DEVELOPMENT OF A DEVICE TO OBTAIN *IN VITRO* THROMBI

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Abstract. *Cardiovascular diseases are nowadays worldwide the main causes of natural death. Most of those diseases are due to occlusion of blood vessels that supply the brain and the heart. Obstruction of these vessels occurs in several ways. Formation of thrombi in the arteries is the most relevant. The thrombus is characterized by blood in its solid form, having defined structural and biomechanical characteristics. It is believed that one of the decisive factors to obtain thrombi is the presence of flow. The influence of flow in the formation of thrombi in vivo is well studied and always mentioned. Most of the studies using thrombi produced in vitro, however, do not consider the characteristics of flow and its possible influence on the structure of the clot formed, and so, don´t present an appropriate model for simulation of arterial thrombus. Observing this gap in the researches of in vitro thrombi our laboratory developed a device for thrombi formation in vitro able to vary the flow's velocity and rotation angle. The device aims to create an instrument for the study of the influence these parameters have on the characteristics thus formed thrombus, creating in vitro, dynamic conditions for thrombus's formation.*

Keywords*: thrombi, formation, in vitro, flow, Chandler*

1. INTRODUCTION

Cardiovascular diseases are nowadays worldwide the main causes of death. In 2005,17,5 million died of cardiovascular disease according to World Health Organization; 43% of the deaths were due to heart attack and 33% to stroke. The numbers will rise to 20 millions in 2015 if nothing is done to repair this situation (WHO, 2008).

Most of those diseases are due to occlusion of blood vessels that supply the brain and the heart. This occlusion occurs by different mechanisms, being the principal one the presence of thrombi. Thrombi are solid formations of blood. They are classified in two types: venous and arterial. Conditions of flow in arteries and veins are different as for their interference in the structure of the thrombus formed. The impact of these different conditions of flow on the structure of thrombi is demonstrated very well *in vivo* where the structural and biochemical characteristics of thrombi are different in arteries and veins. These thrombi with different biomechanical properties have a distinct susceptibility to lysis. Arterial thrombi are more resistant to destruction than venous ones (Cotran *et al.*, 1994).

The influence of flow in the formation of thrombi *in vivo* was very well studied and those studies always took the flow in consideration (Cotran *et al.*1994). Thrombosis *in vivo* occurs in a dynamic rheological environment, where flow conditions regulate the transport of coagulation factors, cells and inhibitors, and strongly influence biochemical mechanisms involved in thrombus's formation (Hathcock, 2006). Most of the studies using thrombi produced *in vitro*, however, do not consider the characteristics of flow and its possible influence on the structure of the clot formed.

Chandler, in 1958 gave a description of the characteristics that distinguish a thrombus formed *in vivo* from one obtained *in vitro* by static methods. He was the first to propose a technique to produce an *in vitro* thrombus in a moving column of blood, simulating blood flow. He studied variations of speed, blood volume and angle, perceiving that flow was the most important factor to obtain a blood clot similar to the arterial thrombus.

The method developed by Chandler consisted of the following steps:

1) Extraction of one milliliter of whole venous blood.

2) Direct infusion in a polyvinyl tube (length 25 cm/internal diameter 0,375 cm)

3) Coupling of the two ends of the tube to form a circle (fastened by a plastic external collar)

4) Arrangement of the so formed loop in a circular disk rotating at 17rpm on a platform tilted 23 degrees to the horizontal plane.

Chandler uses a long player inclined. The rotation angle created a column of blood that moved in the opposite direction to that the tube.

Other researches applied the Chandler methods to obtain thrombi *in vivo* introducing some technical alterations such as adds anticoagulants and different rotation (Tachibana and Tachibana, 1995; Tachibana and Tachibana, 1997, Robbie *et al.*, 1997). Those reports, however, don't give details about the way in which the assembly of bench was performed, and what results were obtained in the thrombi formation.

Observing this gap in the researches of in vitro thrombi the Bioengineering Laboratory of the Federal University of Minas Geraes developed a device for thrombi formation *in vitro* able to vary the flow's velocity and rotation angle. The device aims to create an instrument for the study of the influence these parameters have on the characteristics thus formed thrombus.

2. MATERIAL AND METHODS

2.1. Development of the device

The device was designed with the purpose to answer some key queries:

- Allow the change of flow in the range of values found *in vivo* in arteries and veins;
- Allow the change of angle to study the influence of this variable in the thrombus's formation;
- Allow the formation of several thrombi at the same time;
- Ensure a flow similar in all samples;
- Allow the handling of each individual sample of blood;
- Ensure that the tube where the blood flows has no constrictions that may change the flow.

In accord with the issues above mentioned the device was designed as follows:

The materials used consisted of:

- Two wooden plates;
- Three rectangular steel profiles;
- One aluminum cylinder;
- Nine aluminum profiles;
- Nine discs of nylon;
- The engine DC;
- Potentiometer.
- Nine tubes of PVC;
- Glicerin;

The assemblage of the material was made as follow (fig.1):

- The two wooden plates were arranged in parallel, one over the other in the beginning position;
- The tree rectangular steel profiles were linked to each other to form a sliding mechanism allowing the angular variation between the superior and inferior plate;
- The aluminum cylinder was set over the superior plate;
- The engine was fixed on the side of the cylinder. The transmission engine-cylinder was made by a belt, and the transmission cylinder-discs was performed by contact (pure rotation);
- The nine nylon discs were connected at the other end of the aluminum profiles and so the whole arm disc may enter in contact whit the cylinder when desired. The discs have a circular incision on the face that fits the tube whit fluid (Fig.1);

A power controller attached to the engine allowed change of speed.

A graduated angular scale fixed at the side of the superior platform, executed the measuring of the angle formed by the tube and the horizontal platform.

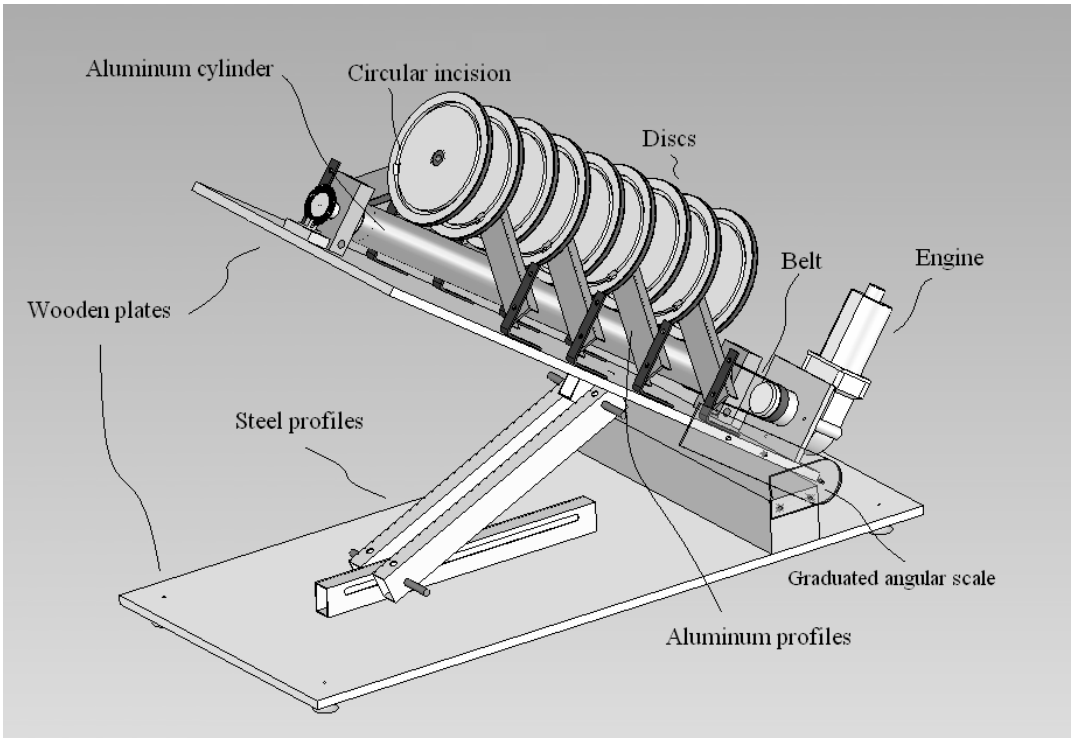

Figure 1: Drawing of the device

2.2. Performance evolution test of the device

The performance evaluation test verified the device's efficiency in the following items:

- Transmission of rotation from the principal cylinder to discs;
- Variations of rotations between the discs;
- Influence of angle change on transmission of rotation.

Using a digital photo tachometer, the number of revolutions per minute of the master cylinder, the first disc (farthest from the engine) and ninth disc (close to the engine) were determined on angular positions of 90, 45 and 20 degrees (angle between the axis of rotation of the disk and the horizontal axis).

We collected 40 values of the rotation in one minute period for each angle tested.

We calculated: the mode, the standard deviation and the error of measurement of each set of recorded data.

$$E = t \times s \tag{1}$$

We calculated also for each angle the rate of rotation transmission of the master cylinder for the first and ninth disc, with the objective of verifying the efficiency of the master cylinder in the forward rotation for the discs.

$$r_t = \frac{n_2}{n_1} = \frac{R_1}{R_2} \tag{2}$$

$$\Delta r_t = \frac{n_2}{n_1^2} E_{n_1} + \frac{1}{n_1} E_{n_2} \tag{3}$$

where r_t is the transmission ratio, *n1 ,R1 , n2 , R2* are respectively the master cylinder and disc rotation and radius.

2.3. Test to define the maximum speed of flow in the device according to the angular.

To simulate the blood viscosity one milliliter of the mixture of 66% water and 44% of glycerin was used as suggested by Horst *et al.* (1997).

To better visualize the flow, two drops of dye were added to the solution.

The column of fluid was direct view. The device's rotation was gradually increase and the maximum speed of flow in the device, for each angle (90, 45 and 20 degrees), was considered the rotation just before the moment that the column of fluid becomes static and moves around in the direction of rotation of the tube.

3. RESULTS

Studies that used similar devices submitting a fluid to a flow used rotations between 12 and 30 rpm (Chandler, 1958, Robbie et al., 1997, Tachibana and Tachibana, 1995; Tachibana and Tachibana, 1997). The device developed in our laboratory was effective for larger variations and able to vary the velocity until 80rpm at angular position of 90 degrees using the solution of glycerin.

There were no articles found using similar devices designed to compare error of measurement (Chandler, 1958, Robbie et al., 1997, Tachibana and Tachibana, 1995; Tachibana and Tachibana, 1997).

Table 1: Experimental results of rotation measurements

Degree	Component	Mode (rpm)	Standard deviation (rpm)	error of measurement (rpm)
90º	Cylinder	267	2,66	5
	1st disc	76	0,91	2
	9th disc	80	1,21	2
45º	Cylinder	261	4,86	10
	1st disc	71	1,04	2
	9th disc	72	1,40	3
20º	Cylinder	259	8,48	17
	1st disc	59	0,77	2
	9th disc	60	1,78	4

Results of transmission ratio are shown in Table 2 while Table 3 presents the results of transmission ratio between real and theoretical values calculated using the projected values of cylinder and disc radius.

Table 2: Transmission ratio results

Degree	disc	Transmission ratio	Error
90º	1st disc	0,28	0,01
	9th disc	0,30	0,02
45º	1st disc	0,27	0,02
	9th disc	0,28	0,02
20º	1st disc	0,23	0,02
	9th disc	0,23	0,03

Table 3: Results of ratio between real and theoretical values

Degree	disc	Ratio real/theoretical
90º	1st disc	0,9851
	9th disc	1,0370
45º	1st disc	0,9415
	9th disc	0,9547
20º	1st disc	0,7884
	9th disc	0,8018

At 90 degrees the device showed good stability and the ratio between the values of ratio transmission real and theoretical were close to 1 (0,985 to 1 st disk, and 1,036 for the 9 th disc) indicating that the cylinder was able to convey adequately the rotation on both discs not existing relevant slipping. The 45 degrees situation was similar as the ratio between the values of the transmission ratio real and theoretical also stood close to 1 (0,951 to 1 st disc, and 0,954 for the 9 th disc). However, when the device was positioned at 20 degrees the ratio of the value of real and theoretical transmission further away from 1 (0,788 to 1 st disk, and 0,801762 for 9 th disc) suggesting that occurred slipping, no proper transmission of the cylinder for the discs in this angular position. A revaluation of the device will be made including locks for fixing the disc in the cylinder and greater stability of the platform when placed in small angles in order to correct the factors that may have influenced the increase of error at 20 degrees.

The maximum speed of flow, for each angle, is shown in "Tab.4".

Table 4: Maximum rotation of fluid column in the device according to the angular position

Degree	Rotation
90º	80 rpm
45º	70 rpm
20º	30 rpm

As expected the maximum speed of flow were reached at 90 degrees. Chandler, in 1958, and then ,Tachibana and Tachibana, in 1995 and 1997, and Robbie *et al.* in 1997, used the angle of 23 degrees to obtain thrombi, but the justification of the choice of this angle was not described. Testing with human blood and variations in angles will be made in the next stage of this project in order to better assess this variable.

As limitation of the device designed there is the fact that the flow is continuous and not pulsate. Nevertheless, the continuous flow use only small amount of blood which allows the use of human blood on the device offering an advantage in simulate the thrombi *in vivo*.

The next step of this project is the testing of whole human blood.

5. CONCLUSION

The device developed was able to vary the flow's velocity and rotation angle, creating an instrument able for the study of the influence these parameters have on thrombus's formation *in vitro*. The device should be improved to achieve a better transmission rate in angles of lesser value.

6. REFERENCES

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