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EXECUTIVE SUMMARY OF THE THESIS

Design of a Test Bench for the Evaluation of Hemolysis Induced by Hemodialysis Catheters

TESI MAGISTRALE IN BIOMEDICAL ENGINEERING – INGEGNERIA BIOMEDICA

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1. Introduction

Hemolysis consists in the rupture of erythrocytes' membrane, leading to the incapacity to transport oxygen and to the release of hemoglobin into the plasma [1].

Mechanical damage to the erythrocytes is the most common cause of hemolysis in cardiovascular devices [2]. Although its incidence has been reduced over the years, sub-clinical hemolysis is still a consequence of the devices' use [3]. Therefore, it is important to evaluate the blood damage induced, with the aim of minimizing it.

During the design process, the prediction of hemolysis through numerical models can be beneficial because it allows a preliminary evaluation of the developed design, leading to a faster development process and a reduction of the costs associated to *in vitro* and/or *in vivo* tests [1], [4]. However, their implementation requires a preliminary experimental validation in order to verify that the numerical results are comparable to those obtained experimentally [5]. Therefore, it is necessary to conduct controlled *in vitro* tests with experimental setups that provide well-documented and reproducible results.

During the last decades, numerous numerical models for hemolysis prediction have been developed. However, their experimental validation is critic, especially because of the complexity of the hemolysis phenomenon, which is due to several factors [4].

The aim of this thesis is to design a test bench for the *in vitro* evaluation of hemolysis induced by hemodialysis tunneled central venous catheters (t-CVCs), whose results could be further used to validate numerical models for the prediction of hemolysis in these devices.

In literature [6]–[8], the experimental setups for the *in vitro* hemolysis testing on CVCs are usually characterized by the same fundamental elements: the catheter to be tested, a pump, connection lines, a reservoir, and sensors.

The developed test bench refers to a previous experimental setup developed at the research

group LaBS – Artificial Organs of Politecnico di Milano [9] for the validation of a numerical blood damage model. The main difference with the other test benches available in literature [6]–[8] consists in the replication of the *in silico* working conditions of the analyzed t-CVCs, positioning them inside an artificial superior vena cava (SVC) with circulating blood.

The design process of the test bench and the execution of the *in vitro* hemolysis tests on the analyzed CVCs are described below.

2. Materials and Methods

The design of the test bench for the *in vitro* evaluation of hemolysis induced by t-CVCs referred to the current regulations (UNI EN ISO 10993-4:2017 [14] and ASTM F1841-19$^{\varepsilon 1}$ [15]) and to the available literature [6]–[9].

Its realization included the development of *custom* components through the combined use of 3D modeling, computational fluid dynamics (CFD) analysis, and 3D printing with biocompatible resins. CFD analysis performed in ANSYS Fluent 2020 R1 (*Ansys Inc., Canonsburg, Pennsylvania, USA*) allowed to determine the components' design studying their inner fluid dynamics. The combined use of the 3D modeling software SOLIDWORKS® CAD (*Dassault Systèmes, Vélizy-Villacoublay, France*) and the 3D printing with Form 3B+ (*Formlabs Inc., Somerville, Massachussets, USA*) allowed the production of customized components, adapted to the necessities of the experimental setup and characterized by complex geometries, which could have not been developed with traditional processing techniques. The use of a biocompatible resin (BioMed Clear V1) as printing material allowed the enhancement of the hemocompatibility of the experimental setup.

The test bench has been used to conduct *in vitro* hemolysis tests on two different t-CVCs currently used in clinical practice: Palindrome™ Precision Symmetric Tip (*Medtronic, Dublin, Ireland*) catheter and Arrow-Clark™ VectorFlow® (*Teleflex, Wayne, Pennsylvania, USA*) catheter.

2.1. Test Bench

To determine the hemolysis induced by the t-CVCs only, the experimental setup consists of two different loops: a *complete loop*, which includes the catheters, and a *white loop*, which excludes them.

The *complete* and *white loops*' components are described below.

2.1.1. *Complete Loop*

The *complete loop* (Figure 2) evaluates the hemolysis induced by the t-CVC and the experimental setup.

Palindrome™ (*P*) and VectorFlow® (*VF*) catheters (Figure 2.1) are inserted into the loop for 25 cm, coaxially to the artificial SVC, which is 48 cm long and characterized by an inner diameter (ID) of 18 mm.

The blood circulation inside the artificial SVC is provided by the Medtronic® centrifugal pump (*Medtronic, Dublin, Ireland*) (Figure 2.5), while the blood circulation inside the t-CVC is provided by the Cobe® Stöckert roller pump (*COBE Cardiovascular Inc., Arvada, Colorado, USA*) (Figure 2.6), set in subtotal occlusion configuration.

During testing, the blood is maintained at the physiological temperature of 37±2°C with the use of a heat exchanger (Figure 2.7).

The *complete loop* also includes the following *custom* components.

Connector for the Catheter

This *custom* component (Figure 2.2 and Figure 1.A) represents the first section of the artificial SVC. It is composed of two parts connected with a sleeve joint. The first (Figure 1.A.1) is inclined of 15° with respect to the artificial SVC and is designed as a 3/8" connector for the pump tubing; the second (Figure 1.A.2) presents a channel (ID 6 mm) for the catheter coaxial insertion inside the SVC.

Support Ring

This *custom* component (Figure 2.3 and Figure 1.B) maintains the coaxiality of the CVC inside the artificial SVC. It consists in a section of SVC which internally includes a vertical support ring in the upper section. It is connected with the adjacent sections of SVC through a double sleeve joint. The support ring is composed of a holder and a ring (ID 6 mm), whose geometries have been designed with a "tear-drop"-like shape to be non-hemolytic, referring to the experimental results of Vorhauer and Taray [10].

SVC Tubes

The remaining artificial SVC has been subdivided into two PMMA tubes. The first (13 cm long) joins the connector and the support ring; the second (27 cm long) the support ring and the reservoir.

Reservoir

The development of this *custom* component (Figure 2.4 and Figure 1.D) referred to the work of Olia *et al.* [11]. It is composed of a 2-l disposable urine bag (Figure 1.D.1) clamped to a reusable base (Figure 1.D.2), which has been adapted to the experimental setup necessities. The reservoir is used in horizontal configuration and presents an inlet with a sleeve joint for the artificial SVC and an outlet with a 3/8" connector for the pump tubing. The urine bag is locked against the reusable base thanks to two clamps made of Grey Resin V4 screwed with threaded rods (Figure 1.D.3).

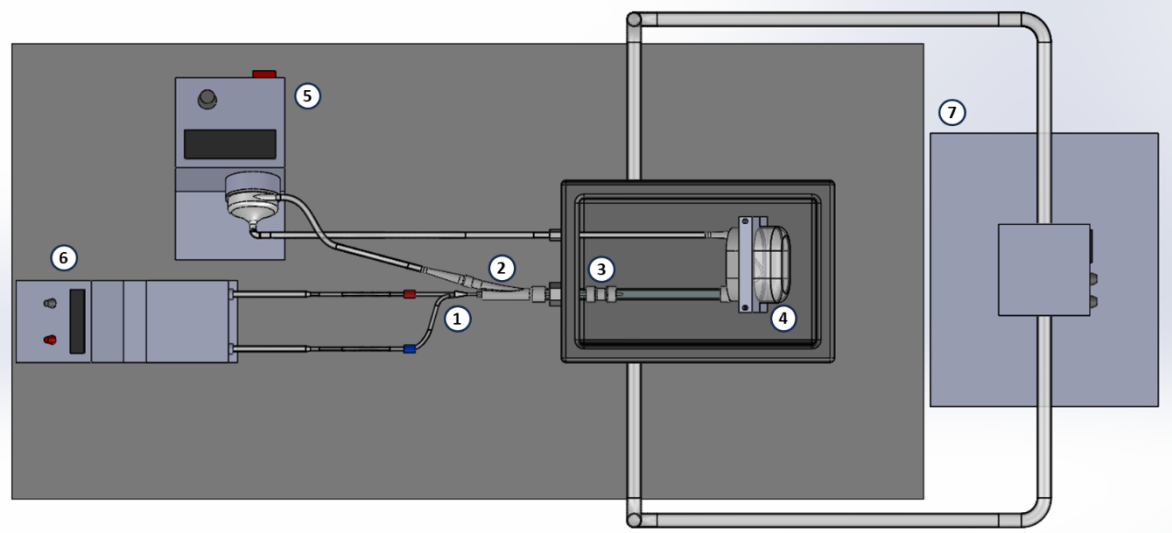

Figure 2 - Schematic representation of the complete loop: (1) t-CVC, (2) connector for the catheter, (3) support ring, (4) reservoir, (5) Medtronic® centrifugal pump, (6) Cobe® Stöckert roller pump and (7) heat exchanger; the t-CVC's delivery line is represented in blue while the suction line in red.

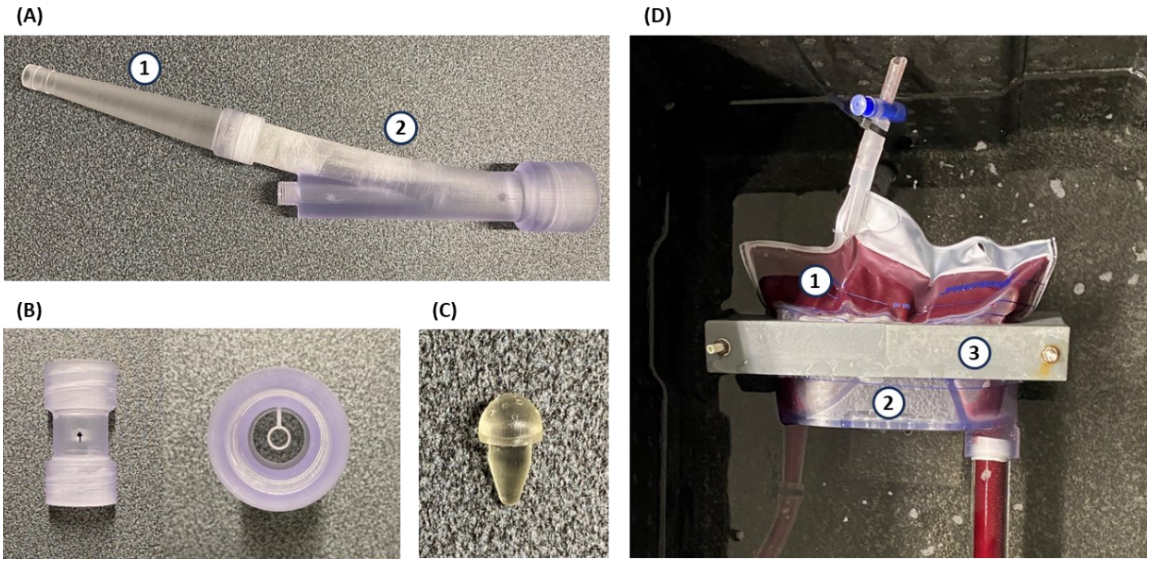

Figure 1 - *Custom* components of the experimental setup: (A) connector for the catheter with a 3/8" connector (A.1) and coaxial insertion for the catheter in the SVC (A.2); (B) support ring; (C) cap for the support ring in the *white loop*; (D) reservoir composed of the disposable urine bag (D.1) locked on the reusable base (D.2) with the clamp set (D.3)

2.1.2. *White Loop*

The *white loop*, which evaluates the hemolysis induced by the experimental setup only, is composed of the same elements of the *complete loop*, except for the catheter, which is excluded. This led to modifications in the Cobe® Stöckert pump tubing and in the support ring.

The pump delivery tube is positioned directly inside the connector for the catheter while the pump suction tube withdraws blood directly from the reservoir outlet through a Y-connector.

The exclusion of the catheter also led to the development of a *custom* deformable cap (Figure 1.C) to close the support ring and avoid clot formation. The cap is made of Elastic 50A Resin and is characterized by a geometry that allows its secure positioning on the ring. In particular, once positioned, the overall geometry resembles a tear-drop, which should be non-hemolytic, as indicated by Vorhauer and Taray [10].

2.2. *In Vitro* Hemolysis Tests

A total of three *in vitro* hemolysis tests have been conducted: two with the *complete loop* including respectively catheters P and VF and one with the *white loop*. The tests referred to the current regulations (UNI EN ISO 10993-4:2017 [12], ASTM F1841-19$^{\varepsilon 1}$ [13] and ASTM F756-17 [14]) and followed the protocol described below.

Blood Collection, Control and Treatment

For each test, fresh bovine blood is collected from an abattoir. Preliminary blood analyses are conducted to verify the physiological hematological parameters (hematocrit, density, and viscosity). The hematocrit is corrected to 30 ± 2% with hemodilution.

Test Execution

Prior to testing with blood, the loop is filled with saline solution. After being recirculated for 5-10 min, the saline solution is substituted with approximately 1500 ml of blood.

The test has a 4-h duration, during which the pumps flow rates are set to 3 l/min for the Medtronic® pump and 0.4 l/min for the Cobe® Stöckert pump.

During the test, pumps flow rates, blood temperature and inlet and outlet pressures of the artificial SVC and of the catheter are monitored with sensors.

To evaluate hemolysis, blood samples are withdrawn following the ASTM F1841-19$^{\varepsilon 1}$ [13] from a sampling port positioned on the venous line of the Cobe® Stöckert pump. A total of 22 blood samples are collected at different time points: four 1-ml *control samples* ($CTRL$) prior to testing; three 1-ml samples after 5, 35, 65, 125, 185 and 245 min since the beginning of the test.

Blood Samples Analysis

Blood samples are analyzed within 48 h from the hemolysis test, preserving them at 2-8°C. The blood samples analysis is conducted in the BioCell Lab of the Department of Chemistry, Materials and Chemical Engineering "Giulio Natta" of Politecnico di Milano.

To evaluate the hemolysis induced by the loops, the plasma free hemoglobin concentration ($freeHb$, mg/dl) is determined for each blood sample. Moreover, the total hemoglobin concentration (Hb, g/dl) is determined for one of the *control samples*. Both concentrations are measured with the cyanmethaemoglobin detection method.

Hemolysis Evaluation

After verifying the linear regression of $freeHb$ for each loop, the hemolysis induced by the t-CVC is evaluated calculating the *modified index of hemolysis* (MIH) [13], following Equation (1):

$$MIH_{t-CVC} = \frac{\Delta freeHb \cdot V \cdot \frac{100 - Ht}{100}}{Q \cdot \Delta T \cdot Hb} \qquad (1)$$

$\Delta freeHb$ (mg/dl) represents the plasma free hemoglobin difference between the *complete loop* and the *white loop*, calculated over a 1-hour period (ΔT). V (ml) represents the total blood volume in the loop, Ht (%) the blood hematocrit, Q (l/min) the blood flow rate inside the catheter, and Hb (g/dl) the total hemoglobin concentration.

Once MIH_{t-CVC} is obtained for each sampling period, the average value is calculated.

In case of linearity of the $freeHb$ data (regression coefficient $R^2 > 0.95$), in the calculation of MIH_{t-CVC}, the term $\Delta freeHb/\Delta T$ is substituted with the difference between the regressions' slopes of $freeHb$ in the *complete loop* and in the *white loop* (named respectively k_{t-CVC} and k_W).

3. Results and Discussion

In vitro hemolysis tests have been conducted on different days using blood collected from the same typology of bovine (age and gender). In Table 1 are reported the hematological parameters measured after the blood hemodilution.

Table 1 - Hematological parameters measured prior to *in vitro* testing

Hematological parameter	White Loop	Complete Loop (*P*)	Complete Loop (*VF*)
Hematocrit	42%	38%	34%
Corrected Hematocrit	30%	30%	30%
Density	1.084 g/dl	1.130 g/dl	1.118 g/dl
Viscosity	2.89 cP	3.06 cP	3.42 cP

After testing, a qualitatively evaluation of the components' inner fluid dynamics has been performed searching for clots formation (Figure 4).

The only *custom* components that showed clots formation were the support ring and the reservoir. While the clots on the support ring were not significant and found on the holder only, the reservoir showed clots on the lower surface of the reusable base and in the 3/8" connector (Figure 4.A,D). In the *white loop*, the clots formed in the Y-connector rather than on the 3/8" connector (Figure 4.B).

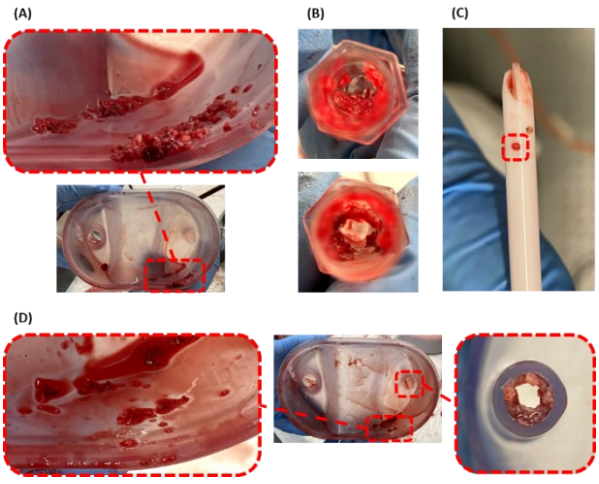

Figure 4 - Clot formation inside the loops components: (A) lower face of the reservoir's reusable base in the *white loop*; (B) Y-connector in the *white loop*; (C) tip of VectorFlow® catheter; (G) lower face and 3/8" connector of the reservoir's reusable base in the *complete loops*

Clots formed also on the head of the Medtronic® pump in all loops. Regarding the analyzed t-CVCs, clots were found only on one lateral opening of the VectorFlow® (Figure 4.C).

Blood Samples Analysis Results

In Figure 3 are reported the $freeHb$ values obtained for the different loops. Hb resulted equal to 7.76 g/dl for the *control sample* of the *white loop*. Since blood is collected from the same typology of bovine (age and gender), the value Hb is kept the same also for the *complete loops*.

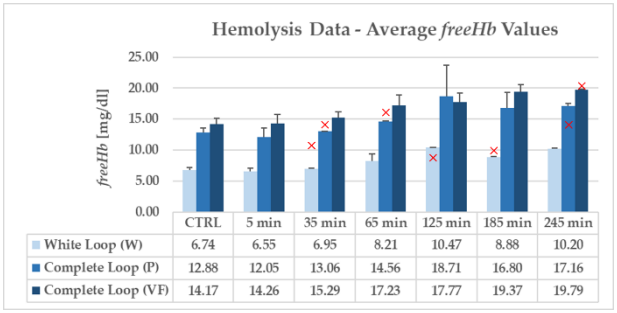

Figure 3 - Average $freeHb$ values for each loop; outliers are highlighted with a red cross

Modified Index of Hemolysis (MIH) Calculation

Since the linear regression of $freeHb$ is not verified ($R^2 \ll 0.95$), the MIH_{t-CVC} has been calculated according to Equation (1), obtaining the following average values: MIH_P = 3.86 and MIH_{VF} = 4.11.

The linearity of $freeHb$ is presumably not verified because of values that modify the increasing trend, not corresponding to a realistic hemolysis trend. If $freeHb$ values at 125 min in the *white loop* and in the *complete loop* with catheter *P* were excluded, the linear regressions of $freeHb$ would be characterized by R^2 close to 0.95, allowing the calculation of MIH_{t-CVC} with their slopes (k_W = 0.0131 mg/(dl·min), k_P = 0.0163 mg/(dl·min) and k_{VF} = 0.0233 mg/(dl·min)). Following this calculation method, the following values of MIH_{t-CVC} would be obtained: MIH_P = 2.47 and MIH_{VF} = 3.01.

Independently of the calculation method, the positive values of MIH_P and MIH_{VF} indicate that the *white loop* is less hemolytic than the *complete loops*, as expected. Therefore, the developed test bench allows the evaluation of the hemolysis induced by the catheters only, excluding the one induced by the experimental setup.

To verify the accuracy of the obtained results, an adequate number of hemolysis testing should be performed. Afterwards, the results should be compared with literature. However, it is critical to conduct this comparison because most of the literature studies evaluate the hemolysis induced by the combination of the catheter and the testing pump. Moreover, different hemolysis indexes are used to evaluate the hemolysis induced.

However, it is reasonable to assume that the obtained MIH values can be considered low and realistic since the analyzed t-CVCs are currently used in clinical practice [15], [16]. This hypothesis is supported by a comparison of MIH indexes of left ventricular assist devices (LVAD) and centrifugal pumps for the extracorporeal circulation, which are generally characterized by higher MIH values [17]–[19]. Since these typologies of devices are currently used in clinical practice too, it is possible to assume that the obtained MIH_P and MIH_{VF} are reasonably low values.

From the obtained results, it is found that the VectorFlow® catheter is characterized by a higher MIH value, suggesting a higher induced hemolysis with respect to the Palindrome™ catheter (as observed also in the previous thesis work [9]). This hypothesis is supported by the finding of clots on one lateral opening of the VectorFlow® catheter (Figure 4.C).

4. Conclusions

In this thesis, a test bench for the *in vitro* evaluation of hemolysis induced by t-CVCs is proposed. Its results could be further used to validate blood damage numerical models.

The test bench presents *custom* components developed with the combined use of 3D modeling, CFD analysis and 3D printing with biocompatible resins. In this way, it was possible to produce customized biocompatible components, adapted to the necessities of the experimental setup and characterized by complex geometries, whose design development was supported by the analysis of the inner fluid dynamics.

To evaluate the hemolysis induced by the t-CVCs only, the test bench consists of two different loops: a *complete loop*, which includes the catheters, and a *white loop*, which excludes them.

Three 4-hour duration *in vitro* hemolysis tests have been conducted with bovine blood: one with the *white loop* and two with the *complete loops* including the catheters. To evaluate hemolysis, $freeHb$ values are determined and used to calculate the MIH for each catheter.

The obtained results indicate that the developed test bench allows the evaluation of the hemolysis induced by the catheters only.

Despite the expected obtained results, it is suggested that the test bench could be further improved. For example, the reservoir could be re-designed to improve the inner fluid dynamics and reduce the clot formation. In this way, the blood damage induced by the experimental setup only could be reduced, allowing a better evaluation of the catheter's hemolysis.

To further verify the accuracy and repeatability of the obtained results, the current regulation [12] requires the conduction of an adequate number of hemolysis testing. A future development could consist in the repetitive execution of the *in vitro* hemolysis tests with the developed test bench to confirm the results obtained in this thesis work. This analysis could also be beneficial to determine the most representative calculation method of MIH with respect to the hemolysis induced.

Once the obtained results are verified, they could be used to validate numerical models for the prediction of hemolysis in the analyzed t-CVCs. Such models could support the design and optimization process of cardiovascular devices.

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