

# Basic evaluation of novel gelatin hemostat

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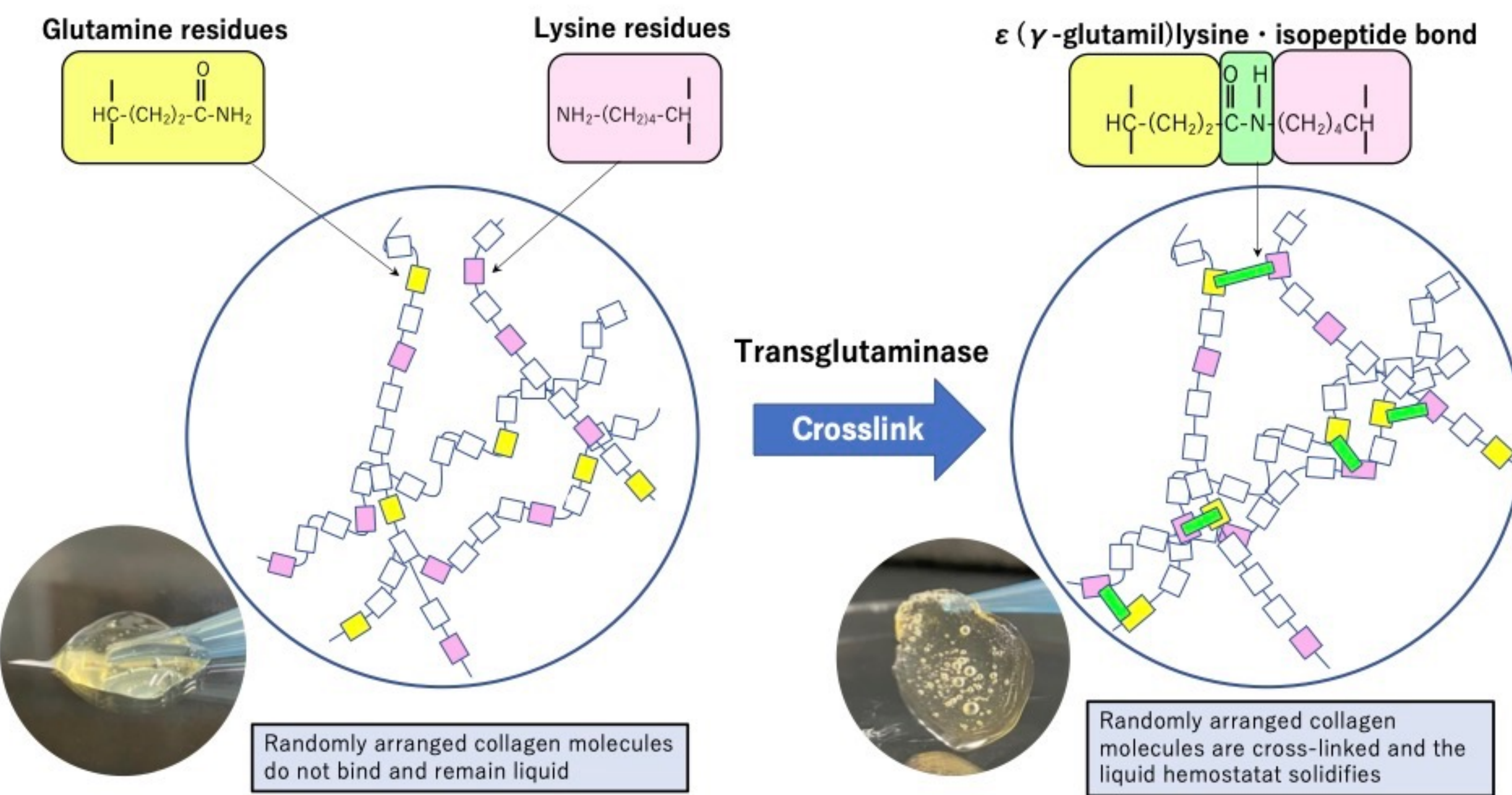
## Purpose

In the field of surgery, hemostasis is especially important for securing clear operative view and avoiding postoperative complications. We have developed a technology that can **instantly induce solidification** by adding a **transglutaminase (TGase)** which cause cross-linking to a **liquid gelatin**.

In this study, we examined the basic physical characteristics, hemostatic ability, and biocompatibility of Cross-linked gelatin (CLG).

## Methods

**Materials:** 20 g of gelatin and 80 g of demineralized water and appropriate concentration of low molecular weight substance were mixed and stirred at room temperature, completely dissolved in a water bath at 55 °C. TGase was dissolved in physiological saline to a concentration of 0.6 wt%.



**Viscoelasticity test:** Sonocrot<sup>®</sup> was used to confirm viscoelasticity. 200  $\mu\text{l}$  of liquid gelatin heated to 37 °C was set in an inspection device. Then, 100  $\mu\text{l}$  of the cross-linking agent (TGase), was added, and the measurement was started immediately.

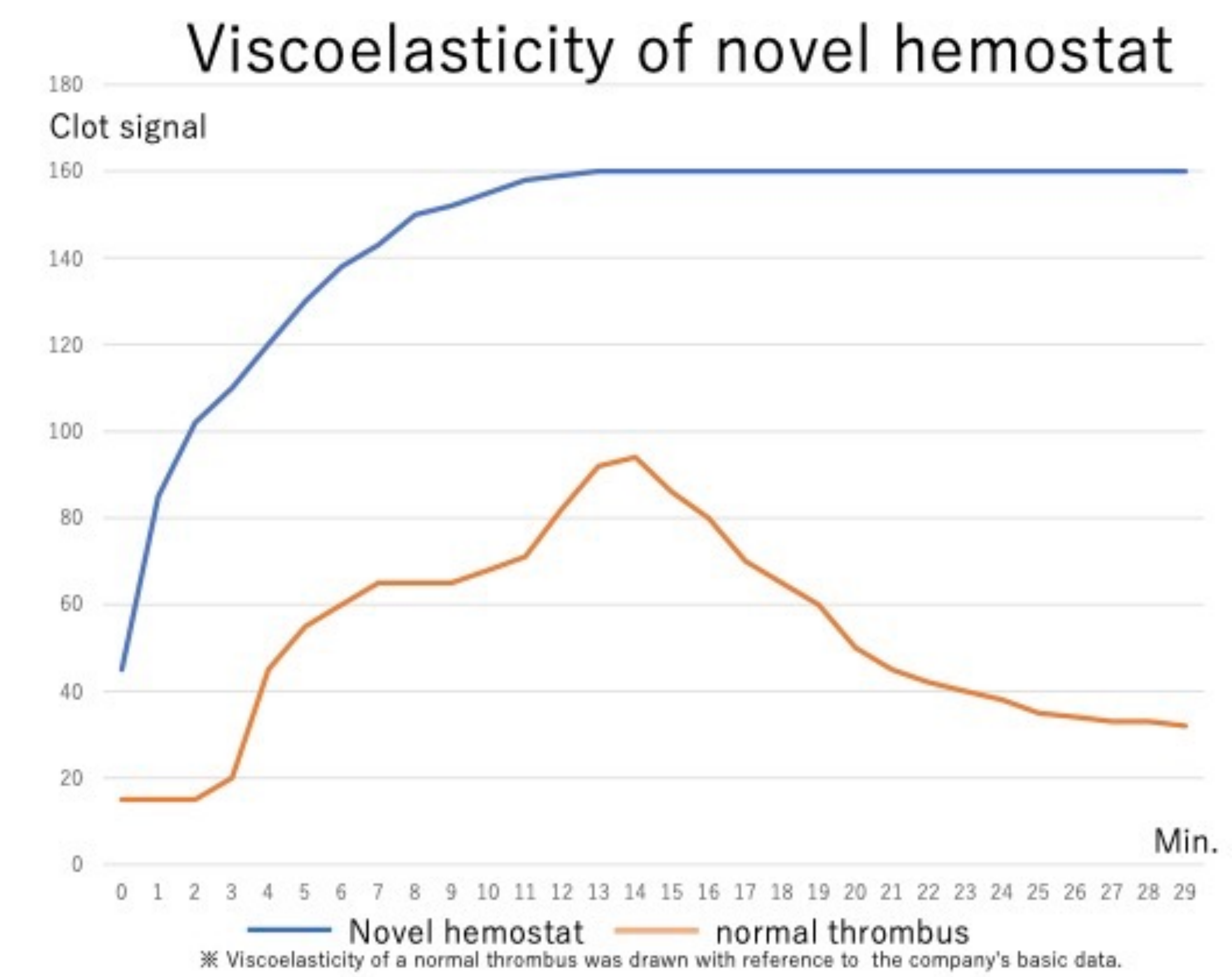
**Pathological examination:** CLG was embedded in the abdominal subcutaneous tissue of 8-week-old male wistar rats, and the skin was sutured after embedding. Procedures were performed in 1st, 2nd, and 4th weeks, respectively. At the 5th week, the appearance of the skin was checked and pathological examination of subcutaneous tissue were performed.

**Hemostatic examination:** Hemostatic examination was performed to 14 weeks-old male wistar rats. Bleeding experiments were performed in the order of the superficial femoral vein (SFV), the common iliac vein (CIV), and the inferior vena cava (IVC), and puncture was performed with a 22G, 22G and an 18G needle, respectively. Videos were taken and measured the time of hemostasis.

## Results

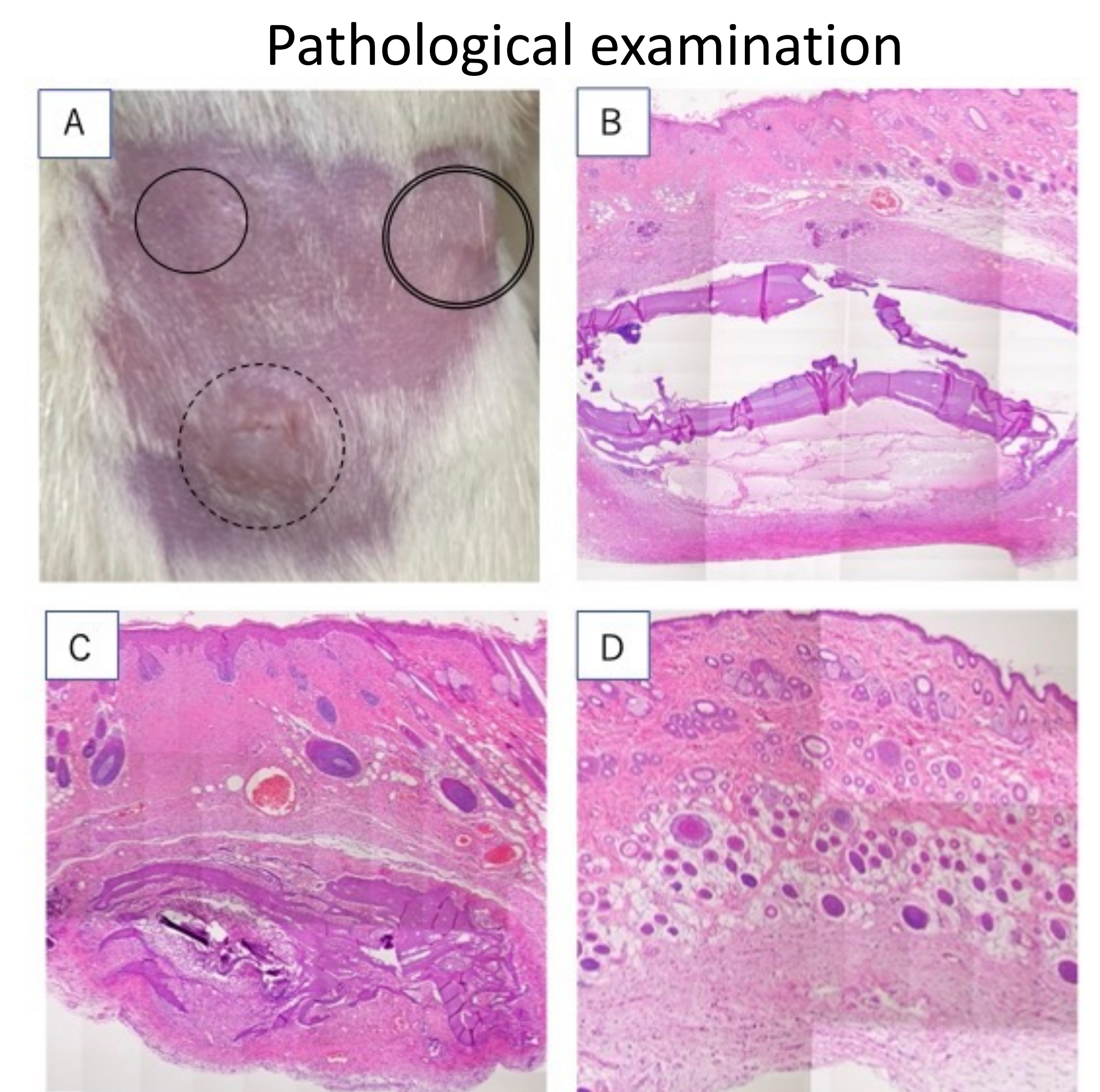
### Viscoelasticity test:

The viscoelasticity **increased immediately after the addition of the cross-linking agent**, and solidification was obtained within several tens of seconds. After that, the viscoelasticity continued to increase over time and reached a constant viscoelasticity in about 5 minutes. Viscoelasticity of novel hemostat was seemed to be always higher than that of normal clot formation.



### Pathological examination:

The skin 1 week, 2 weeks, and 4 weeks after the burial had bulges of 10 mm, 5 mm, and 0 mm due to CLG burial, respectively, and the **progress of absorption could be seen from the surface**. When pathological examination (HE staining) was performed for each, a large CLG was present under the skin one week after implantation, but it shrank slightly after two weeks and was **absorbed to the extent that it could not be confirmed after 4 weeks**.



A: Appearance of skin  
Circle (dashed line): After 1 week, the buried CLG bulge is observed.  
Circle (double): After 2 weeks, a slight CLG bulge can be observed.  
Circle (solid line): After 4 weeks, almost no bulge is observed.

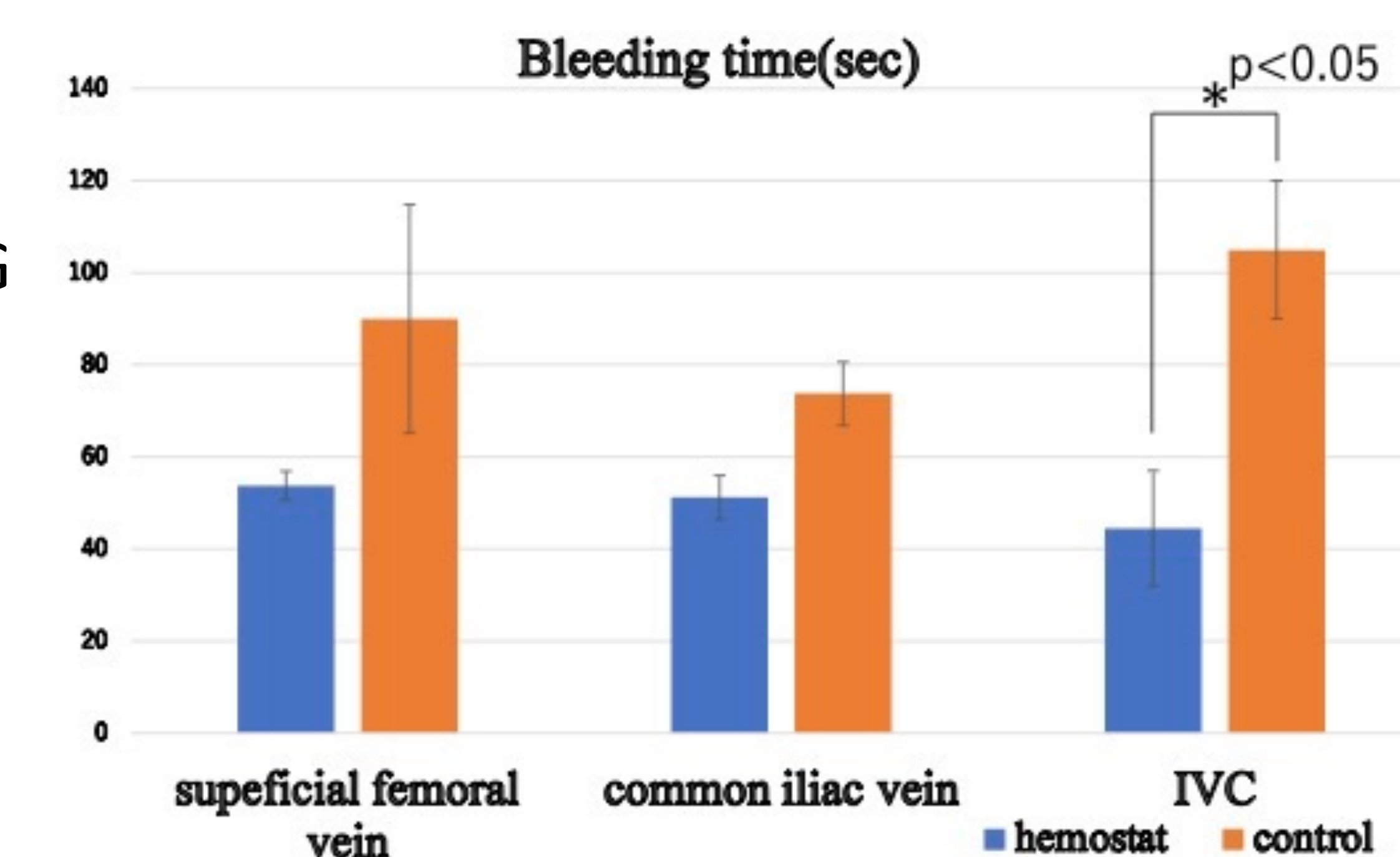
B: After 1 week (weak magnification): Collagen exists under the skin almost as it is.

C: 2 weeks later (Strong magnification): Collagen diameter is halved and gradually absorbed.

D: After 4 weeks (weak magnification): Collagen is absorbed, and no inflammation of surrounding tissues.

### Bleeding experiment:

The average time required from puncture to hemostasis was 90 seconds in the control group and 53.8 seconds in the CLG group in the Superficial femoral vein. In the Common iliac vein, the control group averaged 73.8 seconds and the CLG group averaged 51.3 seconds. In the IVC, the control group averaged 105 seconds and the CLG group averaged 44.5 seconds.



## Conclusions

We developed a novel hemostat made of gelatin with low molecular weight substance, and induced **cross-linking** by TGase. Examination of physical characteristics showed that collagen started to **solidify immediately** after the addition of TGase. Thanks to its immediate solidification, **hemostatic effect was favorable** in animal bleeding model and its **biocompatibility** was also good. Although further examination for its molecular mechanism of solidification or its effect in clinical setting is still needed, CLG has a suitable feature for hemostat.