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Nano/Microbilayer Hemostatic Agents in Diabetic Injury Wound Model in Rat

OUTLINE

1. **Motivation**
   - What is the coagulation mechanism?
   - How does work it and which factors do affect that ?
   - Why the bleeding control in diabetics is important?
2. **Scope of the Study**
3. **Results**
4. **Discussions**

MOTIVATION

COAGULATION MECHANISM

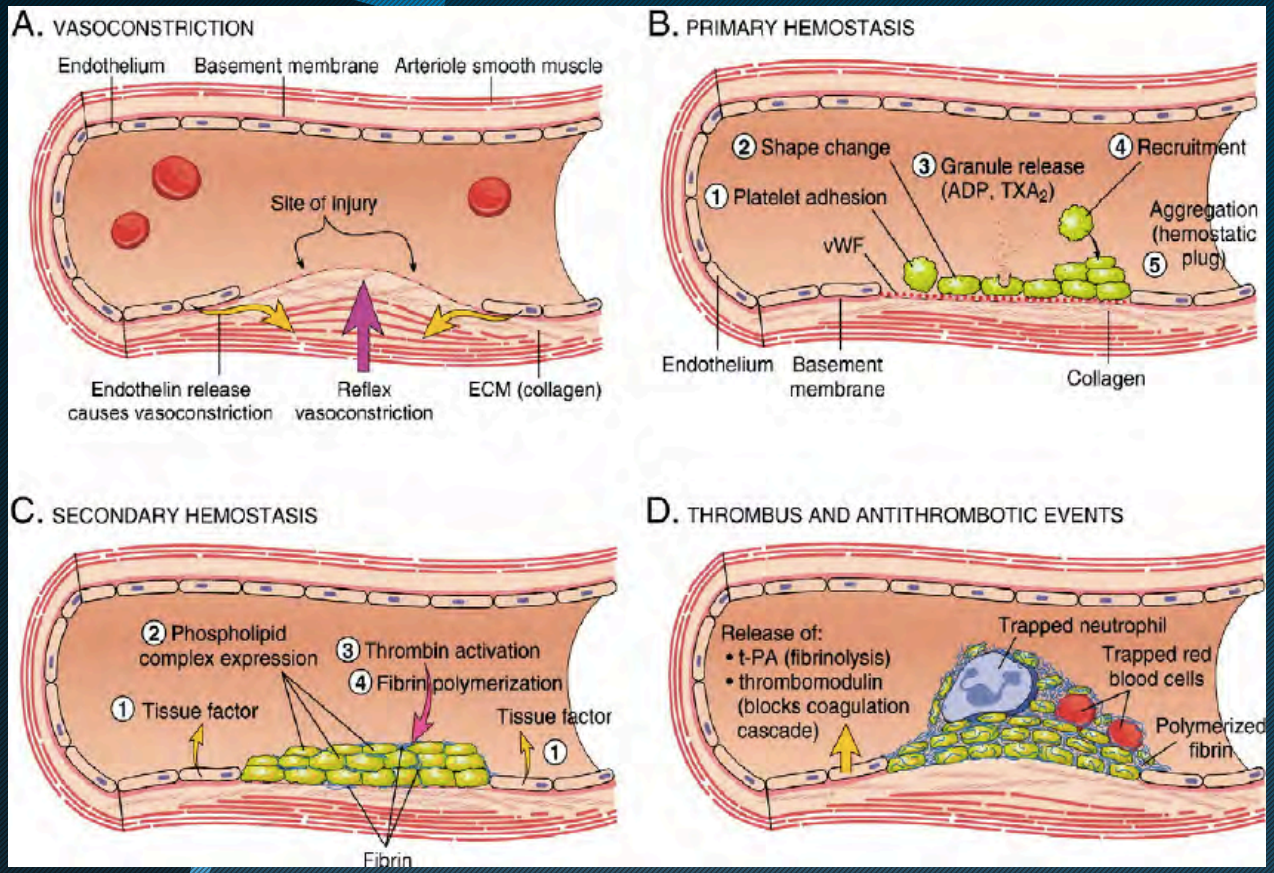


Fig. 1. Illustration of the blood clotting process showing the four main steps of hemostasis (vasoconstriction, primary hemostasis, secondary hemostasis and fi brinolysis).

Coagulation Pathway

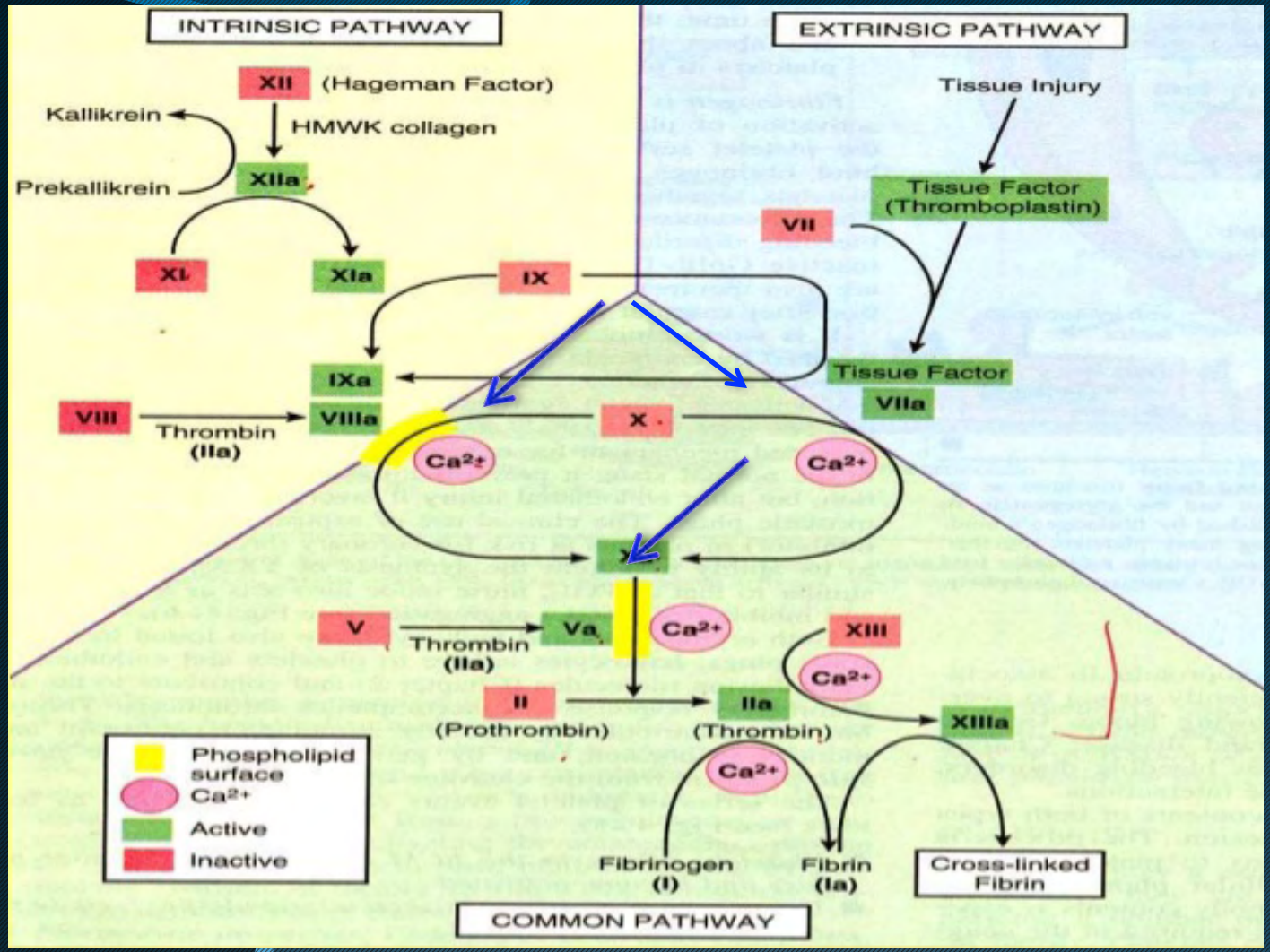
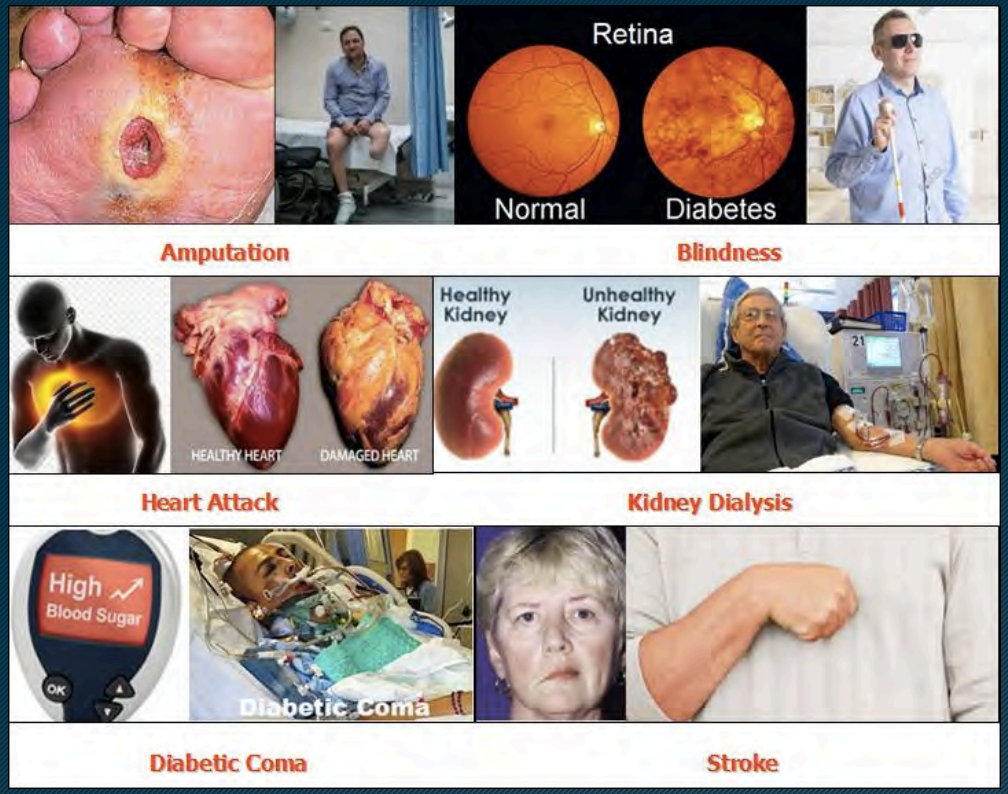


Fig. 2. The intrinsic, extrinsic and common pathways of coagulation cascade.



Hemostasis in Diabetics

- **Premature atherosclerosis and extensive vascular disease because of plaque rupture and thrombus formation**
- **Blood coagulation abnormalities**
- **Disrupted wound healing**

METHODS OF HEMOSTASİS

- Mechanical
- Cauterization
- Chemical

- Vasoconstrictors
- Topical Absorbable Hemostats

Commercial Hemostatic Agents

TABLE I. Commercially Available Hemostatic Agents

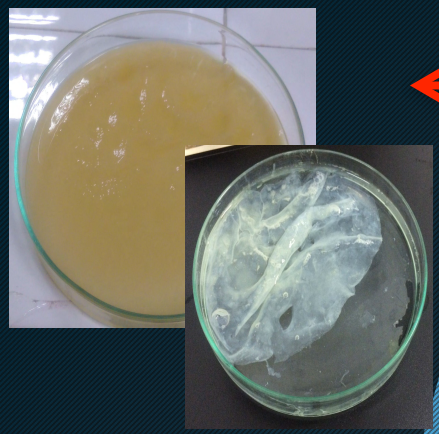
Categories	Types	Pros	Cons
Physical and Absorbable[10,12]	Bone wax, ostene, gelatin foams, sponges, and powders, oxidized cellulose, microfibrillar collagen, bovine and porcine collagen	Tamponades bone surface bleeding, absorbable, and controls small vessel low pressure bleeding	May embolize, prevent bone fusion, reduce structural stability, possible interference with healing process
Biologically Active[10–12,16,25]	Pooled and recombinant thrombin, thrombin and gelatin, fibrin sealants, platelet gels, albumin and glutaraldehyde	Easily applied, rapid response, effective against mild to moderate bleeding, effective in heparinized patients, and broad applications	Immunological response, viral infection, expensive cost per application, short shelf lives, and adverse distal thrombotic events
Synthetic sealants[10–12]	Cyanoacrylates, polyethylene glycol hydrogel	Waterproof barrier, replacement for sutures, full strength within minutes, arterial bleeding	Limited topical usages, dangerous if unreacted, and difficult to apply to irregular wounds
Hemostatic Dressings[10,11,13]	Dry fibrin, chitin, chitosan, alginate, mineral zeolite, kaolin, and smectite	Military and emergency response usage, can stop heavy arterial bleeding, long shelf-life, enhances normal compression treatment, and typically inexpensive	High pressure wounds can expel powders, zeolite causes exothermic reaction, success related to responder training, inconsistent results from animal studies

Behrens AM, Sikorski MJ, Kofinas P. 2013. Hemostatic strategies for traumatic and surgical bleeding. J Biomed Mater Res Part A 2013:00A:000–000.

EXPERIMENTAL

Silk fibroin (SF) or SF/PC nanofibrillar layer

BC/CTS porous layer



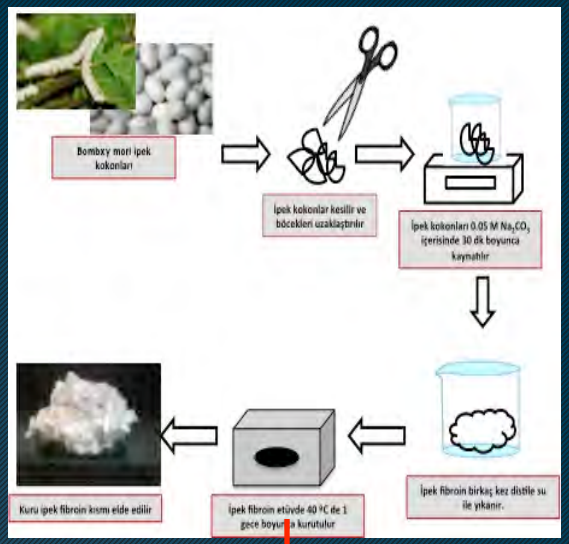


Table I. Native and active agent doped BC/CTS hemostatic agents components

Experimental groups	CTS (mL)	BC (g)	$CaCl_2$ (g)	Kaolin (g)	Vitamin K (mg)	Protamine Sulfate (IU)
BC/CTS	7	14	0.5			
SF coated BC/CTS	7	14	0.5	-	-	-
SF/PC coated BC/CTS	7	14	0.5	-	-	-
SF coated Kao/BC/CTS	7	14	0.5	1		-
SF coated Vit K/BC/CTS	7	14	0.5	-	10	-
SF coated PS/BC/CTS	7	14	0.5	-	-	1000

Karahaliloglu Z., Demirbilek M., Ulusoy İ., Gümüşkaya B., Denkbas E.B. Active nano/microbilayer hemostatic agents for diabetic rat bleeding model. J Biomed Mater Res B Appl Biomater. 2017;105(6):1573:1585.

METHODS

Morphological Characterizations

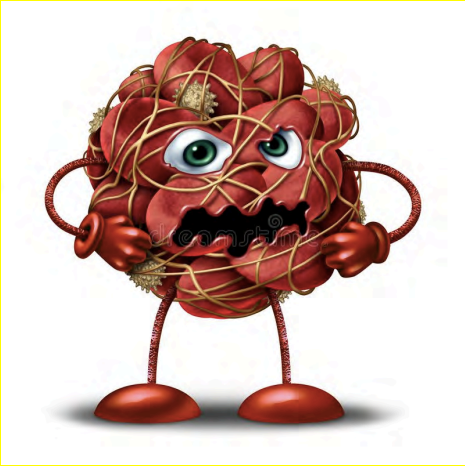
- ✓ Scanning Electron Microscopy (SEM)

In-vitro Tests

- ✓ Whole Blood/PRP Absorption and LDH Activity Test
- ✓ Blood Metabolic, Blood Gas and Blood Chemical Values

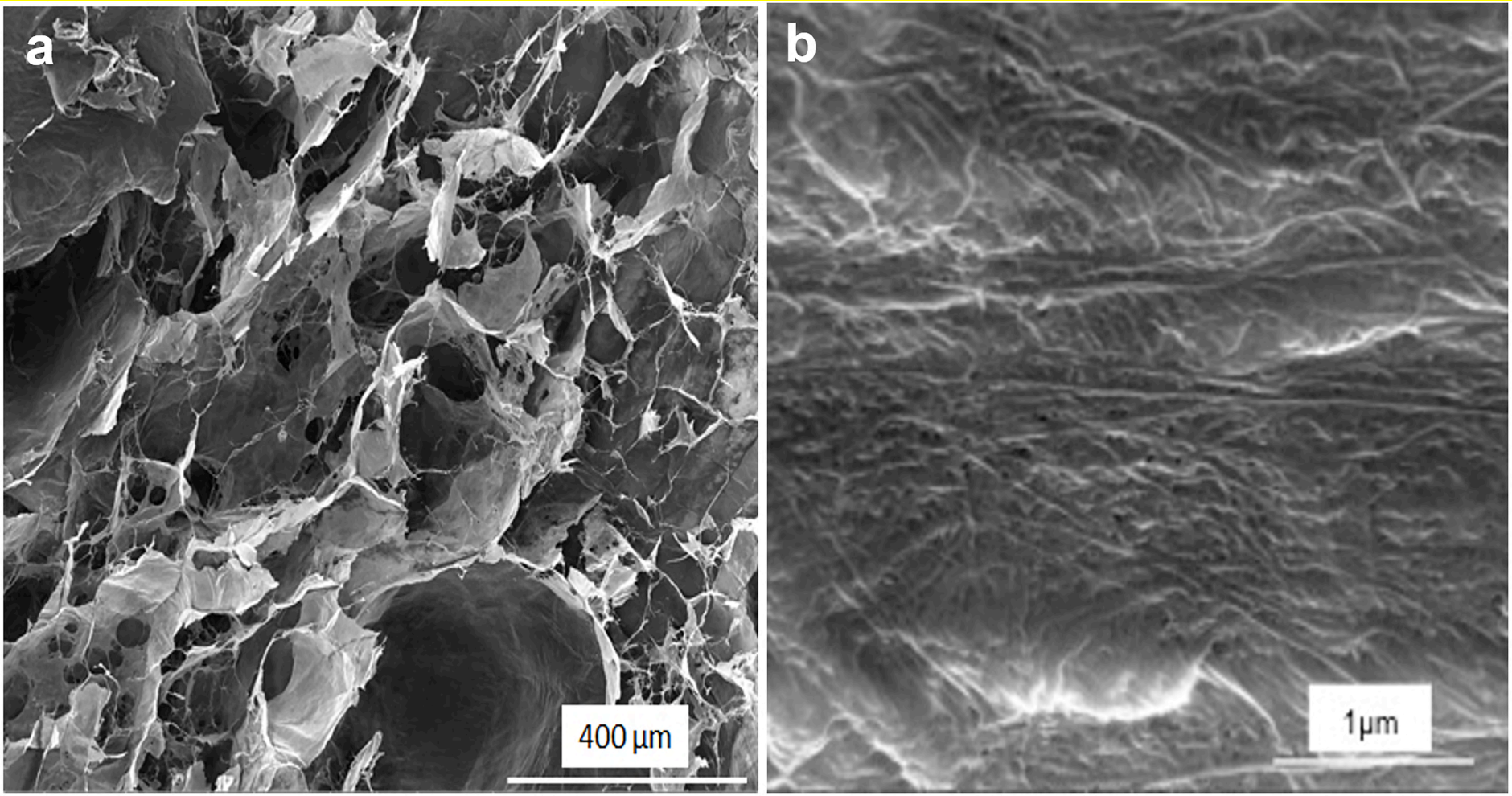
Diabetic Rat Fomeral Artery Bleeding Model

- ✓ Hemostasis Time and Blood Loss
- ✓ Mortality Rate
- ✓ Measurement of Wound Temperature
- ✓ Hematoxylin–Eosin Staining



RESULTS

FIGURE 1. SEM images of native BC/CTS.
(a) Porous structure and (b) inner wall structure
(scale bars: 100 and 2 mm).



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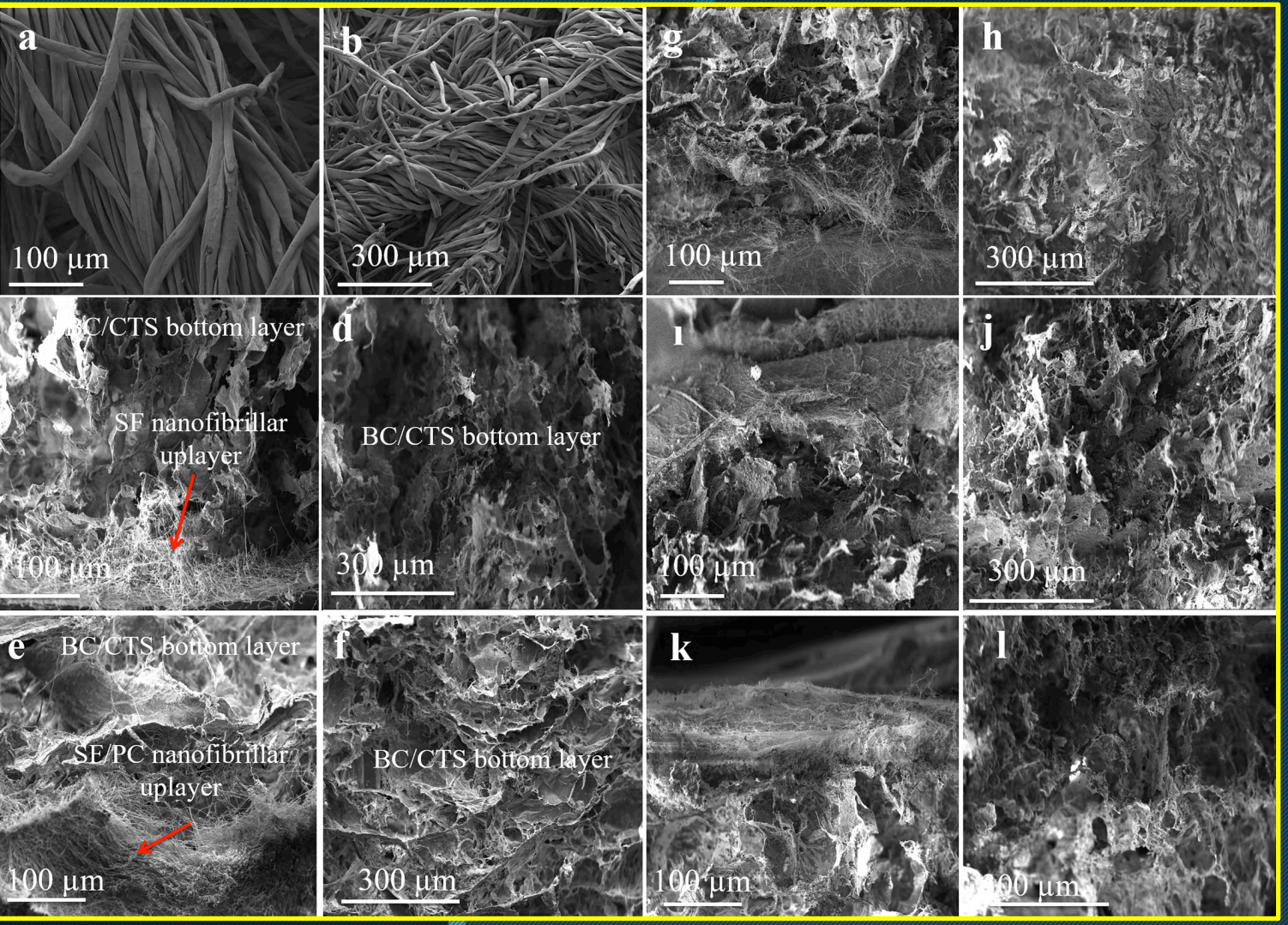


FIGURE 2. (a, b) SEM micrograph of standard gauze and cross-sectional SEM micrographs of (c, d) SF-coated BC/CTS, (e, f) SF/PC-coated BC/CTS, (g, h) SF-coated Kao/BC/CTS, (i, j) Vit K/BC/CTS, and (k, l) PS/BC/CTS hemostatic dressings (left images: bilayer structures, scale bars: 100 mm; right images: sub-porous structures, scale bars: 300 mm).

TABLE I. Porosity Percent of Standard Gauze, Bare, and Composite BC/CTS Hemostatic Dressings

Samples	Porosity (%)
Standard gauze	87.95
BC/CTS	88.34
SF-coated BC/CTS	78.79
SF/PC-coated BC/CTS	85.07
SF-coated Kao/BC/CTS	85.56
SF-coated Vit K/BC/CTS	83.98
SF-coated PS/BC/CTS	67.74

FIGURE 3. Pore histograms of standard gauze, bare, and composite BC/CTS hemostatic dressings.

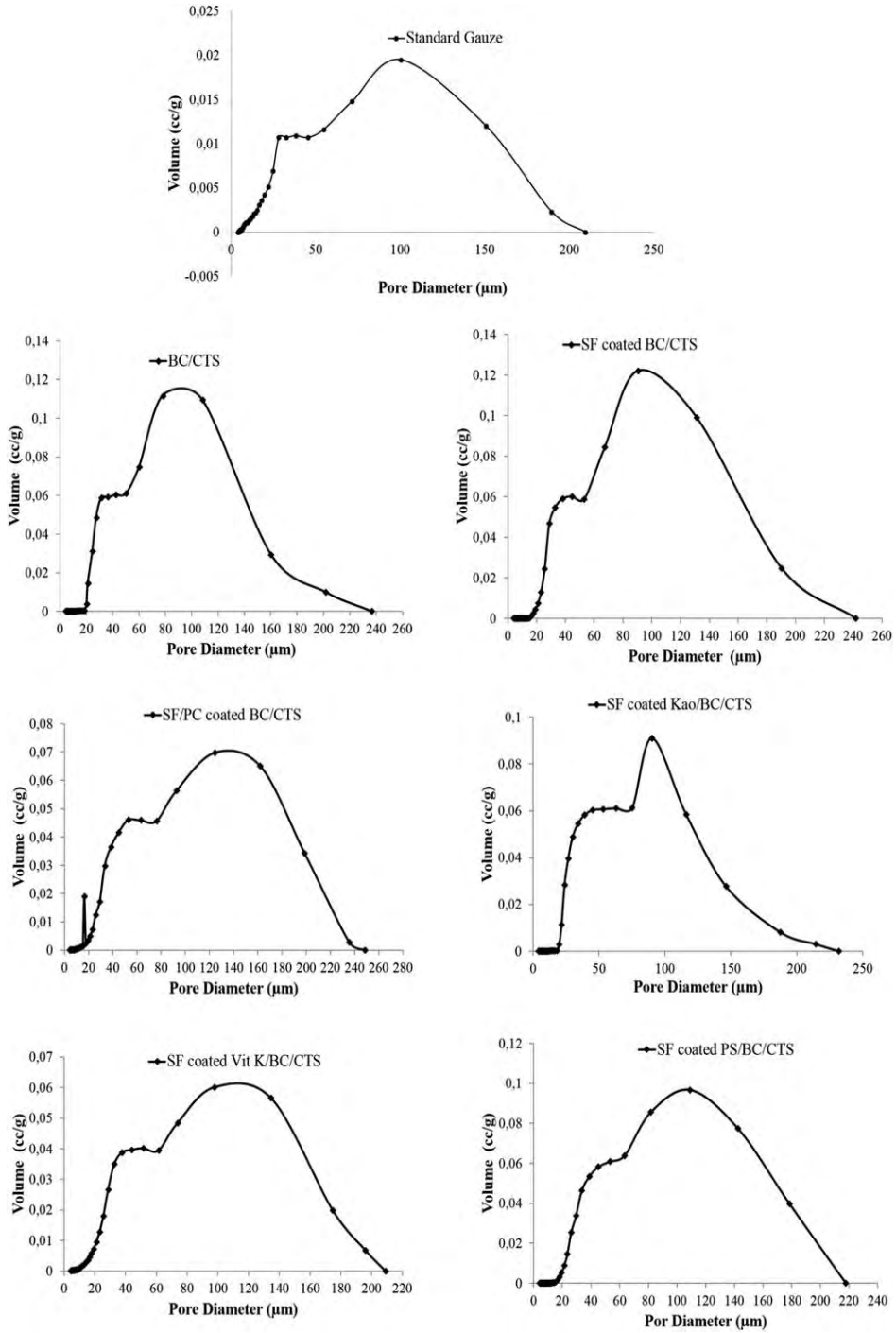


FIGURE 4. Stress–deformation curves of standard gauze, bare, and composite BC/CTS in dry (a) and wet state (b).

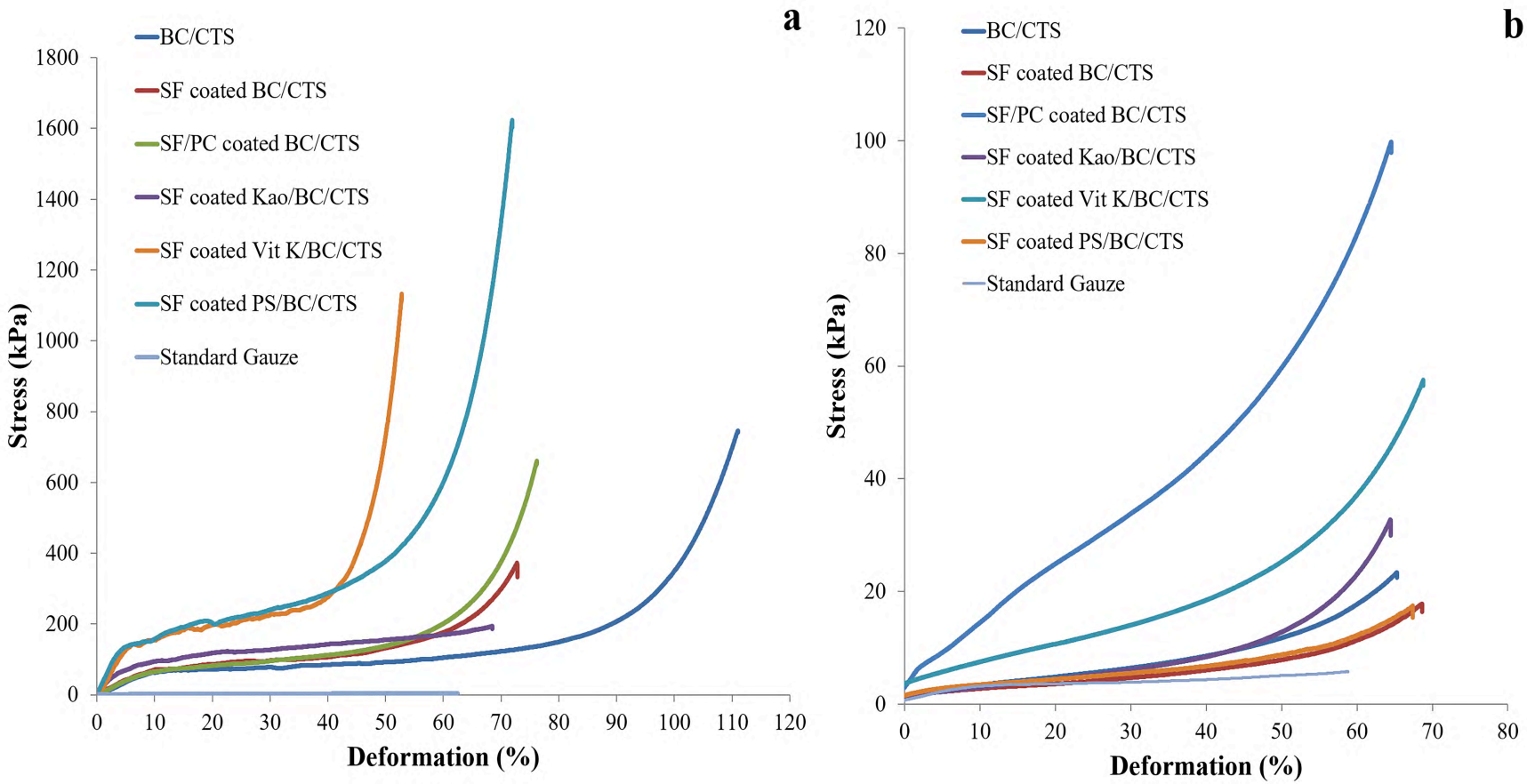


FIGURE 5. Diabetic rat blood and PRP uptake rates of hemostatic dressings (**p<0.005).

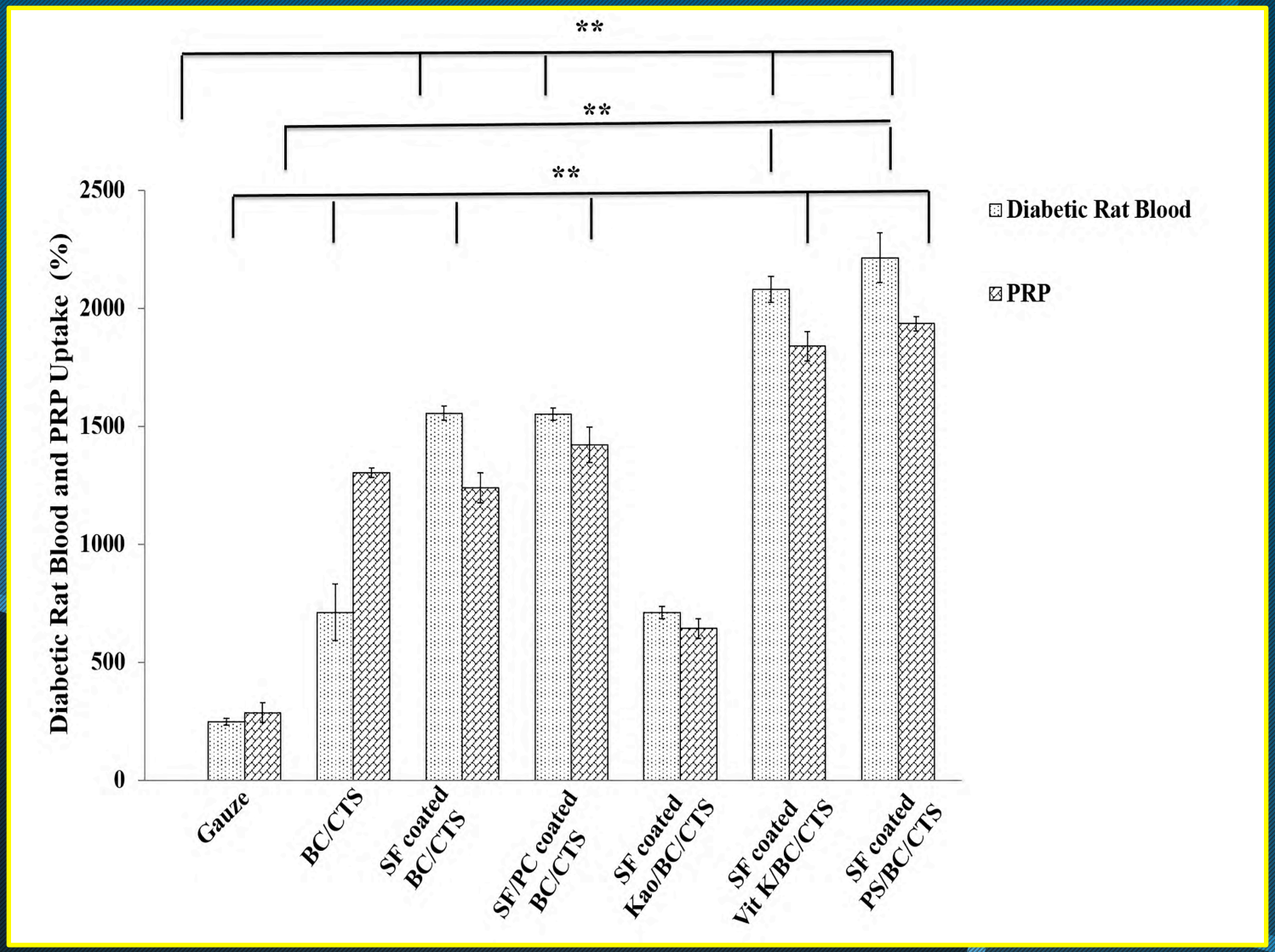


FIGURE 6. LDH activity results of the adhered platelets on the standard gauze, bare, and composite BC/CTS hemostatic dressings (**p<0.005). LDH activity was determined by measuring the OD450 nm spectrophotometrically using the blood plasma of diabetic rats.

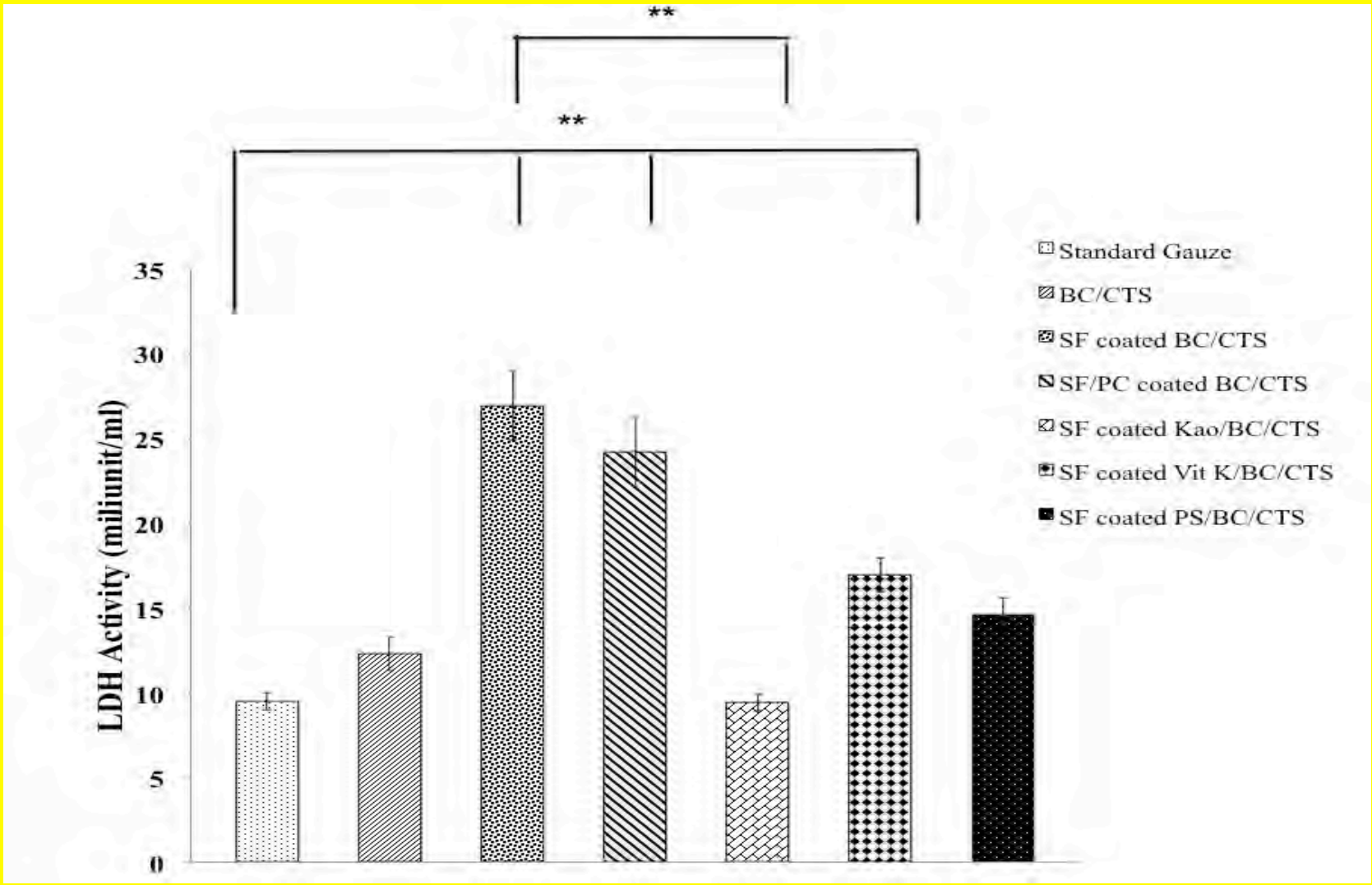


FIGURE 7. The adhered platelets on standard gauze, bare, and composite hemostatic dressings: (a) standard gauze, (b) BC/CTS, (c) SF-coated BC/CTS, (d) SF/PC-coated BC/CTS, (e) SF-coated Kao/BC/CTS, (f) Vit K/BC/CTS, and (g) PS/ BC/CTS (scale bars: 2 mm).

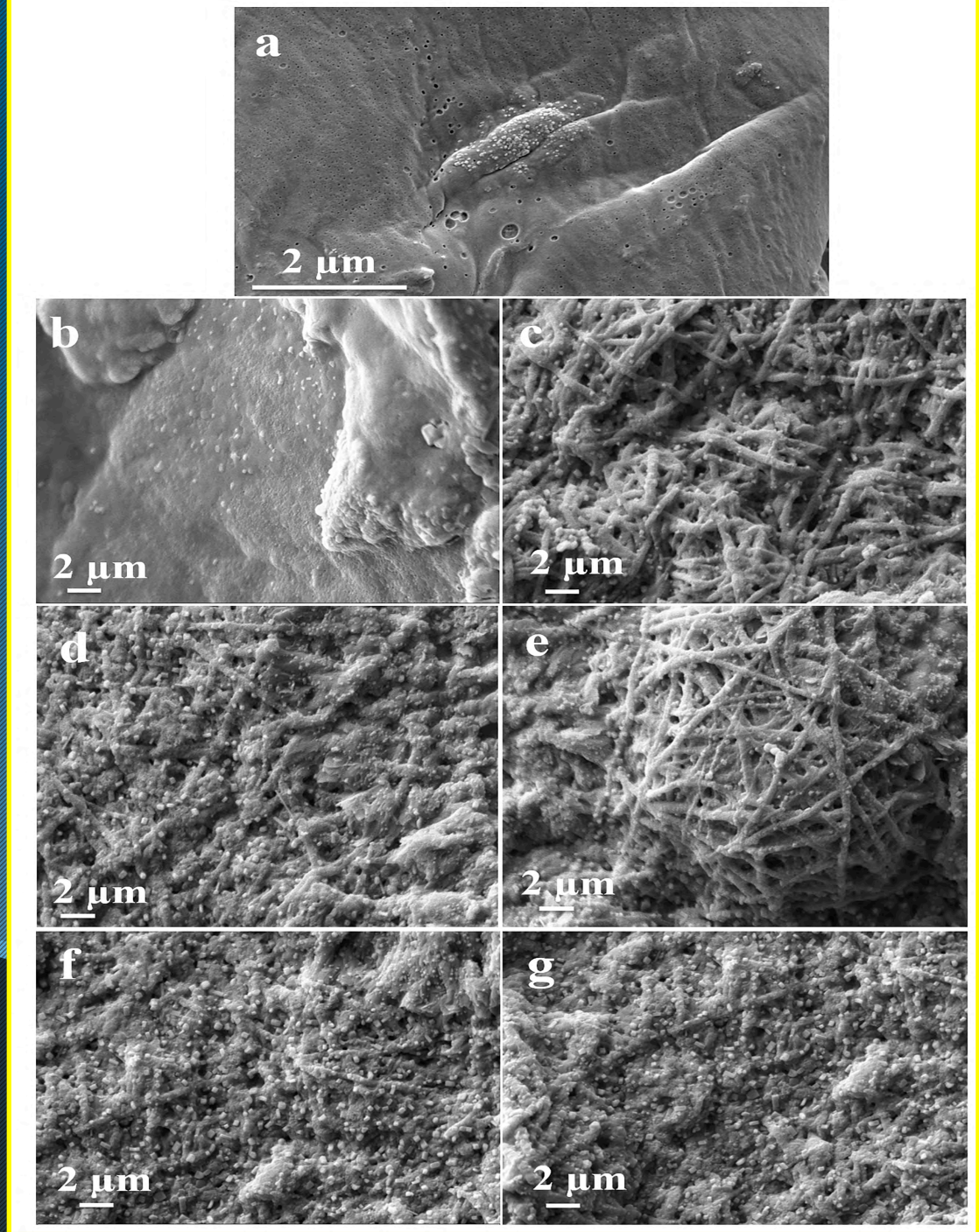


FIGURE 8. Hemostasis time of bare and composite BC/CTS hemostatic dressings in a diabetic rat femoral artery bleeding model (*p<0.05).

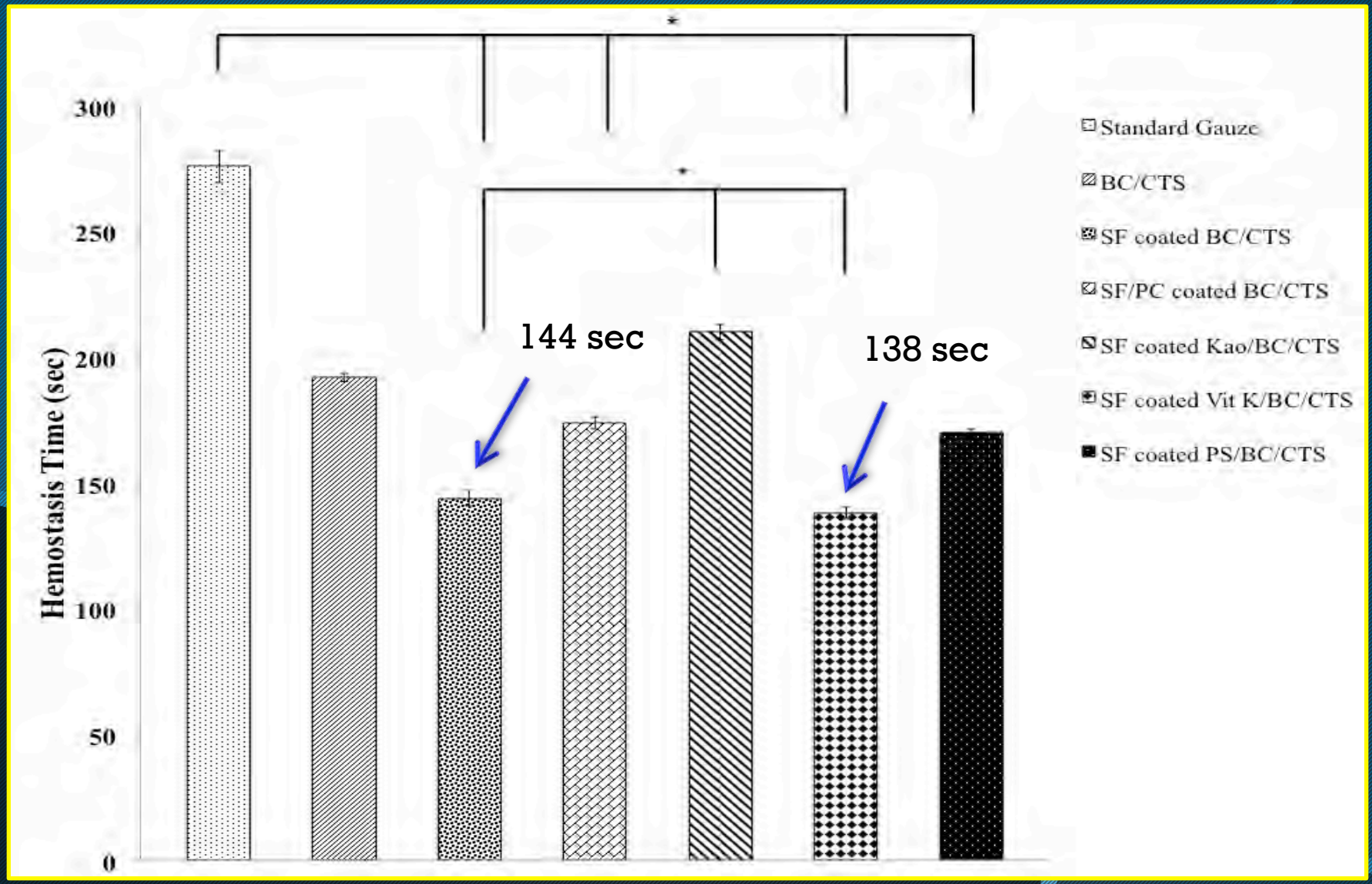
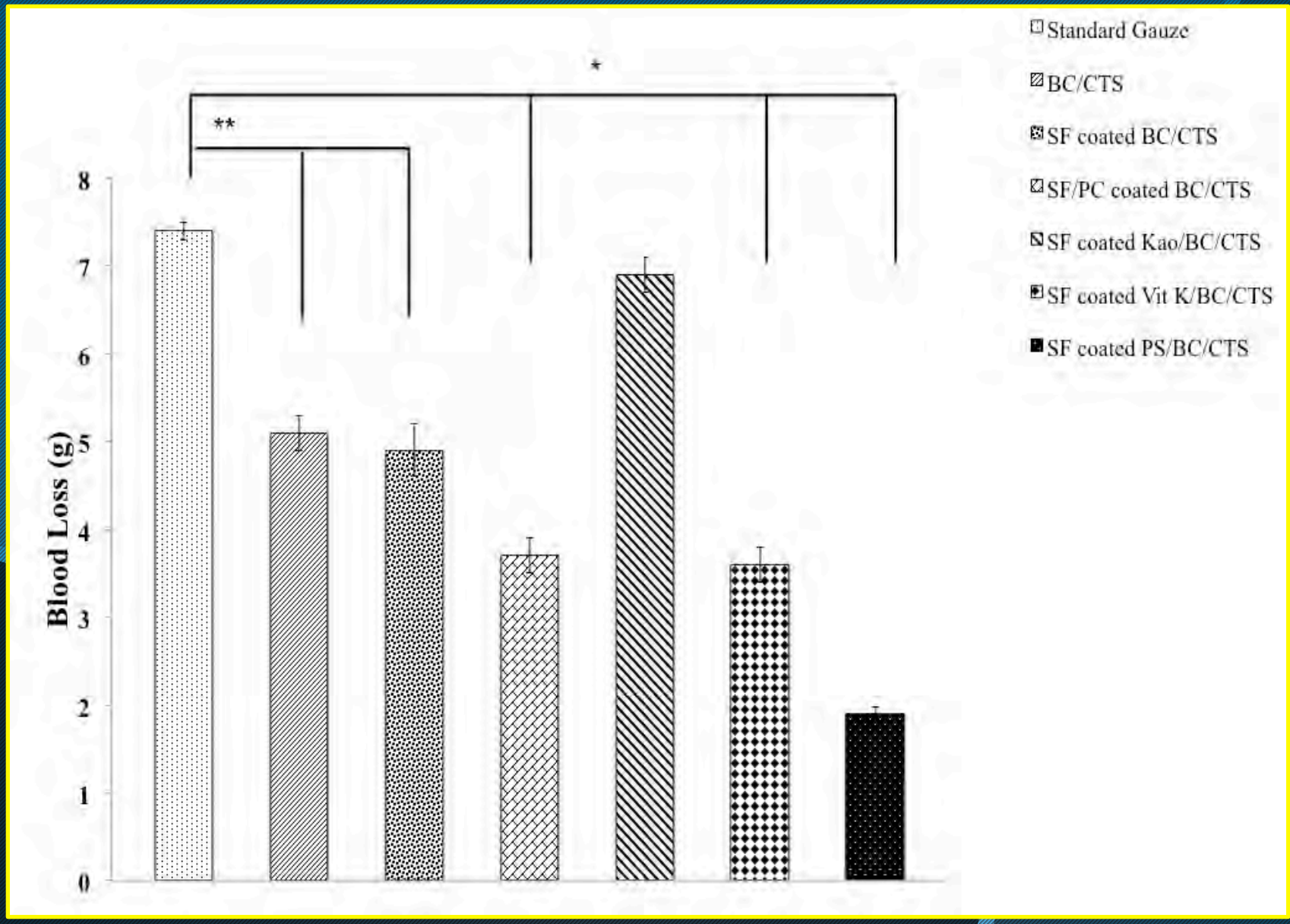


FIGURE 9. Total blood loss in a diabetic rat femoral artery bleeding model (**p<0.005; *p<0.05).



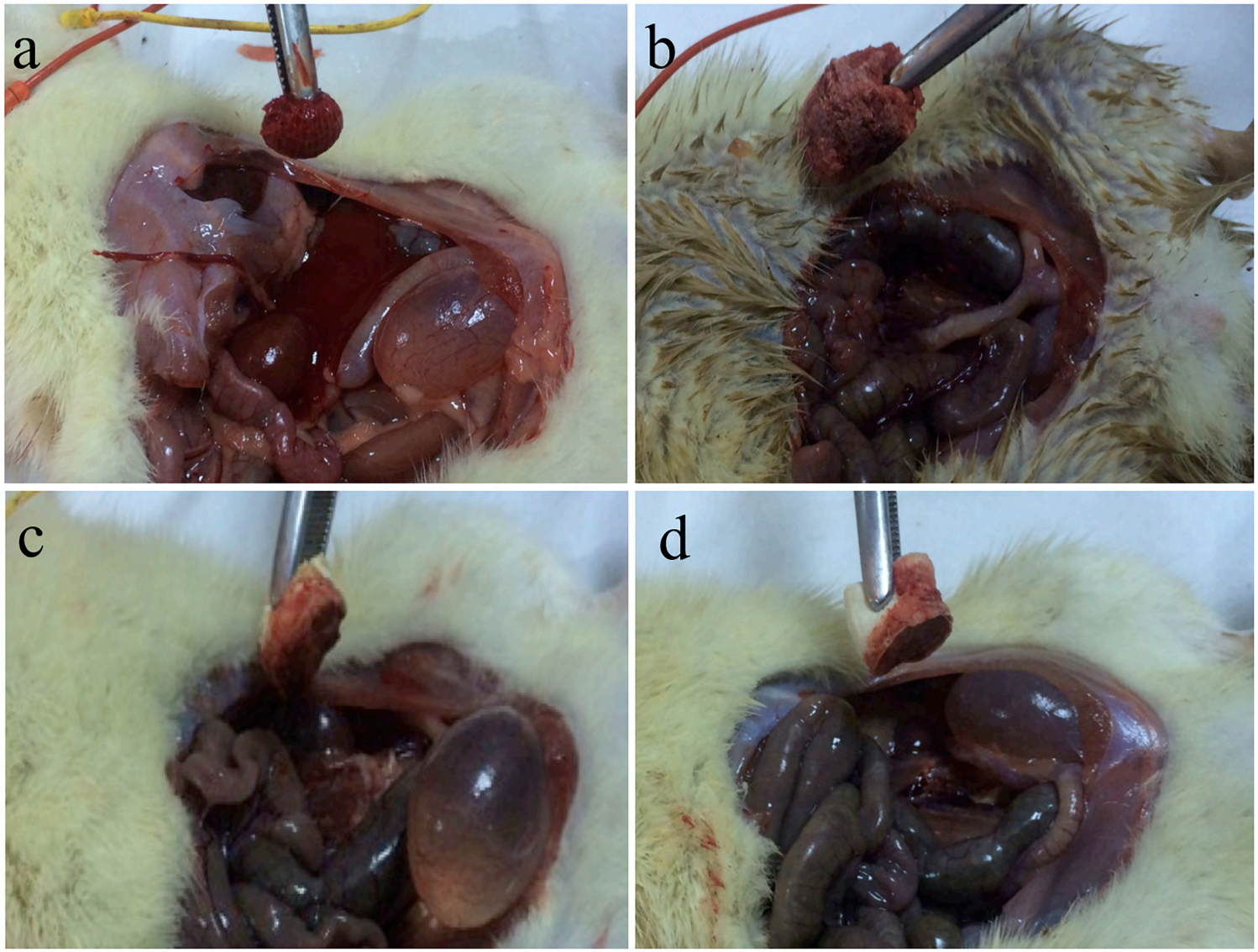


FIGURE 10. Bleeding site after application in a diabetic rat femoral artery model: (a) standard gauze, (b) SF-coated BC/ CTS, (c) SF-coated Vit K/ BC/CTS, and (d) SF-coated PS/BC/CTS.

FIGURE 11. (a) Mortality rates in different groups and (b) in vivo measurement of exothermic reaction after SF-coated Kao/BC/CTS treatment.

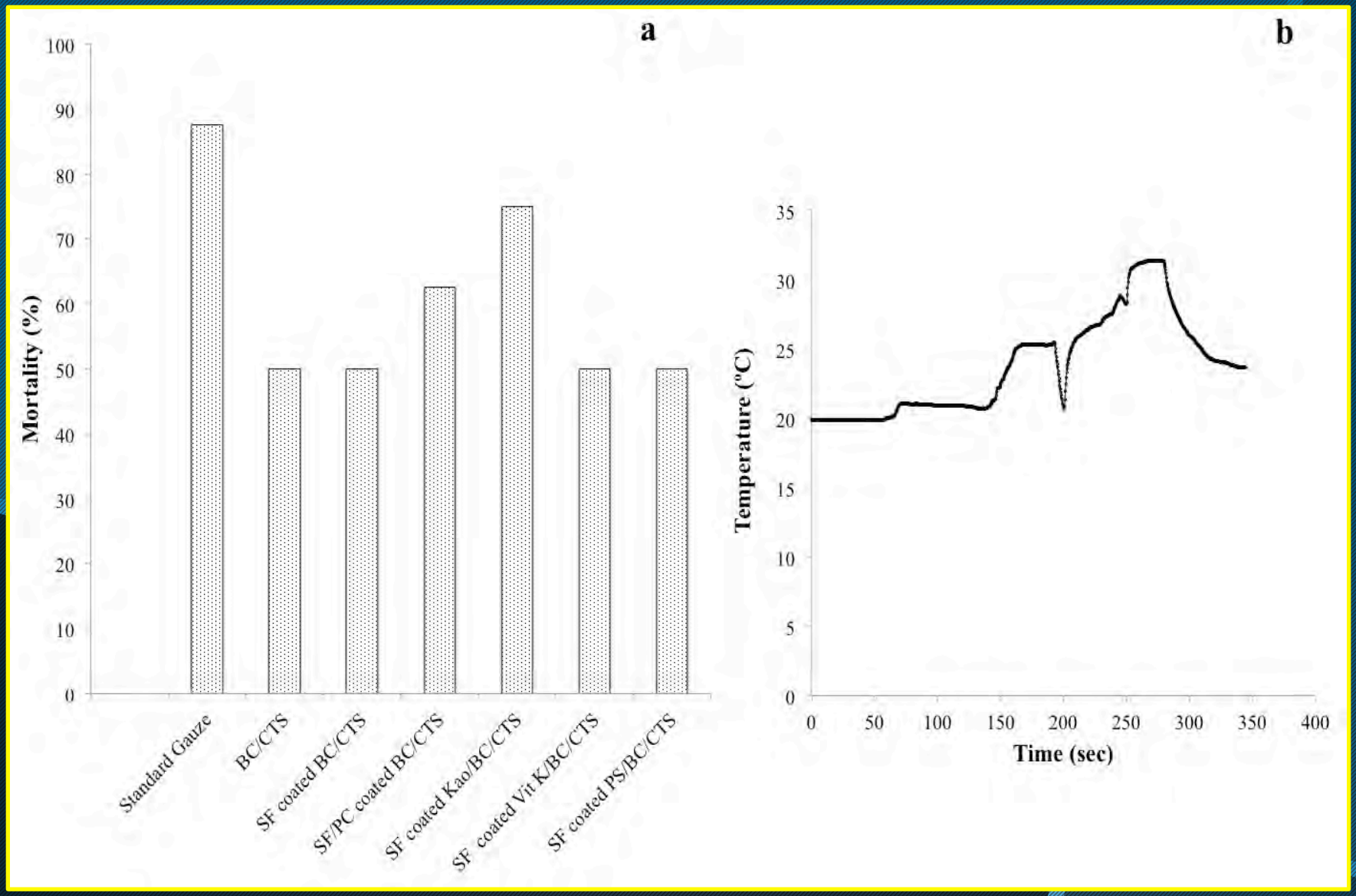
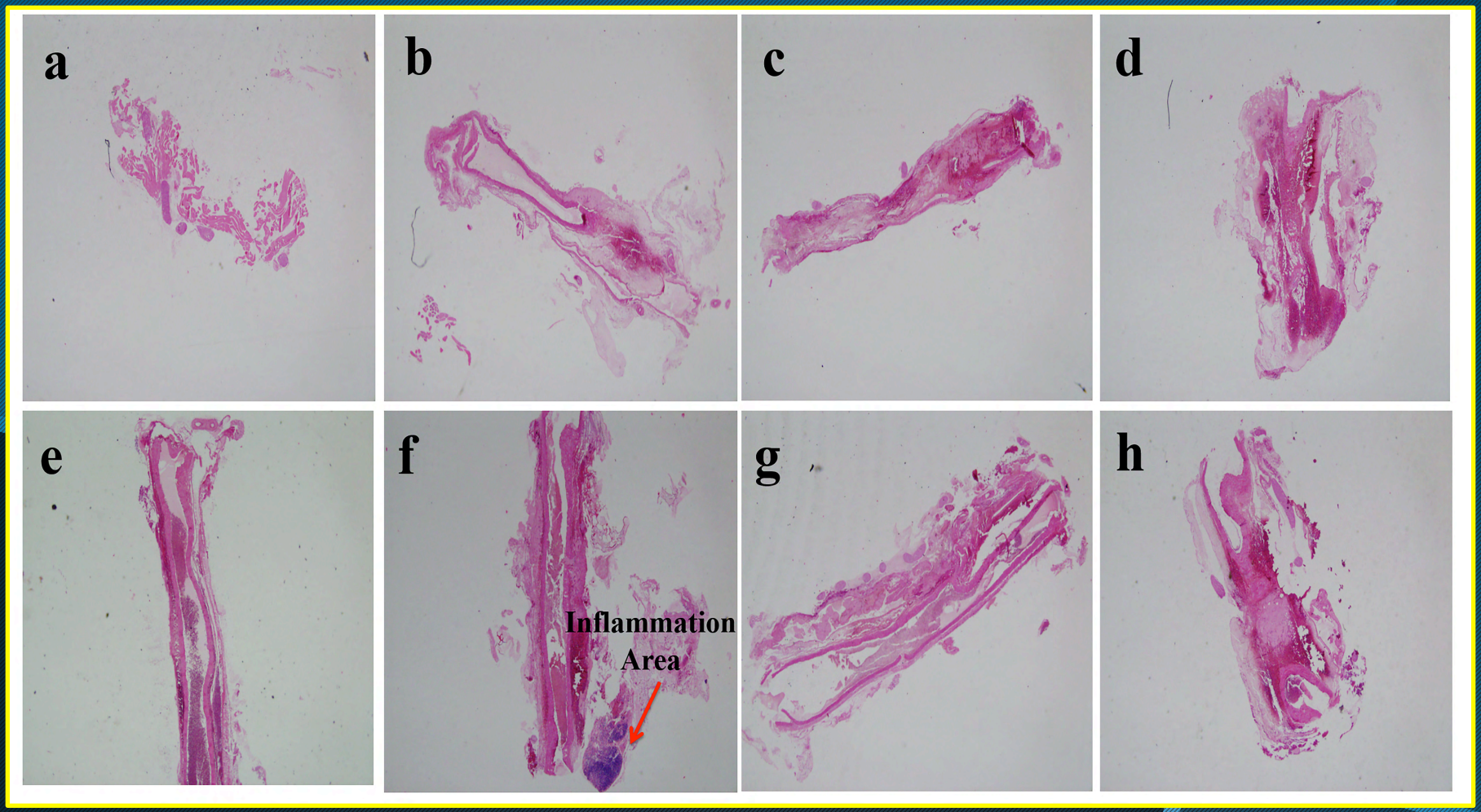


FIGURE 12. 13. Representative femoral artery sections stained with hematoxylin–eosin after application: (a) control, (b) standard gauze, (c) BC/CTS, (d) SF-coated BC/CTS, (e) SF/PC-coated BC/CTS, (f) SF-coated Kao/BC/CTS, (g) SF-coated Vit K/BC/CTS, and (h) SF-coated PS/BC/CTS.



CONCLUSIONS

- The composite hemostatic dressings are statistically superior at controlling hemorrhage compared to the standard gauze and the Kao- reinforced group.
- The application of bilayer hemostatic dressings in the diabetic rat femoral artery model significantly decreased mortality and reduced the bleeding time.
- Moreover, their low cost, easy manufacturing process, and high biocompatibility make them an effective hemostatic agent for use in civilian and military trauma management. Furthermore, this study is significant to understand the hemostatic response in diabetes.

Thank You For Your Attention!!!

HACETTEPE
University

AKSARAY ÜNİVERSİTESİ
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