Technique

An In Vitro Protocol for Evaluation and Comparison of Membrane Oxygenators

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ABSTRACT

With the trend in open heart surgery toward normothermic bypass and warm blood cardioplegia, greater demand is being placed on the perfusionist to select an oxygenator that will perform safely and efficiently under a variety of conditions. While manufacturers report performance parameters for their products, the data is often not comparable due to widely differing conditions. Recent in vitro evaluation techniques employed to characterize membrane oxygenators do not simulate the actual oxygenator conditions observed during cardiopulmonary bypass. Biocompatibility and drug delivery are reported but comparisons of different oxygenator performance parameters are not completely addressed. We have designed a test circuit and an evaluation protocol to simultaneously characterize the performance of multiple oxygenators under identical conditions. The test circuit is designed to simulate clinical conditions and to evaluate gas exchange, blood path pressures, gas path pressures, and hemolysis.

Previously reported studies have relied on a comparison of a single membrane oxygenator and a single bubble oxygenator. Our protocol will compare multiple membrane oxygenators, in vitro, under similar clinically relevant conditions. Such testing would be done prior to animal or clinical trials. Furthermore in vitro tests should be more reproducible and more discriminating than are ex vivo tests.

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INTRODUCTION

Early in the eighteenth century man began his quest to preserve life through artificial circulation. Advances made by Galletti and Brecher ultimately led to the development of the classic perfusion circuit described by vonFrey and Grubber. (1) However, without the advent of heparin, maintenance of viable extracorporeal circulation was difficult if not impossible. (2) Credit has been given to Gibbon for developing the modern concepts of extracorporeal perfusion. His system was first used as a life support system for treating severe pulmonary embolism, and in 1953, was successfully used as the heart-lung apparatus to begin the era of open heart surgery. (3)

Over the past thirty years a flurry of "bigger and different" oxygenators has evolved. The early oxygenators were all direct contact control devices of three major designs: screen, bubble, and disk. (4-9) A short time later the membrane oxygenator was introduced, employing a physiologic membrane for gas exchange rather than direct blood contact. Presently, available oxygenators only include bubble or membrane, yet there are numerous manufacturers producing both. (6,8,9) The fact that oxygenators fall into these two categories has not facilitated the selection of an oxygenator for any given procedure being performed. This selection process is further hampered by the fact that manufacturers report performance parameters, yet data is not comparable between brands due to widely differing testing conditions. This ultimately forces a choice based on price, the influence of marketing strategies by manufacturers, and an empirical assessment of performance based on clinical, ex vivo, and in vitro data. This data is frequently anecdotal and may not relate to current perfusion techniques or allow true comparisons between products from different manufacturers.

Given the plethora of oxygenators available, it is important that a protocol exists to characterize multiple devices. To accomplish this goal we have devised an in vitro testing protocol to evaluate membrane oxygenators (MOs) of different designs and from various manufacturers prior to undertaking expensive animal trials or clinical trials.

We have designed the protocol to simulate the actual oxygenator conditions observed in cardiopulmonary bypass (CPB). In several prior studies only the membrane of the oxygenator was analyzed for drug delivery and complement activation respectively. (10,11) These studies appear only to identify the efficiency of drug delivery and biocompatibility, but do not actually address the issue of comparative MO performance.

The in vitro protocol developed by Kawak, exemplifies these prior limitations in methodology. (12) The circuit employed was designed with the intent of screening membrane oxygenators; however, the study 1) only tested one type of MO (Bentley BOS-CM50); 2) the flow rates used were much lower than those actually employed in adult CPB; and 3) the circuit was not designed for a constant circulation of blood for the duration of the test but only during the actual testing of gas transfer. Drug delivery was studied but the circuit did not approximate the true

Table 1
EQUIPMENT REQUIRED FOR TEST CIRCUIT

1ea	Centrifugal Pump Console
1ea	Roller Pump
1ea	Heater Cooler Unit
1ea	Temperature Indicator
1ea	Mass Spectrometer
1ea	Blood Gas Analyzer
1ea	Oxygen Saturation Meter
2ea	Pressure Manometer
1ea	Co-oximeter
1ea	Air - Oxygen Blender with Flow Meter
1ea	100% O_2 Source*
1ea	100% CO_2 Source*
1ea	100% N_2 Source*
1ea	Air Source*
1ea	6% CO_2/ 94% N_2 Source*
8ea	Tubing Clamps

*All gas sources require appropriate regualtors and flow meters.

Table 2
DISPOSABLE SUPPLIES REQUIRED FOR TEST CIRCUIT

2ea	Bard H-4000 Hollow Fiber Membrane Oxygenators (Deoxygenator)
2ea	5 gallon plastic paint buckets (modified)
1ea	Centrifugal Pump Head and Flow Probe
45ft	PVC Tubing 3/8" X 3/32"
2ea	3/8 X 1/2 Connector
6ea	3/8" X 3/8" Luer Lock Connector
1ea	O_2 Saturation Probe / Connector
24L	Fresh Bovine Blood

Table 3
MODIFIED AAMI STANDARD BLOOD INLET CONDITIONS

Oxyhemoglobin Saturation	65 ±1 %
Hemoglobin Content	12 ± 1gm%
Base Excess	0 ± 5mmol/L
pCO_2	45 ± 2 mm Hg
Temperature	37 ± 1°C

Table 4
TEST FLOW CONDITION PARAMETERS

$Q_G : Q_B$ RATIO	Q_B (L/min)
0.5	2.0
1.0	4.0
2.0	6.0

Table 5
GRAPHICAL ANALYSIS

vs. Q_B	vs. Q_G:Q_B	vs. TIME ON BYPASS
O_2 Transfer Rate	O_2 Transfer Rate	O_2 Transfer Rate
O_2 Saturation	O_2 Saturation	O_2 Saturation
CO_2 Transfer Rate	CO_2 Transfer Rate	CO_2 Transfer Rate
Plasma Free Hgb	Plasma Free Hgb	Plasma Free Hgb

Table 6
OXYGEN TRANSFER

FICK'S PRINCIPLE:

O_2 Transfer Rate =
[(Art. Sat - Ven. Sat) X 1.39 X Hgb] + Sol (pO_2) (ml/min)

Table 7
CO_2 TRANSFER

FICK'S PRINCIPLE:

$$CO_2 \text{ Transfer Rate} = \frac{\text{Exhaust } CO_2(\%)(Q_G)}{100\ Q_B}$$

with:
Exhaust CO_2 = % of Exhaust CO_2 Gas
Q_G = Oxygenator Exhaust Gas Flow Rate
Q_B = Oxygenator Blood Flow Rate

CPB scenario where blood is in constant contact with the MO throughout the CPB procedure. As a result, no valid inference could be drawn with respect to hemocompatibility. Pearson and colleagues then illustrated that various MO's do differ significantly with respect to their haemcompatibility. (13)

METHOD

The specific aim of this circuit and protocol is to characterize the performance of MOs of varying designs and from differing manufacturers. No less than five oxygenators of any given make, model, or design should be evaluated. The order of testing should be randomized among the groups.

CIRCUIT SET-UP

The circuit is designed as a single pass system. The equipment and disposables required for the circuit are listed in Tables 1 and 2 respectively. The blood path of the circuit is a continuous path from a reservoir through a roller pump to a heat exchanger to the deoxygenator to a second reservoir. From this reservoir the blood is pumped through the test unit with a centrifugal pump back to the first reservoir. Two luer lock connectors are placed pre and post test MO; this allows for blood sampling and pressure monitoring on both sides of the test MO. A mass spectrometer is used to analyze the exhaust gas of the test MO (Figure 1).

The reservoirs are constructed using five gallon plastic paint buckets which are modified using 3/8" X 1/2" connectors as the inlet and outlet. We have found that by using silastic tubing on the part of the connectors which are fixed to the bucket and silicone sealant, we are able to achieve a leakless connection.

A deoxygenator is accomplished by utilizing six Bard H-4000 fiber bundles arranged in series. Gas flow is to both oxygenators. Utilizing this design we were able to achieve venous parameters in a single pass through the deoxygenator.

Inlet gas to the deoxygenator is 5% CO_2 / 95% N_2; 100% CO_2 and 100% N_2 are also connected to be used PRN in bursts to achieve venous parameters. From our experience it takes the operator a few runs with this circuit to have a "feel" for the deoxygenator and how to use the gases to adequately attain venous conditions. An O_2 saturation meter is placed in-line after the dexoygenator to aid in regulating the deoxygenator.

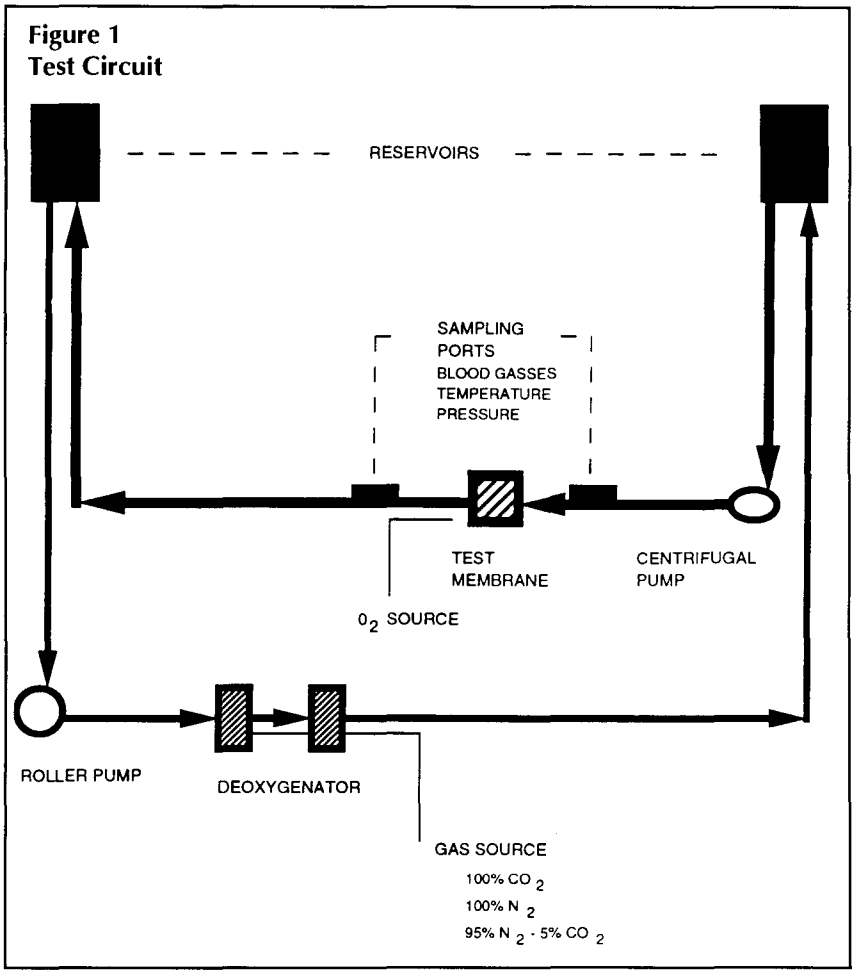
Inlet gas to the test MO can be at any FIO_2 and flow rate desired in order to mimic clinical conditions. The test MO must perform adequate gas exchange such that its output is adequately oxygenated (arterial) blood. During this process, it must induce as little blood trauma as possible. Quantification of gas exchange is performed by sampling inlet and outlet O_2 saturation (Sat) as well as inlet and outlet CO_2 content as well as measuring blood gas values. Rate of hemolysis is determined by measuring plasma free hemoglobin. The circuit is designed to perform at normothermia but can be modified to test the MO at both hyper and hypothermic conditions. The majority of systems described previously employed infrared capnography to measure the percentage of exhaust CO_2. This type of system is problematic as it generates erroneous readings for CO_2 due to unrecognized sample dilution with room air. Conversely, the mass spectrometric technique employed on our system permits identification of N_2, N_2O and other gasses, indicating contamination within the gas stream, and thus allowing more accurate data acquisition.

EVALUATION PROTOCOL

Fresh (less than four hours post collection), filtered , undiluted bovine blood shall be anticoagulated (at the time of collection) with 2,000 u/L heparin and treated with 800 mg Gentamycin to reduce bacterial growth. A hemoconcentrator may be required to arrive at the required hemoglobin (Hgb) of 12 ± 1 gm%.

Once the circuit is primed and debubbled, the blood is conditioned to modified Association for the Advancement of

Figure 1
Test Circuit



Medical Instrumentation (AAMI) venous conditions (see Table 3). Blood flow is initiated at 6 L/min. 100% CO_2 at 15 L/min is administered for 12-15 seconds to replenish the blood CO_2. Turning off the 100% CO_2, 5% CO_2 / 95% N_2 is administered at a flow of 15 L/min to remove the blood O_2 without significantly altering the CO_2 levels. Blood samples will be drawn to determine blood gas values. The O_2-Sat meter is used to monitor O_2-Sat trends and bursts (<12 seconds) of 100% N_2 or 100% CO_2 are administered to adjust the O_2 or CO_2 levels as needed. Blood temperature is maintained at 37°C.

Each test MO should be numbered and selected at random for evaluation by using a random permutation table. Each blood flow (QB) and gas flow (QG):blood flow (QB) combination should also be selected at random for each of the nine possible combinations to invoke the properties of the randomness "theory" and assure the validity of the evaluations performed.

Once venous parameters have been obtained gas flow is started through the test MO; gas flow through the deoxygenator is adjusted to maintain adequate venous conditions. The following data points should be collected: (1) time; (2) gas flow; (3) blood flow; (4) FIO_2; (5) arterial and venous blood gases; (6) temperatures; (7) exhaust CO_2; (8) inlet and outlet pressures; and (9) plasma free hemoglobin (Figure 2). Data should be organized and presented in both a graphical and tabular fashion to facilitate comparison. Relevant graphical analysis is summarized in Table 5. From the data collected one can evaluate O_2-transfer, CO_2-transfer, (see Tables 6 and 7), and hemolysis. Each of the nine combinations of QB and QG:QB should be run for forty minutes and no less than three blood gas measurements will be obtained at ten minute intervals for any QB-QG:QB combination. Venous O_2-Sat is determined with the co-oximeter. All points that appear anomalous should be repeated and comments should be recorded. The total trial duration should not be less than six hours.

STATISTICAL ANALYSIS

The three data sets obtained for any QB and QG:QB are averaged to obtain the mean and standard deviation values for each oxygenator group within each oxygenator type/design. A SAS split-plot analysis of variance (ANOVA), with oxygenators as the main plot and the nine combinations of QB and QG:QB as the subplots, will be performed to determine if at least one of the group means differs by more than would be expected by sampling variations. The assumption of randomness permits comparison between the different group means by examining the ratio of the variance estimated from looking between the group means to variance estimated from looking within the groups.(14) If the resulting F-statistic is large, one can conclude that there exists a statistically significant difference among the groups. The multiple comparison procedure should then be used to help determine which groups are different. A Student-Newman-Keul's T-test may be performed to determine significant differences based on: 1) Membrane material, 2) Oxygenator design, 3) Manufacturer, 4) Blood flow, and 5) Gas to blood flow ratio.

DISCUSSION

This circuit allows for evaluation and comparison of membrane oxygenators in a controlled clinical simulation. The authors are aware that to set up this circuit and to perform the evaluation protocol as described may be cost prohibitive in some hospital settings. However, the time intervals could be shortened and the number of QB-QG:QB could be reduced so that one could do a limited comparison / evaluation before using a MO clinically. It is our intent that industry will adapt this protocol so that the perfusion community will have standardized data to compare when reviewing product literature.

Given the many oxygenators commercially available for use during clinical CPB, an evaluation technique is desirable to

Figure 2
Membrane Oxygenator Evaluation Data Collection Sheet

PRE TRIAL DATA:

DATE:			
OXYGENATOR:		Hct:	
MODEL:		SERIAL #:	
MFG:	START TIME:		STOP TIME:

					BLOOD GAS DATA					PRESSURE DATA			
TIME	GAS FLOW	BLD FLOW	FIO2	A/V	pH	pCO2	pO2	BE	TEMP	Exh CO2	IN PRESS	OUT PRESS	P.F. Hgb
				A									
				V									
				A									
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screen devices prior to use clinically or in animal trials. Oxygenators vary not only in design but in cost. Many times, the choice of product is made on the basis of price and empirical assessment of performance based on clinical, ex vivo, or in vitro data. (15) Frequently, this data is anecdotal and biased by clinical technique employed at one institution or loyalty to a manufacturer. Information provided by a manufacturer will undoubtedly have a prejudice toward their product. An informed decision can now be made when evaluating or selecting a membrane oxygenator by utilizing the circuit and protocol described

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REFERENCES

1. Galletti PM, Breecher GA.Heart-Lung Bypass: Principles and Techniques of Extracorporeal Circulation. New York: Grune & Stratton; 1962.
2. Abel JJ, Rowntree LG, Turner BB. The removal of diffusable substances from the circulating blood by means of dialysis. Trans Assoc Am Phys. 1913; 28: 51-59.
3. Gibbon, JH. Applications of mechanical heart and lung apparatus to cardiac surgery. Minn Med. 1954; 37: 171-180.
4. Webster JG, ed. Encyclopedia of Medical Devices and Instrumentation, Vol. 3. New York: John Wiley & Sons; 1988: 1441.
5. Dawids SG, Engell HC, eds. Physiological and clinical aspect of oxygenator design. Proceedings-Seminar on Advances in Oxygenator Design. New York: Elsevier Scientific Publishing Co.; 1976: 7-12, 45, 60, 213-224, 293-297.
6. Norman JC ed. Cardiac Surgery, 2nd ed. New York: Appleton-Century-Crofts; 1972: 103-121.
7. Dinardo JA, ed. Anesthesia For Cardiac Surgery. Norwalk, CT: Appleton & Lange; 1990: 221.
8. Berger EC. The Physiology of Adequate Perfusion. New York: CV Mosby Co.; 1979: 24.
9. Nose Y. Manual On Artificial Organs: The Oxygenator Vol 2. New York: CV Mosby Co.; 1973: 37.
10. Rosen DA, Rosen KR, Silvasi DL, et al. An in vitro variability in fentanyl absorption by different membrane oxygenators, J Cardiothorac Anesth. 1990; 4(3): 332-335.
11. Videm V, Mollnew TE, Garred P, et al. Biocompatibility of extracorporeal circulation. J Thorac Cardiovasc Surg. 1991; 101(4): 654-660.
12. Kawak AM, Gaylor JD, Simpson K, et al. Gas exchange and artificial lung: An in vitro technique to assess oxygenators potential for respiratory failure therapies. Intnl J Artif Organs. 1991; 14(4): 234-238.
13. Pearson DT. Gas exchange: Bubble and membrane oxygenators. Semin Thorac Cardiovasc Surg. 1990; 2(4): 313-319.
14 Fleiss JL. The Design and Analysis of Clinical Experiments. New York: John Wiley & Sons; 1986: 126, 128, 138, 142-143, 220-227.
15. Kolobow T, Massimo B, Rossana S. Artificial lung (oxygenators). Artif Organs. 1986; 10(5): 370-377.