Characterization of Autologous Sera From the Thrombinator™ System

Arthrex Research and Development

Background

The Thrombinator system employs a 2-stage process to produce autologous, thrombin-rich serum. In the first stage, the Thrombinator system activates the coagulation cascade within the initial aliquot of autologous blood-derived fluid injected into the device; these can include whole blood (WB), autologous conditioned plasma (ACP), or platelet-poor plasma (PPP). The activated device is treated with a second aliquot of a blood-derived fluid to rapidly produce the activated sera on demand. A variety of blood-derived fluids, both with and without anticoagulant, can be used with the Thrombinator system. The purpose of this study was to characterize the initial activation time, thrombin activity, and protein concentration of the sera for various combinations of activation and production fluids. This study also evaluated thrombin degradation over 4 hours using 2 different storage conditions (on ice and at room temperature).

Materials and Methods

Human WB was collected into syringes containing 10%, 13%, or no ACD-A (v/v), depending on the system to be used (WCG IRB #20181429). WB with a 13% concentration of ACD-A was processed in the Angel® PRP system to make PPP. The 10% ACD-A WB and WB without anticoagulant were processed using the Arthrex ACP® double-syringe system to produce ACP. Various combinations of activation and production fluid were tested in the Thrombinator devices.

Thrombinator devices were activated with 4 mL of one of the autologous fluids described previously; 0.1 mL of 10% calcium chloride was used to recalcify any aliquots containing anticoagulant. The activation time (time required for the contents to coagulate following the addition of the first fluid) was measured. Activated sera was produced using an additional 8 mL aliquot of autologous fluid. All activated sera were tested for thrombin activity within 5 minutes of production and after 0.25, 0.5, 1, 2, and 4 hours of storage at room temperature (18 °C) or on wet ice (2-4 °C). After completion of the second step, aliquots of the sera were immediately stored at -80 °C and later analyzed via ELISA for concentrations of IL-1ra, IL-1b, TFG-b1, PDGF-AB, and VEGF.

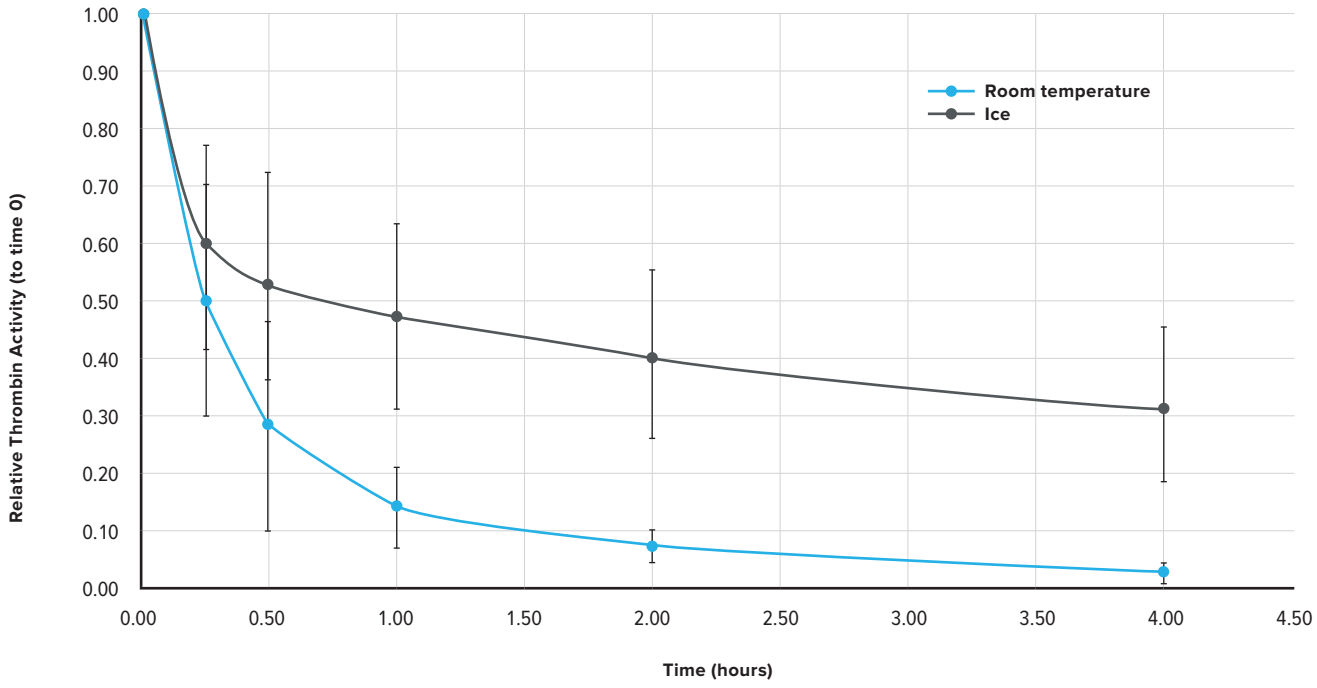
Arthrex®

Results

Table 1. Summary of activation times, initial thrombin activities (T0), and protein concentrations in thrombin sera.[1]

Activation Fluid (4 mL)	Calcium Added (mL)	Activation Time (min)	Activation Fluid (4 mL)	Calcium Added (mL)	Thrombin Activity (U/mL)	IL-1ra (pg/mL)	IL-1β (pg/mL)	TGF-b1 (ng/mL)	PDGF-AB (ng/mL)	VEGF (pg/mL)
WB (-)	0.0	6.0 ± 0.5 (n=14)	Angel® PPP (+)	0.2	21.9 ± 1.8 (n=3)	58.0 ± 69.3 (n=3)	4.6 ± 2.3 (n=3)	5.6 ± 3.8 (n=3)	0.6 ± 0.2 (n=3)	26.2 ± 28.6 (n=3)
WB (-)	0.0		ACP (+)	0.2	19.4 ± 3.9 (n=3)	17.3 ± 11.3 (n=8)	52.5 ± 83.9 (n=8)	9.5 ± 2.3 (n=8)	1.2 ± 0.3 (n=8)	19.6 ± 15.8 (n=8)
WB (-)	0.0		WB (+)	0.2	15.8 ± 0.5 (n=3)	103.6 ± 75.1 (n=6)	60.1 ± 85.3 (n=6)	5.1 ± 2.7 (n=6)	0.6 ± 0.2 (n=6)	28.7 ± 8.3 (n=6)
WB (-)	0.0		ACP (-)	0.0	13.2 ± 4.6 (n=3)	18.4 ± 4.8 (n=6)	12.9 ± 7.2 (n=6)	5.9 ± 1.1 (n=6)	1.0 ± 0.2 (n=6)	26.1 ± 15.3 (n=6)
WB (+)	0.1	13.7 ± 1.3 (n=12)	Angel PPP (+)	0.2	22.3 ± 0.5 (n=4)	22.2 ± 4.5 (n=4)	7.5 ± 5.6 (n=4)	4.0 ± 0.2 (n=4)	0.6 ± 0.1 (n=4)	18.2 ± 2.6 (n=4)
WB (+)	0.1		WB (+)	0.2	21.7 ± 2.2 (n=4)	144.1 ± 10.8 (n=2)	2.1 ± 0.6 (n=2)	4.1 ± 0.1 (n=2)	0.5 ± 0.0 (n=2)	56.2 ± 0.6 (n=2)
WB (+)	0.1		ACP (+)	0.2	19.9 ± 2.0 (n=3)	18.4 ± 11.5 (n=8)	60.7 ± 97.6 (n=8)	10.1 ± 2.7 (n=8)	1.2 ± 0.3 (n=8)	23.2 ± 17.6 (n=8)
ACP (-)	0.0	9.3 ± 1.0 (n=6)	ACP (-)	0.0	13.6 ± 2.8 (n=3)	4.5 ± 2.2 (n=6)	8.5 ± 3.6 (n=6)	8.7 ± 0.7 (n=6)	1.3 ± 0.1 (n=6)	29.7 ± 15.2 (n=6)
ACP (-)	0.0		ACP (+)	0.2	20.3 ± 0.6 (n=3)	4.7 ± 2.1 (n=6)	9.0 ± 4.5 (n=6)	10.5 ± 2.6 (n=6)	1.2 ± 0.2 (n=6)	38.9 ± 21.9 (n=6)
ACP (+)	0.1	12.9 ± 1.0 (n=4)	ACP (+)	0.2	20.2 ± 3.4 (n=3)	4.2 ± 3.5 (n=8)	56.3 ± 81.5 (n=8)	13.7 ± 4.0 (n=8)	1.4 ± 0.2 (n=8)	11.7 ± 15.3 (n=8)
Angel PPP (+)	0.1	22.8 ± 4.4 (n=4)	Angel PPP (+)	0.2	10.3 ± 24 (n=4)	6.3 ± 4.9 (n=4)	21.0 ± 21.4 (n=4)	0.9 ± 0.1 (n=4)	0.1 ± 0.0 (n=4)	1.3 ± 1.3 (n=4)

Figure 1. Relative thrombin activity over time when stored at room temperature vs on ice.[1]



Arthrex®

Discussion and Conclusions

The activation time of the Thrombinator™ system with 4 g borosilicate beads is between 6 and 22.8 minutes depending on the activation fluid used. WB without anticoagulant produced the shortest average activation time, while PPP with anticoagulant produced the longest average activation time. In general, blood-derived fluid without anticoagulant produced faster activation times than the same fluid with anticoagulant. One benefit of using different autologous fluids for each step is the flexibility to activate the device with WB while simultaneously preparing PRP, thus lowering overall processing time.

Average initial thrombin activity ranged from 10.3 - 22.3 U/mL and varied with the combination of activation fluids and anticoagulant. The Angel® PPP(+)/Angel PPP(+) combination that produced the lowest amount of thrombin activity also had the longest activation time. This is attributed to the lack of platelets in the overall system, which act as the primary surface for prothrombin activation, as well as the lack of red blood cells, whose membranes can also act as a surface for prothrombin activation.[2] However, the 10.3 ± 2.4 U/mL produced was well above 1 U/mL, which has been shown in literature to still invoke timely clot formation and platelet activation.[3]

The average concentration of IL-1ra, IL-1b, TGF-b1, PDGF-AB, and VEGF in the thrombin sera was 4.2-144.1 pg/mL, 2.160.7 pg/mL, 0.9-13.7 ng/mL, 0.1-1.4 ng/mL, and 1.3-56.2 pg/mL, respectively, and varied with the combination of activation fluids and anticoagulant. The PPP(+)/PPP(+) combination of activation fluids resulted in the lowest average concentrations of TGF-b1, PDGF-AB, and VEGF. The WB(+)/WB(+) combination of activation fluids resulted in the highest average concentration of IL-1ra. The differences in growth factor content can be attributed to the cellular content of the autologous fluids being injected into the device.

Storage temperature does have an effect on thrombin activity loss for the first 4 hours after production. Fifteen minutes after production, the average relative thrombin activity dropped to 51% and 59% of initial thrombin activity for both the sera stored at room temperature and on ice. After the initial 15 minutes the relative thrombin activity for the sera stored on ice began to plateau and retained, on average, 31% of initial thrombin activity after 4 hours. The relative thrombin activity of the sera stored at room temperature continue to drop and only retained an average of 2% of initial thrombin activity after 4 hours. The decay rate of thrombin can help determine the useable time of the serum depending on the bone grafting application and storage condition used.

References

1. Arthrex, Inc. Data on file (APT-05594). Naples, FL; 2021.
2. Whelihan MF, Zachary V, Orfeo T, Mann KG. Prothrombin activation in blood coagulation: the erythrocyte contribution to thrombin generation. *Blood*. 2012;120(18):3837-3845. doi:10.1182/blood-2012-05-427856
3. Ryan EA, Mockros LF, Weisel JW, Lorand L. Structural origins of fibrin clot rheology. *Biophys J*. 1999;77(5):2813-2826. doi:10.1016/S0006-3495(99)77113-4