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In vitro comparison of two bioartificial liver systems: MELS CellModule and AMC-BAL

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Abstract

Clinically applied bioartificial liver (BAL) support systems are difficult to compare with regard to overall hepatocyte-specific function and clinical outcome. We compared two clinically applied BAL systems, the Modular Extracorporeal Liver Support (MELS) CellModule and the AMC-bioartificial liver (AMC-BAL), in an in vitro set-up. Both BAL systems were loaded with 10 billion freshly isolated porcine hepatocytes, cultured for 7 days and tested on day 1, 2, 4 and 7. Average decrease in hepatocyte-specific functions over 7 days was 9.7%. Three parameters differed between both bioreactors: lidocaine elimination at day 1 and 2 was significantly higher in the AMC-BAL, ammonia elimination showed a significantly higher trend for the AMC-BAL over 7 days and LDH release was significantly lower at day 7 for the MELS CellModule. In conclusion, this first in vitro comparison of two clinically applied BAL systems shows comparable functional capacity over a period of 7 days.
Comparison of MELS CellModule and AMC-BAL

Introduction

Many bioartificial liver (BAL) support systems have been developed since the report of the first clinical application of a BAL system by Matsumura et al. in 1987.\(^1\) The continued interest for improvement is driven by the need to reduce high mortality rates of acute liver failure (ALF) and to shorten long waiting lists for liver transplantation. Therapeutic management of ALF is complex due to its heterogeneity in etiology and disease progression.\(^2\) Research groups from various countries have developed and improved various liver support systems. This has led to a collection of heterogeneous scientific data from which it is difficult to compare one BAL system with another. In particular, large variations in cell type used, preservation method, isolation technique, test set-up, patient population, biochemical parameters, additional non-biological therapies and data presentation hamper the comparison of devices.\(^3\) To overcome these differences and to be able to objectively compare two devices, it is crucial to conduct an experiment in which these two BAL systems are tested along the same lines.

Two European BAL systems that have been applied clinically in a phase I safety trial are the Modular Extracorporeal Liver Support (MELS) CellModule (Fig. 1) of the Charité – Universitätsmedizin Berlin in Germany\(^4,5\), and the Bioartificial Liver of the Academic Medical Center (AMC-BAL; Fig. 1) of the University of Amsterdam in The Netherlands\(^6\) (see for review ref \(^7\).). Hardware configurations of both bioreactors have similar as well as different features (Fig. 1, Table 1). Importantly, both bioreactors are characterized by an internal oxygenation system for efficient and on-site oxygen supply to the hepatocytes. The hepatocytes, however, are located in different compartments in both systems. In the MELS CellModule, the hepatocytes are situated between three independent interwoven capillary membrane bundles; two capillary bundles for perfusion of medium/plasma and one capillary bundle for oxygenation. Because two fluid compartments can be distinguished in the MELS CellModule, \textit{i.e.} a medium/plasma and an extra-capillary cell compartment, the hepatocytes do not come to direct contact with the medium/plasma. In contrast, hepatocytes in the AMC-BAL are attached to a spirally wound non-woven matrix. Between these matrix layers, oxygen capillaries are situated and medium/plasma is perfused through the void spaces. As a consequence, the AMC-BAL consists of one fluid compartment in which the hepatocytes are in direct contact with medium/plasma. Despite these differences, both systems are considered to be the most promising devices because of their internal oxygenation system.\(^8\)

Although both BAL systems have demonstrated their safety in a clinical setting, no direct comparison can be made between these systems on account of secondary outcome parameters. Even pre-clinical \textit{in vitro} or \textit{in vivo} animal studies of these BAL systems are difficult to compare\(^3,9-12\) (Table 1).

We therefore initiated a comparative study in which both bioreactors were assessed for
Chapter 4

Figure 1. MELS CellModule (left) and the AMC-BAL (right). Detail MELS CellModule: a. oxygen capillary, b. inflow medium capillary, c. outflow medium capillary, d. hepatocyte aggregates. Detail AMC-BAL (transverse section): A. polycarbonate housing, B. non-woven polyester matrix, C. oxygen capillary, D. extra-capillary medium perfusion space.

Table 1. Hardware characteristics of the MELS CellModule and AMC-BAL

<table>
<thead>
<tr>
<th></th>
<th>MELS CellModule</th>
<th>AMC-BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioreactor description</td>
<td>Hollow fiber bioreactor with three interwoven, independent capillary membrane systems for decentralized perfusion and oxygen supply of extra-capillary situated hepatocytes</td>
<td>Bioreactor containing a spirally wound three-dimensional non-woven matrix for hepatocytes attachment with internal oxygenator of oxygen capillaries positioned in parallel</td>
</tr>
<tr>
<td>Weight ± 4300 gr</td>
<td>± 650 gr</td>
<td></td>
</tr>
<tr>
<td>Dimensions (L/H/D/V*)</td>
<td>31 / 11 / 31 / 8.3</td>
<td>18 / 11 / 11 / 1.7</td>
</tr>
<tr>
<td>Housing/potting material</td>
<td>Polyurethane (housing and potting)</td>
<td>Polycarbonate (housing and core), Bi-component PUR resin (potting; MG)</td>
</tr>
<tr>
<td>Cell volume/number</td>
<td>up to 600 g</td>
<td>10-20 billion hepatocytes (up to 250 g)</td>
</tr>
<tr>
<td>Priming volume</td>
<td>1.49 L</td>
<td>0.55 L</td>
</tr>
<tr>
<td>Sterilization</td>
<td>Formaldehyde</td>
<td>Ethylene oxide (ETO)</td>
</tr>
<tr>
<td>Matrix</td>
<td>n/a</td>
<td>Hydrophilic, Polyester, TMS 0.58 m², USCS 8.7 m²</td>
</tr>
<tr>
<td>Medium capillaries</td>
<td>Polyethersulphone, MWC 400kDa TMS 2.11 m² (two bundles)</td>
<td>n/a, direct plasma/medium-cell contact</td>
</tr>
<tr>
<td>Perfusion rate</td>
<td>Recirculatory, 250 mL/min</td>
<td>Recirculatory, 150 mL/min</td>
</tr>
<tr>
<td>Medium feed</td>
<td>50 mL/h</td>
<td>n/a</td>
</tr>
<tr>
<td>Oxygen capillaries</td>
<td>Hydrophobic, Multilaminate, TCS 2.22 m²</td>
<td>Hydrophobic, Co-extruded PP+PMP, TCS 1.1 m²</td>
</tr>
<tr>
<td>Oxygenation</td>
<td>Air at 600 mL/min, CO₂ at 15-25mL/min</td>
<td>Culture gas (95% air / 5% CO₂) at 200 mL/min</td>
</tr>
<tr>
<td>Acid-base regulation</td>
<td>Via partial CO₂, pressure adjustment</td>
<td>Via bicarbonate addition</td>
</tr>
<tr>
<td>Ports</td>
<td>4 medium ports (2x I/O)</td>
<td>2 medium ports (I/O; also for CS)</td>
</tr>
<tr>
<td></td>
<td>2 oxygen ports (I/O)</td>
<td>2 oxygen ports (I/O)</td>
</tr>
<tr>
<td></td>
<td>2 CS ports</td>
<td>1 extra port for CS</td>
</tr>
<tr>
<td></td>
<td>3 ports for pressure sensors</td>
<td>1 port for temperature probe</td>
</tr>
<tr>
<td></td>
<td>1 port for temperature probe</td>
<td></td>
</tr>
</tbody>
</table>

L, length in cm; H, height in cm; D, depth in cm; V, volume in L; MWC, molecular weight cut-off; TMS, total membrane surface; TCS, total capillary surface; USCS, useful surface for cell seeding; PP, polypropene; PMP, polymethylpentene; PUR, polyurethane; MG, medical grade; CS, cell seeding; I/O, inflow and outflow; n/a, not applicable
Comparison of MELS CellModule and AMC-BAL

their hepatocyte-specific function and metabolic activity. To allow accurate comparison, both bioreactors were loaded with an equal amount of freshly isolated porcine hepatocytes from an identical source, cultured with the same culture medium and tested with the same methods. BAL system specific operational properties, e.g. cell culture maintenance, were not changed.

Materials and Methods

Compartment volume analysis of MELS CellModule

Prior to charging the bioreactors, the volumes of both compartments of the MELS CellModule were determined by spiking experiments. This information was essential as 1. hepatocyte-specific function was determined by calculating changes in concentration of compounds either produced or eliminated by the hepatocytes in the total volume of test medium, and 2. production and elimination of metabolites and substrates are influenced by the wash-out/release kinetics as the result of the number of fluid compartments in a bioreactor. As the AMC-BAL has only one fluid compartment, a change in parameter concentration in time is solely caused by the activity of hepatocytes. Since the MELS CellModule has two fluid compartments, i.e. the medium/plasma compartment (MC) and cell compartment (CC), convection/diffusion (C/D) mass transfer will influence parameter concentration in each compartment. We analyzed the C/D kinetics by injecting 3 mL 2.5M NH₄Cl (Merck, Darmstadt, Germany) and 1.5 mL 2M D-glucose (VWR Int. Ltd, Poole, England) in MC, and 1.5 mL 2M L-lactate (Sigma-Aldrich, Steinheim, Germany) and 1.5 mL 2M KCl (Sigma-Aldrich, Steinheim, Germany) in CC at t=0 with a medium recirculation rate of 250 mL/min and standard oxygenation. Samples of 0.5 mL were then taken from both compartments at t=1; 2.5; 5; 10; 15; 20; 25; 30; 35; 40; 45; 50; 55, 60; 70; 80; 90 min. and t=16 hours, the next day. The concentrations of NH₄Cl, D-glucose, L-lactate and KCl in both compartments were determined and plotted against time. We selected the three best curve estimation regression models using software package SPSS 12.0.1, by ranking mean $R^2$, mean significance $R^2$, and visualisation of the curves, to calculate average y-axis intercepts of each injected compound of each compartment as a measure of the MC/CC ratio. Total bioreactor volume was calculated from the dilution fraction of all four injected compounds at t=16 hours. Both compartment volumes were calculation from the total bioreactor volume by using the MC/CC ratio.

Hepatocyte isolation

All animal experiments were conducted in the Surgical Laboratory of the Academic Medical Center according to the institutional guidelines of the local Animal Ethical Committee.
Hepatocytes were isolated from livers of female pigs (20-24 kg) by a two-step collagenase perfusion technique according to a modified protocol of Seglen\textsuperscript{13} at day 0, as previously described.\textsuperscript{3} Two livers were harvested to obtain a sufficient number of viable cells for both bioreactors. Porcine hepatocytes were suspended in ice-cold culture medium, consisting of William’s E medium (Cambrex, Verviers, Belgium) supplemented with 10\% (v/v) heat inactivated fetal bovine serum (Cambrex, Verviers, Belgium), 2 mM glutamine (Cambrex, Verviers, Belgium), 1 μM dexamethasone (Centrafarm, Etten-Leur, The Netherlands), 20 mU/mL insulin (Actrapid\textsuperscript{®}, Novo Nordisk, The Netherlands), 2 mM ornithine (Sigma-Aldrich, Steinheim, Germany), 100 μg/mL streptomycin, 100 U/mL penicillin and 0.25 μg/mL fungizone mix (100x; Cambrex, Walkersville, MD, USA). Total yield of isolated hepatocytes was estimated by determination of the cell pellet volume after three times of centrifugation at 50xg for three minutes. Viability of the isolated hepatocytes was determined by trypan blue exclusion test. The first hepatocyte suspension was kept on ice for approximately 3.5 hours and was used to replenish the yield of the second isolation to gain a final cell number of 20 billion hepatocytes. This suspension was then divided into two aliquots of ten billion hepatocytes for each bioreactor. The AMC-BAL was charged unprimed, whereas the MELS CellModule was charged after priming.

**Hepatocyte culture**

Each bioreactor was placed in their standard culture cabinet at 37°C and operated according to its standard protocol to ensure optimal bioreactor-specific culture conditions. The MELS CellModule was continuously perfused with recirculating culture medium at 250 mL/min. Fresh medium was continuously added at a flow rate of 150 mL/h for the first day and 50 mL/h for the remaining culture period, except for the test periods. Air was perfused at 600 mL/min and mixed with CO\textsubscript{2} at a variable flow rate of 16-24 mL/min to control acid-base balance. The AMC-BAL was rotated for two hours to ensure optimal cell attachment to the matrix and even cell distribution within the bioreactor following the inoculation procedure. The integrated oxygenator was perfused with sterile 95\% air and 5\% CO\textsubscript{2} at a flow rate of 200 mL/min. Two hours after the attachment period, dead and unattached cells were removed by flushing 1 L of fresh culture medium through the bioreactor at 150 mL/min. The bioreactor was then continuously perfused with 2 L recirculating culture medium at 150 mL/min.

**Testing of bioreactor function**

Both bioreactors were tested at day 1, 2, 4 and 7. Oxygen consumption was determined by measuring the decrease in oxygen tension at the medium outflow port during 15 minutes after closure of the oxygen supply to the bioreactor. The oxygen consumption test was
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performed for both bioreactors in the standard culture mode and O₂ consumption was calculated as described by Van de Kerkhove et al. Subsequently, culture medium was replaced by test medium which was composed of normal culture medium supplemented with 1.85 mM lidocaine hydrochloride (Sigma-Aldrich, Steinheim, Germany), 2 mM L-lactate and 5 mM NH₄Cl. Culture medium was flushed out the MELS CellModule with 3 L test medium. To ensure complete replacement, both medium containing capillary bundles were alternately opened and closed to redirect remaining culture medium out of the bioreactor. After flushing, test medium was continuously recirculated at 300 mL/min in a closed mode for three hours without continuous addition of fresh medium. The AMC-BAL was flushed with 1 L test medium. Thereafter, test medium was continuously recirculated at 150 mL/min for three hours. Both bioreactors were tested with the same volume of test medium. Samples were taken from all compartments at t=60, t=90, t=120, t=150 and t=180 and analyzed for ammonia, urea, lidocaine, glucose and lactate concentrations as well as activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Total sample volume drawn at each time point was equal between both bioreactors.

Ammonia and lidocaine elimination, urea and albumin production, AST and LDH release, glucose and lactate consumption and/or production of the AMC-BAL were determined by calculating the changes in concentration in test medium per hour per billion cells. For the MELS CellModule, we first determined the concentration of each compound per time point for the whole bioreactor ([C]ₜₜ) according to the following equation:

\[
[C]_{tot} = \frac{[C]_{MC} \cdot V_{MC} + [C]_{CC} \cdot V_{CC}}{V_{tot}}
\]  

[1]

[C] is the concentration of the compound, V is volume (L), subscripted MC, CC and tot are medium compartment, cell compartment and total, respectively. The final function was then determined by calculating the changes in [C]ₜₜ per hour per billion cells.

After each test, both bioreactors were flushed again with new culture medium to remove all test medium. The AMC-BAL was flushed with 1 L culture medium after which a total end culture volume of 2.2 L was continuously recirculated at 150 mL/min. The MELS CellModule was flushed with 3 L culture medium in the same manner as prior to the test and subsequently continuously perfused at 250 mL/min with addition of culture medium at a flow rate of 50 mL/min.
**Statistical analysis**

Statistical analysis was performed by using SPSS 12.0.1 for Windows software (SPSS Inc., Chicago, IL, USA). Results are reported as means ± standard error of the mean (SEM). Repeated measurement ANOVA rank tests were used to compare both bioreactors at all days to discriminate time dependent trends, as well as each test day to distinguish significant daily differences. Significance was reached if $p < 0.05$. Graphical presentation of the data was performed using Prism version 3.0 (GraphPad Prism Inc, San Diego, CA, USA).

**Results**

**Compartment volume analysis**

Total volume of the MELS CellModule was 1.49 L ± 0.04 L, calculated from the dilution fraction of four metabolites injected in the medium (MC) and cell (CC) compartment at t=16 hours (n=3). The three best curve estimation regression models for the changes in concentration of all injected metabolites in both compartments in time were the logarithmic, power and cubic model with an average $R^2$ of 0.74 ± 0.042 ($p = 0.048 ± 0.013$). The average MC/CC ratio, calculated from the y-axis intercepts of these three models for each metabolite, was 0.76/0.24 resulting in an average MC volume of 1.13 L and an average CC volume of 0.36 L.

![Figure 2](image-url). AST and LDH release of the MELS CellModule (white bars) and the AMC-BAL (black bars). A: AST release, B: LDH release. A-B are expressed in U/hour/10^9 viable hepatocytes (mean ± SEM; * = $p < 0.05$).
Isolation outcome and bioreactor tests

Ten porcine livers were used for cell isolation with an average yield of $12.0 \times 10^9 \pm 2.9 \times 10^9$ vital hepatocytes per liver and an average viability of $98.0 \% \pm 1.1 \%$ to obtain sufficient amounts of viable cell mass to load five CellModule and five AMC-BAL bioreactors with 10 billion hepatocytes each. The MELS CellModule encountered gas/medium leakages during the first (day 2), third (day 0), and fifth experiment (day 6) and, as a consequence, was terminated at these respective days. To allow equal comparison, the corresponding AMC-BAL was also terminated in the first and third experiment. As a result, data are presented as a n=4 for day 1 and 2, n=3 for day 4, n=2 for day 7, respectively, for the MELS CellModule, and n=3 for day 7 for the AMC-BAL. We used the repeated measurement ANOVA test to analyse the data of all test days (n=13) for time-dependent differences between both systems, as well as daily differences between the MELS CellModule and the AMC-BAL.

Cellular damage

AST and LDH release (Fig. 2) were determined to assess hepatocellular damage in both bioreactors. Only at day 7, a significantly higher release of LDH was observed for the AMC-BAL. No significant differences were found for AST and LDH release at day 1, 2 and 4 between both bioreactors, although a tendency towards a higher AST and LDH release of the AMC-BAL was observed at day 1.

Hepatocyte-specific and metabolic functions

Ammonia and lidocaine elimination as well as urea and albumin production were determined to assess liver-specific functionality of both bioreactors (Fig. 3). In general, liver-specific functionality was stable for both bioreactors during 7 days, except for urea production that decreased in time for both bioreactors. The average decrease of all four hepatocyte-specific functions over 7 days for both bioreactors was 9.7%. Ammonia elimination of the AMC-BAL was higher at all days as compared to the MELS CellModule with a mean ammonia elimination of $97.6 \pm 5.9$ and $68.8 \pm 7.6 \mu mol/hour/billion hepatocytes, respectively. Although daily differences between both bioreactors were not significant, the overall higher trend for ammonia elimination of the AMC-BAL was significant. In contrast, no significant differences were observed between both bioreactors for urea production. Lidocaine elimination was significantly higher at day 1 and 2 for the AMC-BAL when compared to the MELS CellModule with means of $42.0 \pm 3.4$ and $22.8 \pm 0.2 \mu mol/hour/billion hepatocytes, respectively. Albumin production of both bioreactors did not differ significantly at all time points, but varied to a large extent between separate experiments with an average coefficient of variation of $73.1\% \pm 13.4\%$ for MELS CellModule and $46.8\% \pm 5.5\%$ for AMC-BAL.
Glucose and lactate concentrations were determined to assess carbohydrate metabolism within each bioreactor over 7 days (Fig. 4). In the first two days, hepatocytes in both bioreactors consumed lactate. Both bioreactors produced glucose during the first days, except for MELS CellModule at day 1 showing glucose consumption. Thereafter, carbohydrate metabolism was reversed into glucose consumption and lactate production in both bioreactors. Although differences were not statistically significant, distinct trends were observed. The overall conversion rate of glucose of the MELS CellModule, calculated by the slope over 7 days, increased with 19.3 μmol/hour/billion hepatocytes per day ($r^2 = 0.87$) and was lower than the overall glucose conversion rate of the AMC-BAL that increased with 49.3 μmol/hour/billion hepatocytes per day ($r^2 = 0.97$). The same tendency was observed for lactate showing an overall conversion rate in the MELS CellModule of 39.1 μmol/hour/billion hepatocytes per day ($r^2 = 0.96$) and in the AMC-BAL of 71.9 μmol/hour/billion hepatocytes per day ($r^2 = 0.98$). Overall carbohydrate conversion rate, calculated by dividing the average overall glucose conversion rate by the
average overall lactate conversion rate per experiment, was 1.5 ± 0.2 for the AMC-BAL and 1.9 ± 0.1 for the MELS CellModule.

Although absolute oxygen consumption values differed significantly between both bioreactors at all days (Fig. 5), no valid conclusion can be made since both bioreactors differ considerably in their hardware characteristics, in particular with regard to fluid dynamics and oxygenation. Despite these differences, oxygen consumption of the AMC-BAL increased in time by 2.9 μmol/hour/billion hepatocytes per day ($r^2 = 0.98$) to 144 ± 13 % at day 7 relative to day 1, whereas oxygen consumption of the MELS CellModule decreased in time by 0.8 μmol/hour/billion hepatocytes per day ($r^2 = 0.91$) to 77 ± 37 % on the last day relative to day 1.
Discussion

This study reports the first in vitro comparison of two BAL systems, i.e. the MELS CellModule and the AMC-BAL. Both bioreactors have been investigated intensively in pre-clinical in vitro and in vivo studies as well as in phase I clinical studies\(^\text{(3-6,9-12)}\), but from these data no direct device comparison can be made. Because both devices are distinct in operating features, we included the following three essential experimental conditions in our study protocol: 1. both BAL systems were loaded with the same amount of freshly isolated porcine hepatocytes originating from the same cell pool; 2. liver specific parameters for cell function and integrity of both BAL-systems were tested with an identical test solution under comparable conditions; and 3. all operational procedures for each bioreactor were performed by its own experienced team.

After reviewing all end-point parameters, we conclude that both bioreactors are comparable in function over a period of 7 days. Average decrease in hepatocyte-specific functions over 7 days was 9.7%. However, distinct differences and trends were observed. The AMC-BAL eliminated significantly more lidocaine at day 1 and 2. A significantly higher trend over 7 days was observed for ammonia elimination in the AMC-BAL. LDH release was significantly lower at day 7 in the MELS CellModule. Both BAL systems showed a comparable decline in urea synthesis over 7 days. During the whole experiment, the AMC-BAL showed higher oxygen consumption than the MELS CellModule. This was probably associated with a higher trend of lactate production in the AMC-BAL at day 7, suggesting an increased anaerobic glycolysis. Both bioreactors changed from gluconeogenesis in the first days to glucose consumption in the last days. This effect was more pronounced in the AMC-BAL.

The minor differences in outcome parameters of the MELS CellModule and AMC-BAL are not easily clarified, but are not unexpected in view of the different characteristics of both BAL systems. For example, mass transfer for certain compounds may be more efficient in the AMC-BAL, because of the direct contact between the test medium and the hepatocytes.\(^\text{14,15}\) This might explain the higher ammonia elimination rate over 7 days and the higher lidocaine elimination during the first 2 days in the AMC-BAL. In contrast, increased lactate production and higher LDH release in the AMC-BAL at day 7 suggest anaerobic glycolysis to occur. This effect might also counteract the more efficient mass transfer for certain metabolites of the AMC-BAL. An alternative explanation for the higher enzyme leakage in the AMC-BAL at day 7 might be the higher shear stresses caused by contact of medium with the cells. Cells inside the MELS CellModule are probably less exposed to shear stress owing to their position on the other side of the semipermeable membrane. Despite small differences, we conclude that the functionality of both bioreactors is comparable. Therefore, both systems should function equally in a clinical study. However, for clinical application of a bioartificial liver, other features should also
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be considered, \textit{i.e.} costs, logistics of BAL availability\textsuperscript{16,17}, reliability, convenience and the source of the biocomponent.

In summary, we have demonstrated that a device comparison of two BAL systems, \textit{i.e.} the MELS CellModule and the AMC-BAL, is feasible. We conclude after reviewing all end-point parameters, including parameters for metabolic activity, cell damage and hepatocyte-specific functions, that both BAL systems are comparable over a period of 7 days. Although the MELS CellModule and the AMC-BAL differ in their hardware characteristics, the hepatocyte-specific functions of both systems are high. In addition, since both BAL systems are equally functional, we conclude that the applied cell type, instead of the device itself, is the crucial factor for final performance of the BAL system. Since prohibition of application of xenogeneic cells is a fact in many countries and is further advancing in Europe, development of an immortalised human liver cell line has therefore the highest priority for clinical use of BAL systems. Consequently, this study suggests that both BAL systems will potentially show life supporting capacity when charged with such a human cell line.

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