SUPPLEMENTARY INFORMATION

A cysteine enzyme hemostat for efficient heparin-tolerant blood coagulation

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Supplementary Figures

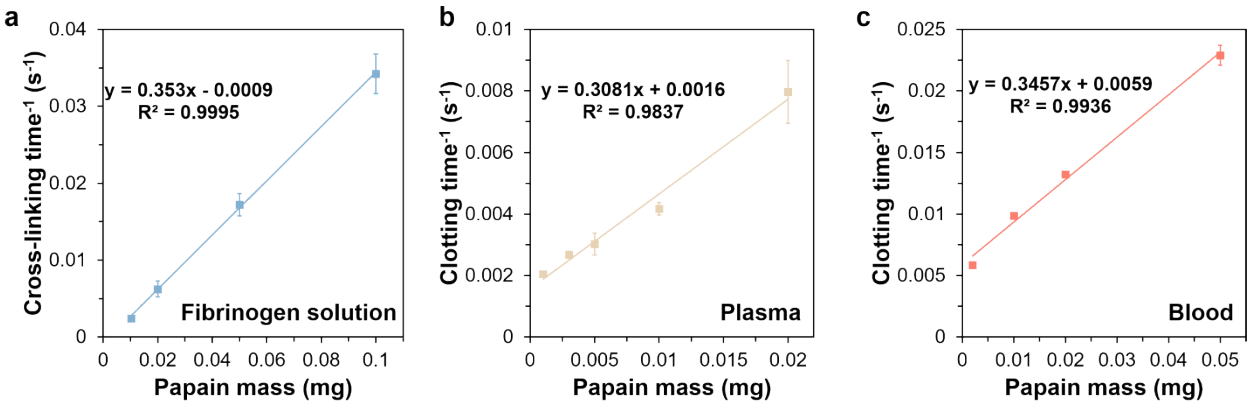

Fig. S1 The reciprocal of cross-linking/clotting time is in linear relationship with concentration of papain when the amount of substrate is larger than the enzyme under certain pH and temperature. The slope exhibits procoagulant activity of papain in the (a) fibrinogen solution, (b) normal plasma and (c) whole blood. Data values corresponded to mean ± SD, n = 3. Error bars represent SD.

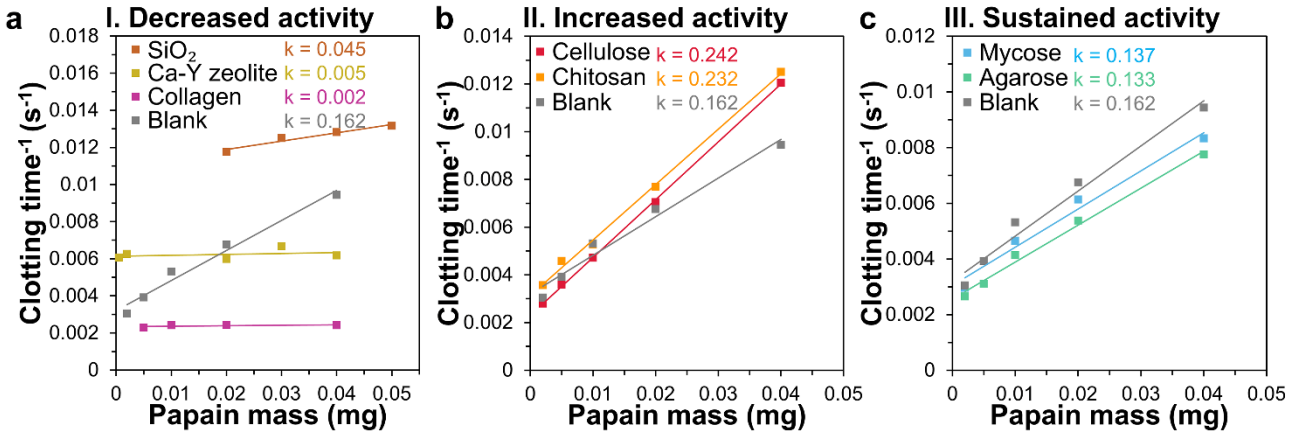

Fig. S2 The slope of reciprocal of clotting time to papain mass exhibits procoagulant activity of papain immobilized on (a) SiO_2, Ca-Y zeolite, collagen, (b) chitosan, cellulose, (c) mycose and agarose, respectively.

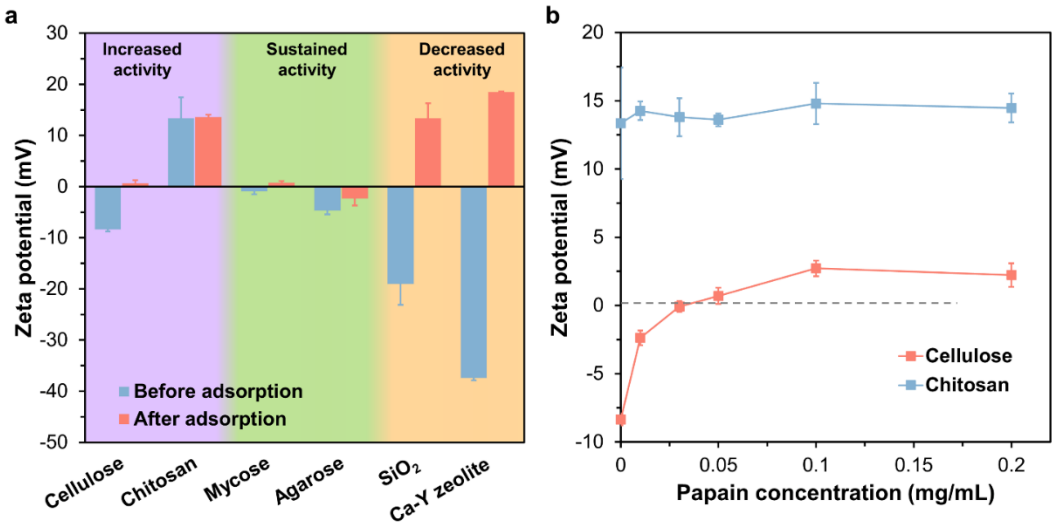

Fig. S3 Zeta potential change of different supporters after loading papain. (a) Zeta potential of cellulose, chitosan, mycose, agarose, SiO_2 and Ca-Y zeolite with or without loading papain. (b) Zeta potential change of cellulose and chitosan with different concentrations of papain was adsorbed. Data values corresponded to mean ± SD, n = 3. Error bars represent SD.

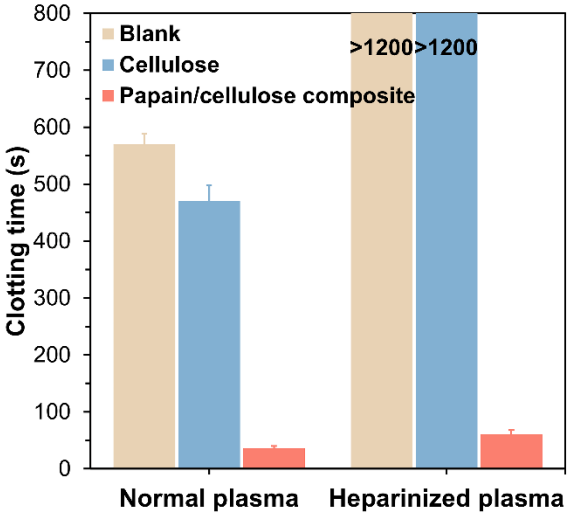

Fig. S4 Procoagulant performance of cellulose and papain/cellulose composite in the normal and heparinized plasma. Data values corresponded to mean ± SD, n = 3. Error bars represent SD.

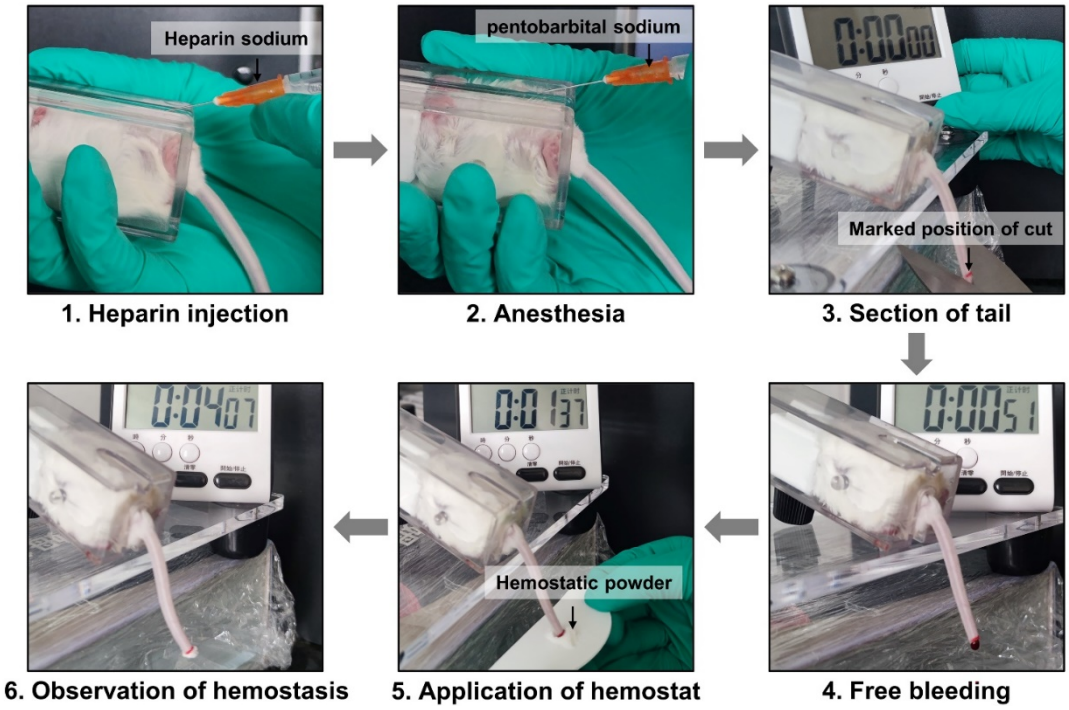

Fig. S5 Operation process of mice tail amputation bleeding model. Firstly, mice were injected intraperitoneally with heparin to construct an anticoagulant model. After 15 min, pentobarbital sodium was injected to induce anesthesia. Then we cut off the tail at marked position to establish bleeding model. After free bleeding for 1 min, we added hemostatic powder on the wounded site without pressure for 2 min and observed hemostatic performance.

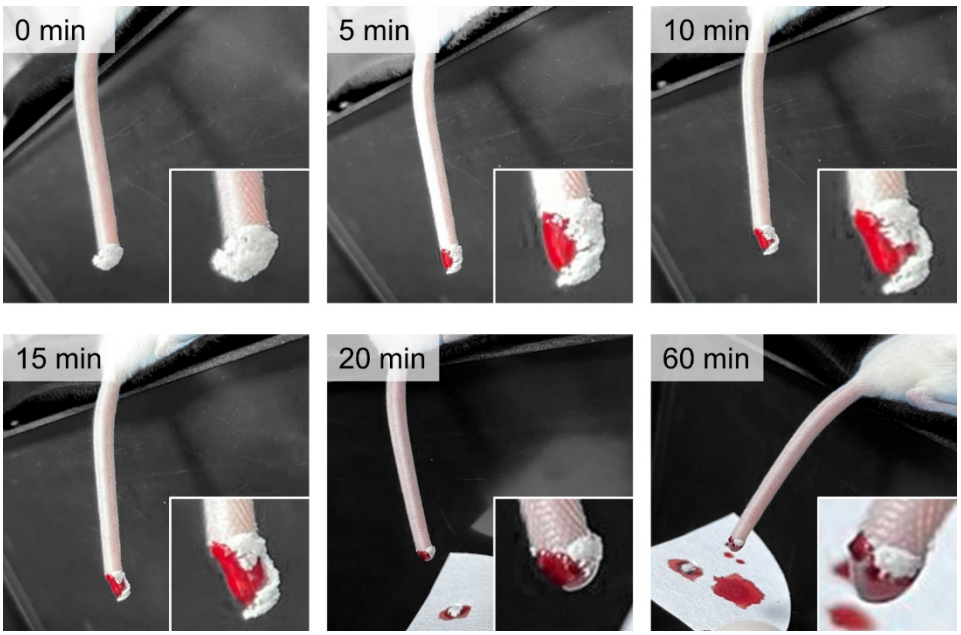

Fig. S6 Bleeding state of heparin-treated mice tail amputation model treated with papain alone at different times.

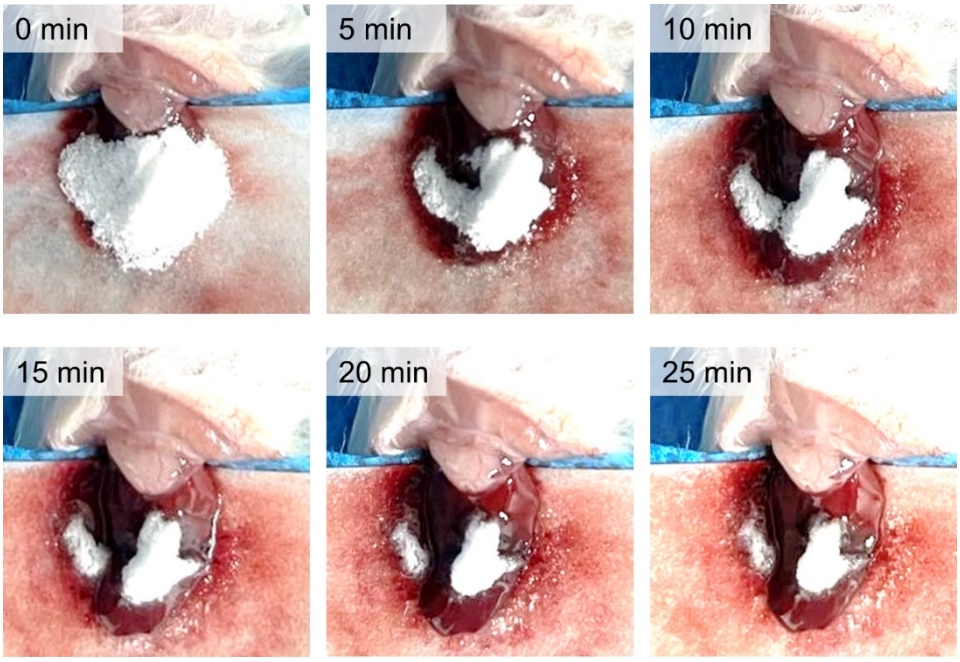

Fig. S7 Bleeding state of heparin-treated mice liver injury model treated with papain alone at different times.

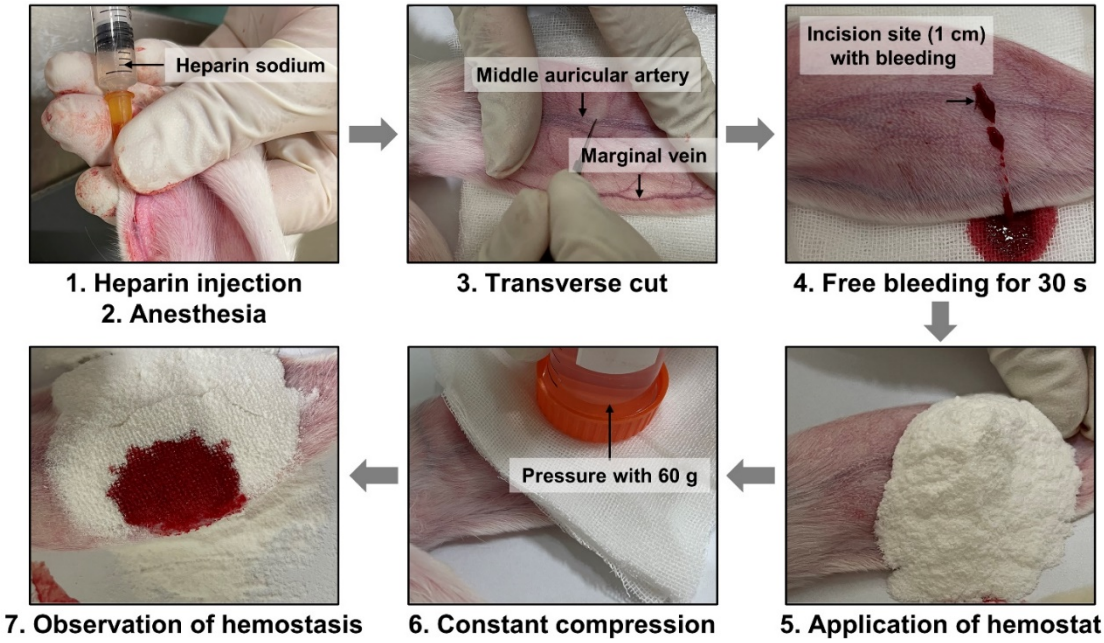

Fig. S8 Operation process of a rabbit auricular artery injury model. Firstly, heparin sodium was injected through marginal vein to construct a heparinized model. Then we cut off the middle auricular artery transversely followed by free bleeding for 30 s. Hemostatic agents (cellulose or papain/cellulose composite) were applied to the wound with constant compression. After 2, 3, 4, 5, 6 and 10 min, we removed the compression and observed the hemostatic performance of hemostatic agents.

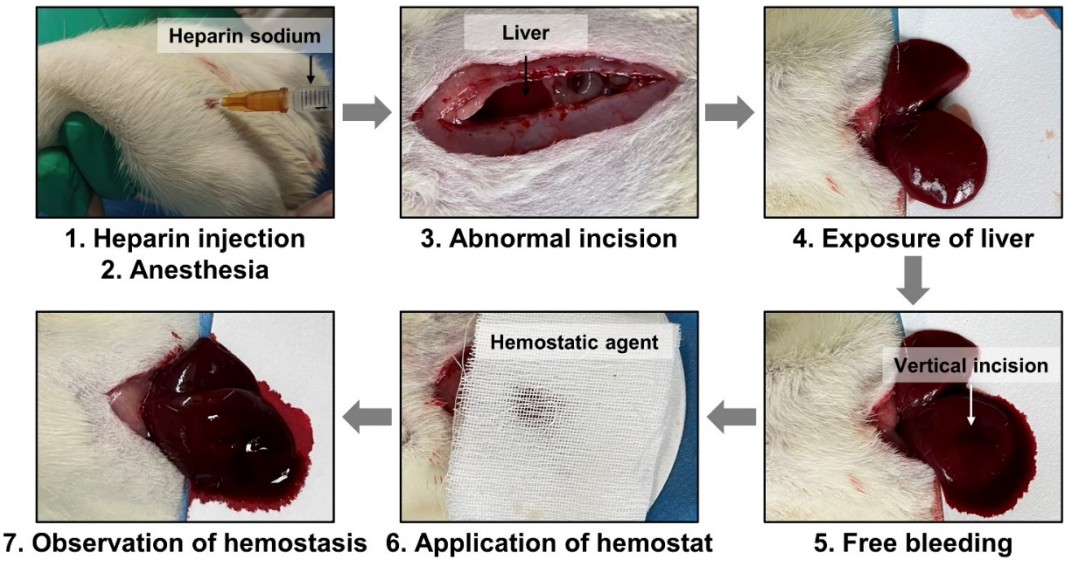

Fig. S9 Operation process of a rat liver injury model. Firstly, heparin sodium was injected intraperitoneally to construct a heparinized model. Then the liver of rat was exposed entirely with abnormal incision. A vertical incision was made to establish bleeding model followed by free bleeding for 10 s. The injured site was treated with hemostatic agents, and hemostatic time and blood loss were recorded.

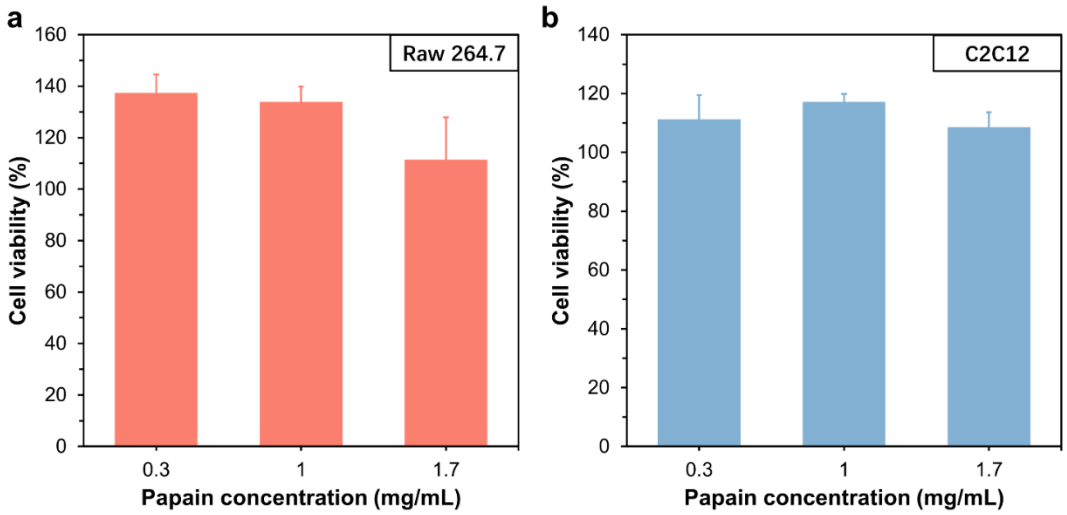

Fig. S10 Cytotoxicity of papain/cellulose composite. Cell viability of (a) Raw264.7 and (b) C2C12 treated with papain/cellulose composite for 2 h. Data values corresponded to mean ± SD, n = 3. Error bars represent SD.

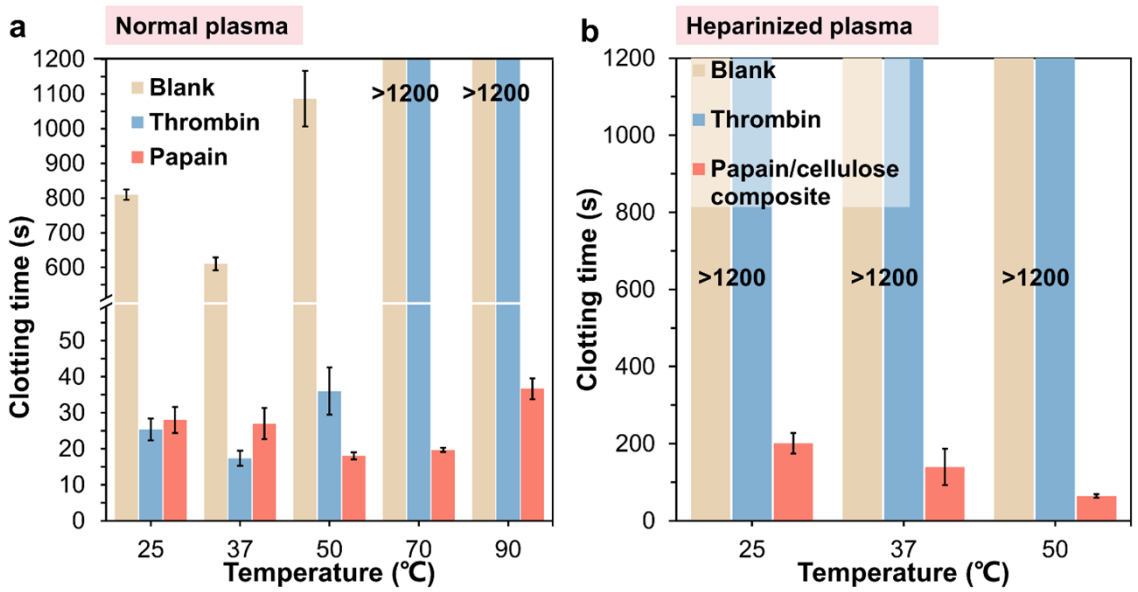

Fig. S11 Thermostability of papain. (a) Clotting time of papain and thrombin in normal plasma clotting assay. (b) Clotting time of papain/cellulose composite in heparinized plasma clotting assay. Data values corresponded to mean ± SD, n = 3. Error bars represent SD.

Supplementary Tables

Table S1 Hemostatic time and blood loss of papain alone in heparin-treated mice tail amputation model.

	Hemostatic time (s)	Blood loss (g)
1	330	0.2805
2	>1800	0.5826
3	263	0.1157
4	437	0.2837
5	>1800	0.6241

Table S2 Hemostatic time and blood loss of rabbit auricular artery bleeding model.

No.	Cellulose		Papain/cellulose composite	
	Hemostatic time (s)	Blood loss (g)	Hemostatic time (s)	Blood loss (g)
1	>600	>29.36	120	8.11
2	>600	>25.68	210	6.73
3	>600	>13.29	120	1.52
4	360	6.36	180	2.79
5	300	5.26	240	2.41
6	270	3.70	240	1.96
Mean ± SD	-	-	185 ± 55	3.92 ± 2.78