area of the wound at each time point is outlined. The dots were permanent marker pen marks on the outside of the wells to ensure the same location was imaged. Scale bar = 1000 μ m. Serum Auto, serum autologous; Serum Allo, serum allogeneic; Trima D2, Trima day 2 (fresh) platelet lysate; Trima D8, Trima day 8 (expired) platelet lysate; Haemo D8, Haemonetics day 8 (expired) platelet lysate; FCS, fetal calf serum.

concentration of TGF- β 1 is delivered to the ocular surface, the traditional anti-inflammatory outcomes could be observed. These results emphasize that the methods used to produce platelet lysate components require further study.

Nanoparticles most likely represent platelet-derived extracellular vesicles (PL-EVs), which contain proteins, lipids, and RNAs, and are a newly discovered way by which cells communicate to their neighbours.¹⁶ PL-

EVs contribute to processes such as clotting, angiogenesis, inflammation, immunoregulation, cellular prion protein transport, tumor progression, and platelet lysate-mediated wound healing.^{16,17} An increase of PL-EV concentrations due to increasing time of platelet concentrations in storage is associated with loss of platelet function, measured by externalization of CD62P in response to thrombin receptor-activating peptide 6 activation.¹⁴ In the current study, there was

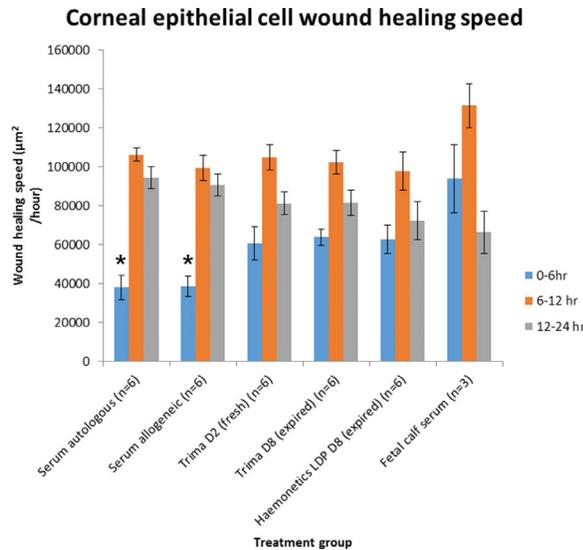


Figure 3. The speed with which corneal epithelial cells migrated to close a scratch wound in culture medium supplemented with 10% of serum, platelet lysates, and fetal calf serum. One-way ANOVA showed a significant difference between groups at 0 to 6 hours. Tukey's comparison showed significant differences between serum autologous and fetal calf serum (*) and between serum allogeneic and fetal calf serum. There was no significant difference in other pairwise comparisons at 0 to 6 hours. There was no significant difference between groups in corneal epithelial cell wound-healing speed by one-way ANOVA at 6 to 12 hours and at 12 to 24 hours.

no difference in nanoparticle radius and concentration between serum and platelet lysates, suggesting no change in platelet lysate function due to PL-EVs. Platelet lysates were comparable to serum in promoting the proliferation of corneal epithelial cells in vitro and appear to be better in promoting initial corneal epithelial cell migration to close a wound compared with serum.

Fibronectin, a major platelet adhesive ligand, is a plasma protein not dependent on platelets. Observed reductions in fibronectin levels in Trima D2 and D8 can be attributed to the manufacturing method. The Trima system stored platelet components in PAS, with a final composition of approximately 70% PAS-E and 30% plasma, hence reducing plasma and fibronectin levels by 70%. In comparison, there was no reduction of plasma or fibronectin in Haemonetics D2, D8, and autologous and allogeneic serum.

This study differs from previous comparisons of serum eye drops in that it assessed the characteristics of fresh (day 2) and expired (day 8) platelet lysate produced from freeze-thawing of apheresis platelets collected in either PAS (Trima) or 100% plasma (Haemonetics) as a potential source of growth factors

for production of a new class of eye drops to be used as an innovative alternative to current serum eye drop treatments. It is clear that fresh and expired platelet lysate did not uniformly yield high concentrations of all growth factors of interest in enhancing corneal epithelial repair in a clinical setting.

Results of the in vitro corneal epithelial cell proliferation test confirmed the efficacy with which platelet lysates promote epithelial cell proliferation. Results of the scratch wound assay suggest that all three platelet lysates tested (fresh and expired Trima, expired Haemonetics) were as effective, if not better, than autologous and allogeneic serum in closing corneal epithelial wounds in the initial 0 to 6 hours of injury. These results suggest the future application of platelet lysates for the treatment of corneal surface diseases.

This study provides valuable data regarding the ability of both fresh and expired platelet lysates collected in both the Trima system and the Haemonetics system to replicate or enhance growth factor levels found in autologous and allogeneic serum components, and it confirmed their ability to promote proliferation and heal corneal epithelial wounds. Furthermore, we have shown that platelet lysates derived from fresh and expired Haemonetics MCS+ apheresis platelets collected in 100% plasma were most able to replicate the growth factors found in serum. The results provide a new avenue for platelet lysate manufacture within blood service operations. Platelet lysates could be prescribed by clinicians in the same way as serum eye drops, with an enhanced growth factor profile compared with autologous serum formulations. The ability to manufacture platelet lysate from expired platelet components will likely result in a reduced overall cost of production of the drops compared with serum eye drops and will not compete with the requirements of patients for fresh platelet component access for transfusions. Furthermore, the expired platelet units have been manufactured according to the EDQM standards around the preparation, use, and quality assurance of blood components providing blood banking operations additional manufacturing opportunities to manufacture novel transfusion materials for human patients. It will also avoid the need for collection of whole blood, for serum, for production of the eye drops. Indeed, the current study has demonstrated the potential suitability of using expired platelet components as a source of platelet lysate. Therefore, platelet lysates derived from expired platelet units constitute a feasible and potential novel source of human growth factors for the treatment of ocular surface diseases. Further in vivo studies to assess the wound-healing nature of

platelet lysate on the corneal epithelium will provide confirmation of the efficacy of these platelet lysate products.

Conclusions

In conclusion, fresh and expired Trima and Haemotronics platelet apheresis concentrates could be used to produce platelet lysates with enhanced concentrations of some growth factors and equivalent corneal epithelial healing properties compared with autologous or allogeneic serum, providing an efficient and reliable alternative for the treatment of challenging cases of ocular surface disease.

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* JZ and DC contributed equally to this article.

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