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ORIGINAL ARTICLE

An *in-vitro* study on corn-shaped balloon-enhanced thrombolysis

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Abstract

Background: Glossy balloons were used to enhance deep vein thrombolysis. However, thrombi tend to yield in the balloon dilatation due to their smooth surface. It may be preferable to enhance thrombolysis by using balloons with a rough surface, instead of balloons with a smooth surface. **Material and methods**: Four rabbits were used for the experiment and 60 blood clot samples were obtained, and the samples were randomly separated into one control group and four balloon-solubilised groups. Urokinase solution (5000 U/ml) was used as the thrombolytic drug. The balloon-solubilised groups were enhanced respectively by 1 atm and 2 atm pressurised glossy balloons, and 1 atm and 2 atm pressurised corn-shaped balloons. Thrombolysis rates and residual rates for different granularities of blood clots were calculated for comparasion. **Results**: Thrombolysis rates of the groups using corn-shaped balloons were higher than those of the groups using glossy balloons ($p = 0.003$ and $p = 0.002$). Residual rates of $\Phi \geq 3.7$ mm blood clots for the groups using corn-shaped balloons were lower than those for the groups using glossy balloons ($p < 0.001$ and $p < 0.001$). **Conclusion**: Balloons could be used to enhance thrombolysis; under the same balloon dilatation pressure, thrombolysis rates when using corn-shaped balloons are better than those when using glossy balloons.

Key words: *Deep vein, thrombus, thrombolysis, balloon solubilisation*

Introduction

Deep vein thrombosis (DVT) may break and travel to the lungs, thus causing pulmonary embolism (PE) (1–6) and post-thrombotic syndrome (7). Several treatment approaches have been developed, including anticoagulation therapy with therapeutic agents (8–10), catheter-directed intra-thrombus thrombolysis (CDIT) (11,12), and pharmacomechanical catheter-directed thrombolysis (PCDT) (13–15). Clinical trials have proved that the combined use of a balloon catheter and thrombolytic drugs helps to reduce thrombolytic drug dosage and improve thrombolytic effect (16–20). The thrombi tend to slip and yield in the dilatation process of using a conventional glossy balloon due to their smooth surface. Sometimes, this makes it difficult to capture and crush thrombi effectively, thus affecting the final thrombus solubilisation results. It may be preferable to enhance thrombolysis by using balloons with a rough surface. This study was conducted using a kind of corn-shaped balloon. *In-vitro* experiments were performed to compare the solubilisation performance of the corn-shaped balloon with that of a glossy balloon. It is expected that this study will provide a constructive reference for the research and development of new PCDT devices.

Material and methods

Experimental setting

The clots used for experiments were made of the blood of four healthy Chinese rabbits. In order to observe the thrombolysis processes and compare effects of the two different types of balloons, 60 segments of transparent silicone tube of 10 mm lumen

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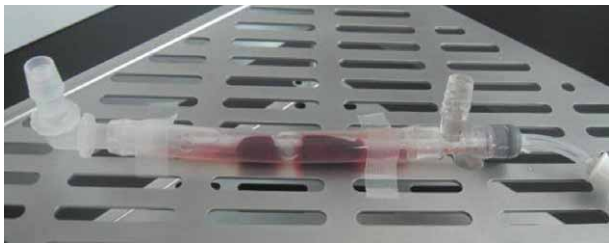

Figure 1. The experimental setting.

and 130 mm length were used to simulate deep vein vessels. A similar simulation was used by Andrew (21). We plan to simulate human deep vein vessels with porcine vessels in the future. A right-angle siphon was connected to the proximal end of a simulated vessel, in order to prevent liquid overflow. A T-shaped three-way connector was connected to the distal end of the simulated vessel by one of its long arms. The short arm of the T-shaped three-way connector was also used to prevent liquid overflow. Another long arm of the T-shaped three-way connector was connected to a short segment of silicone tube of 10 mm lumen and 20 mm length. Another end of the short tube was connected to a rubber plug for closure. The simulated vessel was fixed on a base (Figure 1), with the protruding end of the right-angle siphon and the short arm of the T-shaped three-way connector in an upright position.

Harvesting and treatment of blood clots

The rabbit blood (120 ml) was randomly divided into 60 samples of 2 ml each. Each sample was injected into a simulated vessel and kept at room temperature for 30 minutes, then moved to a 4°C refrigerator and kept for two days. Each clot was washed gently with 0.9% sodium chloride solution, and roll-dried with neutral filter paper. Each clot was measured to obtain the net weight (w_0), and put into a simulated vessel again.

Preparation of balloons

Two types of balloons made of medical silicone were used in this study. One was a conventional glossy balloon and the other was a corn-shaped balloon, made by sticking 0.3-mm diameter cone-like particles on the surface of the glossy balloon. The length and

Figure 2. (A-F) Dilatation process of the corn-shaped balloon.

diameter of each type of balloons were 35 mm and 3 mm in the natural state (without dilatation). The dilatation processes of the two types of balloons in air are shown in Figure 2. Due to the smooth surface of the glossy balloon, the thrombi tended to slip and yield during balloon dilatation (Figure 3A–E), which made it difficult to crush the thrombi effectively. For corn-shaped balloons, in contrast, the particles on the surface could embed into the thrombus surface, helping to capture and crush the thrombi effectively (Figure 3F–J).

Experimental methods

The blood clot samples were divided into five experimental groups. Group 1 (control group) samples were treated with thrombolytic drug and without balloon solubilisation. Group 2 samples were treated with thrombolytic drug and glossy balloon solubilisation. The balloons were dilated to 1 atm pressure. Group 3 samples were treated in the same way as those of group 2, except that the balloons were dilated to 2 atm pressure. Group 4 and group 5 samples were treated in the same way as those of group 2 and group 3, except that corn-shaped balloons were used instead. A similar experimental method was used by Andrew et al. (21). The use of 1–2 atm dilatation pressure was in accordance with the method reported by Bagan et al. (16). Urokinase solution (5000 U/ml) was used as the thrombolytic drug for all blood clot samples. All experiments were performed in the experimental setting as shown in Figure 1.

The experiment was performed as follows: Using a syringe, the thrombolytic drug was injected into the simulated vessel slowly via the short end of the T-shaped three-way connector until the simulated vessel was filled with the thrombolytic drug, and the protruding end of the right-angle siphon and the short arm of the T-shaped three-way connector were left with some room for overflow. The balloon was dilated with a medical balloon dilatation pump. After each experiment, the mixture of clot fragments and thrombolytic drug was filtered with six sieves of different apertures, which were stacked one onto another. The apertures of the six sieves were 4.5 mm, 3.7 mm, 2.5 mm, 1.8 mm, 1 mm, and 100 μm,

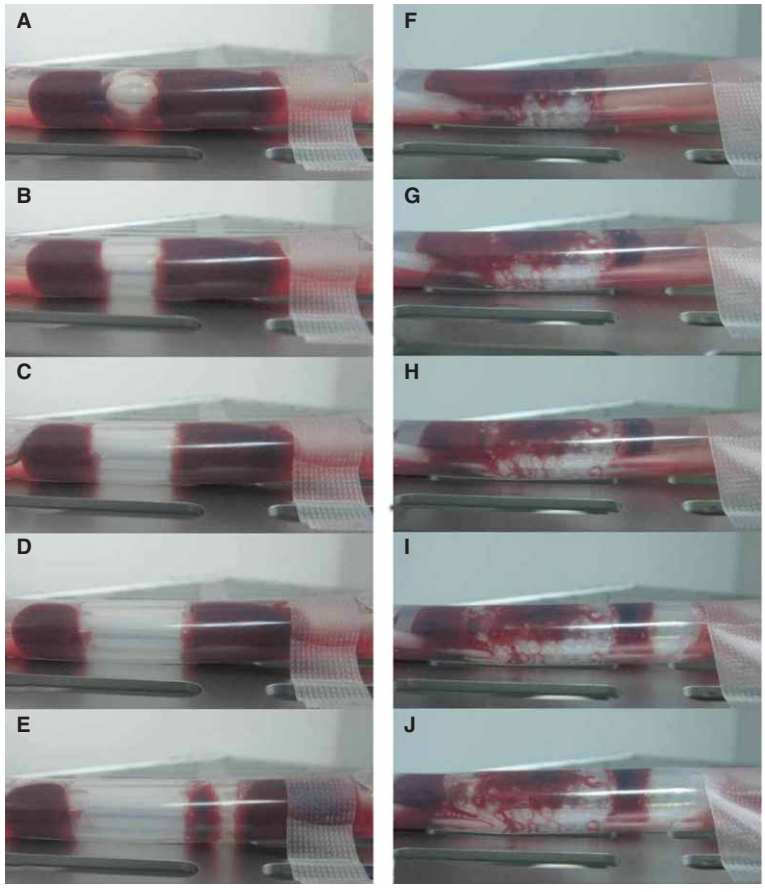

Figure 3. Thrombolysis processes using two different types of balloon solubilisation. (A–E) Thrombolysis process using glossy balloon solubilisation, (F–J) thrombolysis process using corn-shaped balloon solubilisation.

respectively, decreasing gradually from top to bottom. After each filtration, the sieves were kept in air for five minutes to drain the liquid.

After that, different sizes of clot fragments on sieves were collected and weighed (w_k, k = 1,2,3,4,5,6). The thrombolysis rates were calculated with the equation $((w_0 / \sum_{k-1}^{7} w_k) / w_0) \times 100\%$. The residual rates for different sizes of clot fragments were calculated with the equation $(w_k / \sum_{k-1}^{7} w_k) \times 100\%$.

Measured parameters and statistics

For all five experimental groups, the thrombolysis rates and residual rates of clot fragments were expressed in the form of mean ± standard deviation, and compared with three different statistical methods. The four balloon-solubilised groups were compared with the control group using the method of one-way analysis of variance (ANOVA). Experimental groups using the same dilatation pressure but different types of balloons were compared with the method of independent-sample *t* test. Experimental groups using the same type of balloon but different dilatation pressures were compared with the method of Pearson bivariate correlation analysis. The statistics software SPSS 19.0 was used. *p* values ≤ 0.05 were considered statistically significant.

Results

Observation

Before injecting the thrombolytic drug, the clot samples of all experimental groups were of a dark red colour and smooth surface. For group 1, after injecting the thrombolytic drug, the reagent in the analogue vessels turned from transparent to some sort of red slowly, and the colour became darker over time. At the end of the thrombolysis process, the colour of clots became brighter than before thrombolysis, the sizes became smaller, and the surface became rougher, but each clot sample still remained a solid piece, and it was not crushed into fragments.

For each sample of group 2 or 3, the reagent in the simulated vessel turned from transparent to a colour of light red and the clot slipped and yielded obviously, but still could be cleaved from a single piece to two or three pieces. At the end of the thrombolysis process, we filtered the mixture of clot fragments and thrombolytic drug with the six sieves. It could be observed that the clot fragments remained mostly on the top two sieves of large apertures (for some samples, clot fragments remained only on one of the two top sieves); a few fragments remained on the four sieves below. The clot fragments remaining on each sieve are shown in Figure 4A–F. The fragments were from one typical sample of group 2.

For each sample of group 4 or 5, the reagent in the analogue vessel turned from transparent to red, and the clot was crushed from a single piece into many small pieces. Compared with group 2 or 3, clot yielding was significantly inhibited. At the end of the thrombolysis process, we filtered the mixture of clot fragments and thrombolytic drug with the six sieves. The clot fragments were visible evenly on all six sieves. The clot fragments remaining on each sieve are shown in Figure 4G–L. The fragments were from one typical sample of group 4.

Data measurement

Thrombolysis rates. The thrombolysis rates for all five experimental groups are shown in Table I. According

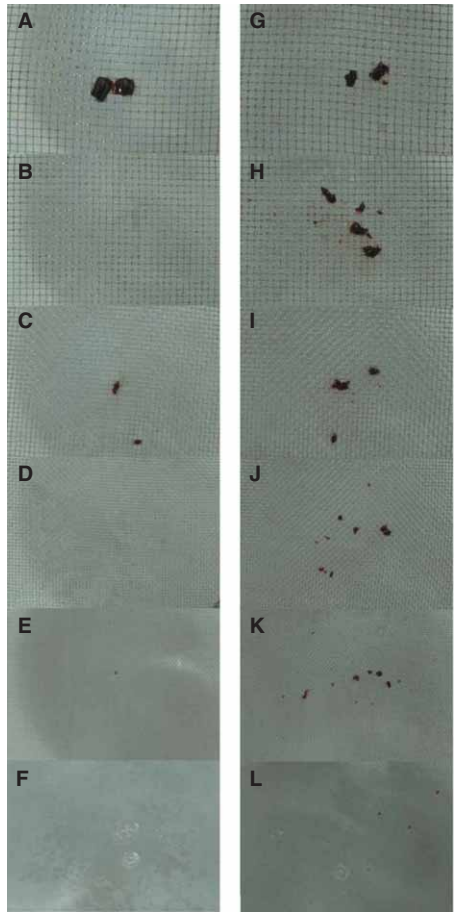

Figure 4. Thrombus lysis conditions. (A–F) Residual clot fragments of grain sizes of 4.5 mm, 3.7 mm, 2.5 mm, 1.8 mm, 1 mm, and 0.1 mm after glossy balloon solubilisation. (G–L) Residual clot fragments of grain sizes of 4.5 mm, 3.7 mm, 2.5 mm, 1.8 mm, 1 mm, and 0.1 mm after corn-shaped balloon solubilisation.

Table I. Thrombolysis rates and residual rates of clot fragments for all five experimental groups.

Group	Thrombolysis rate (%)	Residual rate of $\phi \geq 3.7$ clot fragments (%)	Residual rate of $\phi < 3.7$ clot fragments (%)	Number of samples
Only thrombolytic drug	20.87 ± 6.26	100 ± 0.00	0 ± 0.00	12
Thrombolytic drug + 1 atm glossy balloon	28.60 ± 6.00	90.60 ± 4.36	9.40 ± 4.36	12
Thrombolytic drug + 2 atm glossy balloon	31.39 ± 3.76	87.87 ± 8.45	12.13 ± 8.45	12
Thrombolytic drug + 1 atm corn-shaped balloon	35.91 ± 4.33	51.58 ± 5.46	48.42 ± 5.46	12
Thrombolytic drug + 2 atm corn-shaped balloon	38.12 ± 4.90	50.65 ± 4.28	49.35 ± 4.28	12

to the outcome of one-way ANOVA analysis, the thrombolysis rates for groups 2, 3, 4, and 5 were higher than those for the control group [F = 17.018 between groups, $p < 0.05$ ($p < 0.001$)]. There was a significant difference between the control group and each of the other groups. It was shown that the thrombolytic effect when using balloon solubilisation and thrombolytic drug simultaneously was superior to that when using only the thrombolytic drug.

According to the outcome of independent-sample *t* test analysis, the thrombolysis rates for group 4 were higher than those for group 2 [$p < 0.05$ ($p = 0.003$)]. A significant difference was observed between group 4 and group 2. The thrombolysis rates for group 5 were also higher than those for group 3 [$p < 0.05$ ($p = 0.002$)]. A significant difference was observed between group 5 and group 3. It was shown that, under the same balloon dilatation pressure, the solubilising effect of the corn-shaped balloons was superior to that of the glossy balloons.

According to the outcome of Pearson bivariate correlation analysis with the experimental data of groups 2 and 3, the correlation between thrombolysis rates and balloon dilation pressure was weak and of no statistical value [correlation coefficient was 0.269, $p > 0.05$ ($p = 0.204$)]. Likewise, according to the outcome of Pearson bivariate correlation analysis with the experimental data of groups 4 and 5, the correlation between thrombolysis rates and balloon dilation pressure was also weak and of no statistical value [correlation coefficient was 0.233, $p > 0.05$ ($p = 0.273$)]. It was shown that no matter which type of balloons was used for solubilisation, dilatation pressure influenced the thrombolytic effect to a small extent. The distribution of thrombolysis rates for the five experimental groups is shown in Figure 5.

Residual rates of blood clot fragments. The residual clot fragments were divided into two grades, $\phi \geq 3.7$ mm grade and $\phi < 3.7$ mm grade, according to their

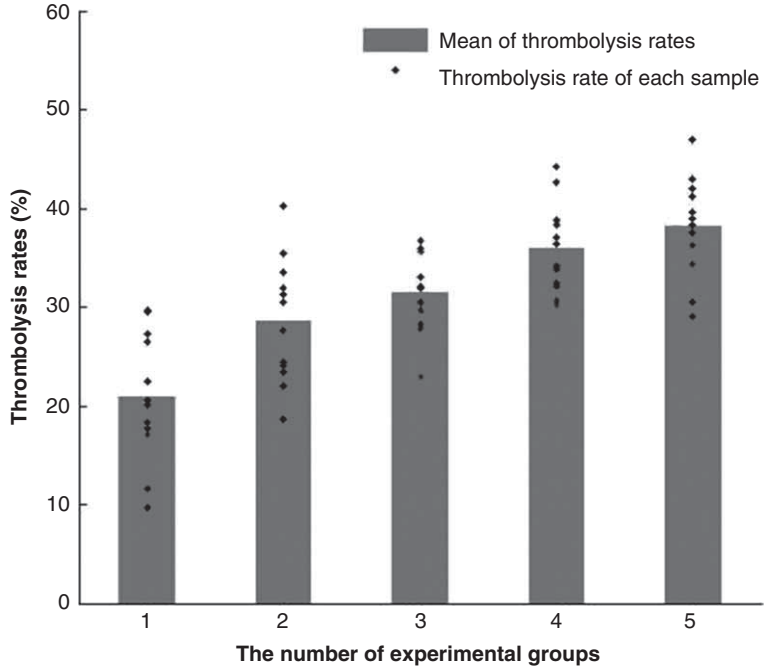

Figure 5. The distribution of thrombolysis rates for all five experimental groups.

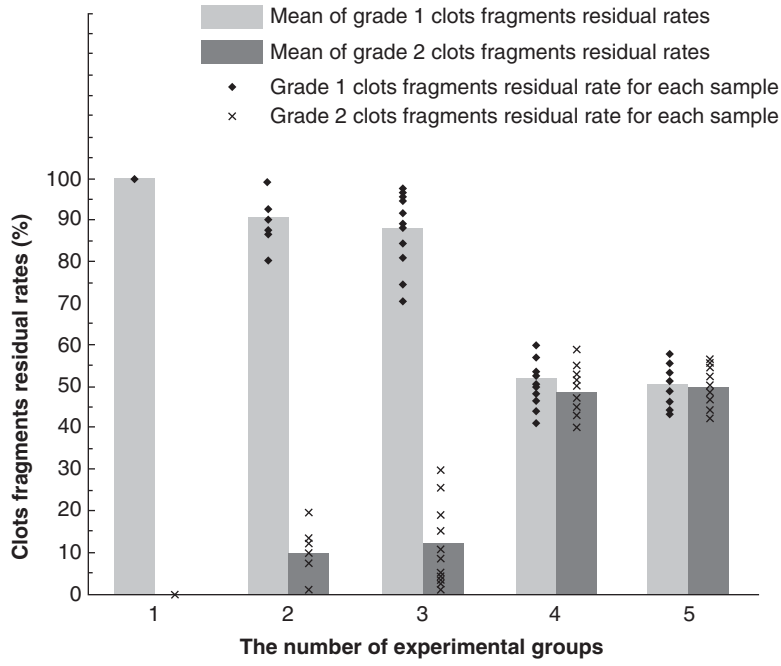

Figure 6. The distribution of residual rates of two grades of clot fragments for all five experimental groups. Yielding could be inhibited to some extent; thrombolysis could be improved; and thrombus solubilisation could be enhanced.

distribution. In grade 1, the size of clot fragments was ≥3.7 mm. In grade 2, the size of clot fragments was <3.7 mm. The residual rates for the two grades of clot fragments for each experimental group are shown in Table I. According to the outcome of one-way ANOVA analysis, residual rates of clot fragments of grade 1 for groups 2, 3, 4, and 5 were lower than those for the control group [F = 215.136 between groups, $p < 0.05$ ($p < 0.001$)]. There was a significant difference between the control group and each of the other groups. It was shown that the thrombolytic effect when using balloon solubilisation and thrombolytic drug simultaneously was superior to that when using only the thrombolytic drug.

According to the outcome of independent-sample *t* test analysis, the residual rates of clot fragments of grade 1 for groups 4 and 5 were lower than those for groups 2 and 3 [group 4 vs group 2, $p < 0.05$ ($p < 0.001$), group 5 vs group 3, $p < 0.05$ ($p < 0.001$)]. On the contrary, the residual rates of clot fragments of grade 2 for groups 4 and 5 were higher than those for groups 2 and 3 [group 4 vs group 2, $p < 0.05$ ($p < 0.001$), group 5 vs group 3, $p < 0.05$ ($p < 0.001$)]. A significant difference was observed between group 4 and group 2. A significant difference was also observed between group 5 and group 3. It was shown that, under the same balloon dilatation pressure, the thrombolytic effect when using corn-shaped balloon solubilisation was superior to that when using glossy balloon solubilisation. According to the outcome of Pearson bivariate correlation analysis with the experimental data of groups 2 and 3, the correlation between residual rates of clot fragments of grade 1 and balloon dilatation pressure was weak and of no statistical value [correlation coefficient was -0.199, $p > 0.05$ ($p = 0.351$)]. Likewise, the correlation between residual rates of clot fragments of grade 2 and balloon dilatation pressure was weak and of no statistical value [correlation coefficient was 0.199, $p > 0.05$ ($p = 0.351$)]. In the same manner, according to the outcome of Pearson bivariate correlation analysis with the experimental data of groups 4 and 5, the correlation between residual rates of clot fragments of grade 1 and balloon dilatation pressure was weak and of no statistical value [correlation coefficient was -0.094, $p > 0.05$ ($p = 0.661$)]. The correlation between residual rates of clot fragments of grade 2 and balloon dilatation pressure was also weak and of no statistical value [correlation coefficient was 0.094, $p > 0.05$ ($p = 0.663$)]. It was shown that, no matter which type of balloons was used for solubilisation, the dilatation pressure influenced thrombolytic effect to a small extent. The distribution of residual rates of the two grades of clot fragments for all five experimental groups is shown in Figure 6.

Discussion

The application of ultrasound to improve intravenous thrombolysis has been investigated. Clinical trials

have shown the low efficacy of this technique (22). Moreover, ultrasound possesses a potential, especially at higher intensities, to lead to platelet adhesion (23) and blood vessel damage (24). Low frequency vibro-percussion(LFVP) may offer an acoustic alternative in the treatment of DVT due to its known clot disruptive abilities (25) and unique internal transmission characteristics through body tissue (26). Nevertheless, it is necessary to search for proper LFVP parameters to obtain ideal clot clearing effects (21).

The use of balloon-enhanced thrombolysis has two main benefits:

- Balloon dilatation enhances thrombolysis, which helps to improve the thrombolytic effect; and
- the balloon opens vein occlusion, which helps with local dispersion of thrombolytic drug, reducing the incidence of haemorrhagic disease.

Using conventional glossy balloons for solubilisation in the process of dilatation, thrombi tend to slip and yield, thus affecting thrombolysis, and restricting the overall solubilisation effect. When using corn-shaped balloons instead, the small particles on the surface could partially embed into the thrombus surface, thus easing thrombus capture; during balloon dilatation, the distances between these particles could be expanded, easing thrombus tearing.

Thrombus migration is a common phenomenon occurring during balloon-enhanced thrombolysis for DVT, and this increases the risk of distal embolization (27,28). This phenomenon may happen, no matter which ballons are used, conventional glossy balloons or corn-shaped balloons. However, embolic filters could be used to capture the debris and prevent distal embolization.

Conclusion

In this paper, the solubilisation effect of corn-shaped balloons and that of conventional glossy balloons was studied and compared. The results of *in-vitro* experiments using animal blood clots show that

- use of balloon solubilisation helps to enhance the thrombolytic effect;
- under the same dilatation pressure, the thrombolysis rates using corn-shaped balloons are higher than those using glossy balloons;
- under the same dilatation pressure, the thrombolysis using corn-shaped balloons is better than that using glossy balloons; and
- no matter which type of balloon is used, the dilatation pressure influences thrombolysis rates and thrombolysis to a small extent.

As the thrombi could be crushed more finely by using corn-shaped balloons, the overall surface area of thrombi could be increased. From the perspective of long-term solubilisation, corn-shaped balloons should be better than glossy balloons. For complications, the use of corn-shaped balloons may also trigger pulmonary embolism, therefore, an inferior vena cava filter should be used routinely. As all data used in this study were from *in-vitro* experiments with animal blood clots, the conclusion is only for reference. Further study based on *in-vitro* experiments of human thrombi or *in-vivo* experiments of animal thrombi is required in the future.

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Declaration of interest: The authors have no conflicts of interest or financial ties to disclose.

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