Hybrid membranes for blood-contacting surfaces: preliminary characterization

M. Todesco[1,2], G. Merigliano[1,2], V. Candela[1,2], L. Iop[2,3], T. Palmosi[2,3], G. Gerosa[2,3] and A. Bagno[1,2]
[1] *Department of Industrial Engineering, University of Padua, via Marzolo 9, 35131, Padova, Italy*
[2] *L.i.f.e.L.a.b. Program, Padova, Italy*
3 Department of Cardiac, Thoracic and Vascular Sciences, University of Padova, via Giustiniani 2, 35127, Padova, Italy

***Abstract* — The hemocompatibility of any mechanical circulatory support device is mostly conditioned by the nature of the blood-contacting surface. Hybrid membranes as the inner surfaces of the artificial ventricle were produced by coupling a polymeric material (polycarbonate urethane) with decellularized biological tissues (animal pericardia). Physicochemical and mechanical characteristics of the hybrid membranes were carefully evaluated confirming satisfactory features in terms of composition and mechanical resistance.**

***Keywords*— pericardium, hybrid membrane, blood contacting surface, hemocompatibility.**

I. INTRODUCTION

NOWADAYS, heart transplantation represents the optimal solution for refractory end-stage heart failure [1]. However, patients' mortality is increasing due to the prolonged waiting list time.

The quest for alternative therapeutic options resulted in the development of mechanical circulatory support devices that can replace one ventricle or the whole heart performances. These supports are respectively defined as: Ventricular-Assist Devices (VADs), which have the potential to increase patients' survival and quality of life mostly replacing the left ventricle, and Total Artificial Hearts (TAHs), which are the most sophisticated mechanical circulatory supports. These latter replace both ventricles and heart valves with a double pneumatically or electromechanically driven pump.

Over the years, several TAHs have been developed and implanted, but currently only one is still in clinical use: the CardioWest TAH [2]. At present, another implantable TAH, named Carmat, is under evaluation in clinical trials.

Several drawbacks are still limiting the extended application of TAHs: geometry, power supply, mechanical and biological factors [3]. These latter can be summarized as "blood compatibility" concerns: they can result in hemorrhages, hemolysis, thrombosis and thromboembolism. In addition, calcification and infections can cause the device failure.

Hemocompatibility involves several aspects (from platelet activation to immune system recruitment) and engineering requirements (from design to hemodynamics) [4]. The selection of appropriate biomaterials does not necessarily imply the compatibility of the whole device [5], but the progression of blood–material interactions is surely determined by blood-contacting surfaces.

The present work aims at producing and preliminarily characterizing innovative hybrid membranes for the construction of blood-contacting surfaces, which are intended for the production of the inner surfaces of novel mechanical circulatory supports. Membranes were obtained by coupling a polymeric material (commercial polycarbonate urethane, available in two different formulations with and without added silica), which is responsible for the mechanical resistance, with biological decellularized tissues (porcine and bovine pericardia), which are in direct contact with blood, to assure high hemocompatibility. The biological layer will also be prone to endothelialization by circulating cells.

The hybrid membranes were characterized from physicochemical and mechanical point of views.

II. MATERIALS AND METHODS

A. Tissue preparation

Fresh pericardia of healthy animals (calves and pigs) were supplied from local abattoirs. Pre-pericardial fat was removed and each pericardium was dissected free from its attachment at the base of the heart. Samples from the anterior left ventricular region of each pericardium were used for the fabrication of the hybrid membrane.

B. Decellularization

Tissues were treated according to the TriCol procedure [6]. The procedure was carried out under constant agitation and following 8 hour-long cycles. The inactivation of the cell proteases was followed by alternated hypo- and hypertonic solutions, combined with increasing concentrations of Triton X-100 (0.1-1%, Sigma-Aldrich). Cellular components were extracted from the tissue by 10 mM bile salt anionic surfactant sodium cholate (Sigma-Aldrich).

C. Membrane fabrication

Decellularized tissue samples were washed in deionized water and gently dried with filter paper. The samples were then placed on the serosa side and fastened into a customized metallic frame (Figure 1). On the other side (fibrosa), a thin layer of liquid polycarbonate urethane was poured (ChronoFlex AR and ChronoFlex AR-LT, AdvanSource Biomaterials, Wilmington, MA, US). Both polymers were supplied as 22% (w/v) solution in N,N-dimethylacetamide (DMAc). The membranes were dried for 24 hours at 40°C in a vacuum oven (Raypa, Barcelona, Spain).

Therefore, four different hybrid membranes were produced by coupling porcine and bovine pericardia with the two polymers.

D. Analytical techniques

The following analytical techniques were utilised to characterize biological samples, polymers themselves and hybrid membranes: Fourier Transform Infrared spectroscopy

Attenuated Total Reflection FTIR-ATR (Nicolet iS-50, Thermo Fisher Scientific, Waltham, Massachusetts, USA), Differential Scanning Calorimetry DSC (Q200, TA Instruments, New Castel, Delaware, USA), Thermo-Gravimetric Analysis TGA (SDT Q600, TA Instruments, New Castel, Delaware, USA).

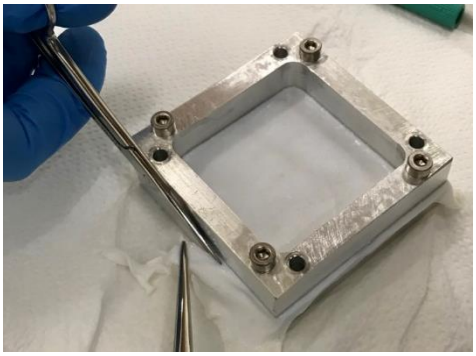

Fig. 1: Pericardium is fastened into the metallic frame (50 x 50 mm^2) before being covered with the polymeric solution

E. Mechanical characterization

For the mechanical assessment, samples were shaped into dog bone specimens by means of an in-house designed cutter. Sample thickness was measured using a Mitutoyo digital caliber (model ID-C112XB, Aurora, IL, USA) (Table I). Mechanical tests (uniaxial tensile loading tests) were performed by means of the Bose Electroforce System (Bose Corporation, Eden Prairie, Minnesota, USA). The system was equipped with two Bose linear actuators (200 N) and one loading cell (225 N). Tests were performed at room temperature; biological samples were continuously wetted with 0.9% NaCl solution to prevent dehydration. The gauge length of each sample was 5 mm.

Samples were elongated (elongation rate set at 0.5 mm/s) to rupture for measuring the Ultimate Tensile Strength (UTS) and the Failure Strain (FS); the elastic modulus was calculated as the slope of the stress-strain curves in the linear region.

III. RESULTS

A. FTIR-ATR, DSC and TGA

FTIR-ATR spectra confirmed the chemical composition of the polycarbonate urethanes. FTIR-ATR spectra were also acquired on the pericardial side of the hybrid membranes: these spectra allowed ascertaining that the polymers do not diffuse throughout the biological tissues because of the solvent casting procedure (data not shown).

DSC analysis allowed determining the glass transition temperature of both polymers: – 31.5°C for ChronoFlex AR-LT, and – 28°C for ChronoFlex AR (Figure 2).

TGA analysis confirmed the presence of a 9% inorganic residue in the ChronoFlex AR-LT (Figure 3): this is due to the microsilica added to make the polycarbonate urethane less sticky.

B. Mechanical characterization

Mechanical tensile tests were performed on polymeric membranes, on decellularized animal pericardia, and on hybrid membranes.

Table II summarizes the results obtained from the mechanical tests on native bovine (NBP) and native porcine (NPP) pericardia, on the membrane fabricated by coupling decellularized bovine pericardium with Chronoflex AR (DBP-AR) and AR-LT (DBP-ARLT), and by coupling decellularized porcine pericardium with Chronoflex AR (DPP-AR) and AR-LT (DPP-ARLT).

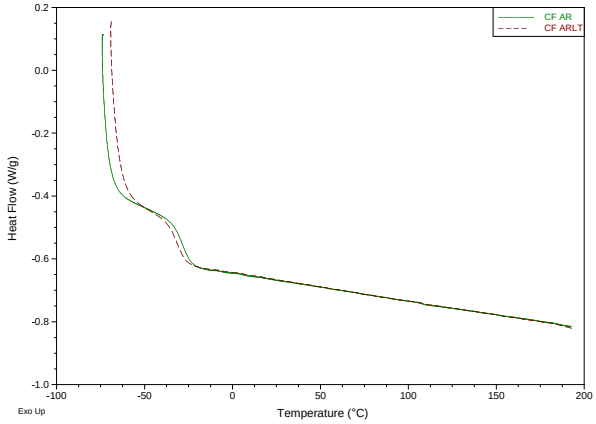

Fig. 2: DSC analysis of ChronoFlex AR and ChronoFlex AR-LT: glass transition temperatures are -28°C and -31.5°C, respectively

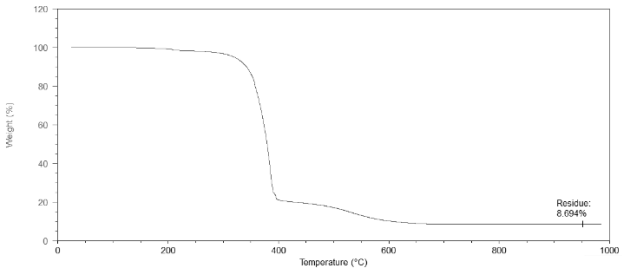

Fig. 3: TGA analysis of the ChronoFlex AR-LT identified the presence of 9% inorganic residue (silica)

TABLE I
SAMPLES THICKNESS

Sample	*Thickness [mm]*
NBP	0.23 ± 0.17
NPP	0.12 ± 0.07
DBP-AR	0.53 ± 0.05
DBP-ARLT	0.62 ± 0.1
DPP-AR	0.42 ± 0.02
DPP-ARLT	0.59 ± 0.04

It is worthy to mention that hybrid membrane failure is mainly influenced by pericardium mechanical resistance: the application of tensile load causes the elongation of collagen fibres within tissue matrix; therefore, collagen fibres lose their crimped configuration until they break. Since pericardium and polymer are well adhered to each other, collagen fracture results in the entire membrane failure.

Mechanical data showed a distinct decrease of stiffness when biological tissues are decellularized and coupled with

polymers, which is more evident for the ChronoFlex AR than the ChronoFlex AR-LT. Similarly, a decrease in UTS values appeared, which is larger for porcine pericardium than for bovine one. Interestingly, FS values increased up to more than 120% when both decellularized pericardia are coupled with the ChronoFlex AR. On the other hand, ChronoFlex AR-LT, which contains 9% microsilica, did not largely modify FS values of native tissues. This outcome is of particular interest in the perspective of the foreseen application: in line of principle, the hybrid membrane is required to be compliant with respect to the movements of the pulsatile chamber.

As to the membrane thickness (see Table I), different values are due to pericardia variability (considering both different species and different animals within the same specie): it is possible to control membrane thickness by adjusting the amount of polymer.

TABLE II
MECHANICAL DATA

Sample	*E [MPa]*	*UTS [MPa]*	*FS [adim]*
NBP	51.38 ± 13.85	17.27 ± 2.45	0.696 ± 0.1
NPP	71.59 ± 27.20	24.88 ± 7.88	0.617 ± 0.2
DBP-AR	8.64 ± 1.83	13.45 ± 5.51	1.21 ± 0.5
DBP-ARLT	10.21 ± 0.29	9.55 ± 0.26	0.74 ± 0.06
DPP-AR	6.21 ± 0.77	8.72 ± 0.66	1.29 ± 0.1
DPP-ARLT	13.65 ± 0.82	8.39 ± 2.62	0.69 ± 0.3

IV. CONCLUSION

This preliminary evaluation of the hybrid membranes fabricated by coupling decellularized biological tissues with polycarbonate urethanes allowed assessing their composition and mechanical properties. At present, morphological studies are in progress by using SEM and two-photon microscopy to investigate both the surface and the cross-section of the hybrid membranes. Moreover, further investigations are ongoing to assess fatigue resistance under cyclic loading and the capacity of the hybrid membranes to evoke (or not) platelet activation and thrombin formation. Eventually, cytocompatibility tests (in vitro) and calcification test (in vivo) will be carried out for an exhaustive characterization of the hybrid membranes.

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