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Design and Characterization of a 3D-Printable Membrane Aeration Module for Small-Scale Bioprocess Prototyping

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ABSTRACT

Oxygen transfer is a critical design parameter in laboratory-scale bioprocess systems used for prototyping, process development, and scale-down studies of mammalian cell cultures, particularly when cultivating shear-sensitive mammalian cells. In this work, we present the design and characterization of a 3D-printed modular, membrane-based aeration module that enables bubble-free oxygen transfer in laboratory reference cell-cultivation systems. The aeration module was developed as an external, small-scale unit intended for flexible integration into laboratory bioreactors and perfusion setups. Fabricated via fused deposition modeling, the final design features a three-chamber membrane-stacking architecture that ensures mechanical stability, tightness, and biocompatibility, while allowing for straightforward adaptation through editable CAD files. The system was experimentally evaluated with respect to oxygen transfer performance under varying relative liquid flow rates and membrane configurations (PTFE and PVDF), each with two different pore sizes (0.22 μm and 0.45 μm). Key performance parameters of the aeration module were determined and include dissolved oxygen (DO) profiles, volumetric oxygen transfer coefficients (7.26 h^{-1}), oxygen transfer rates (OTRs) (max. 61.4 $\text{mg L}^{-1}\text{h}^{-1}$), and the pressure-normalized oxygen mass transfer rate (0.87 $\text{g m}^{-2}\text{bar}^{-1}\text{h}^{-1}$). Overall, the modular design and quantified performance provide a versatile tool for rapid iteration and evaluation of membrane-based oxygenation strategies in early-stage bioprocess development.

1 | Introduction

In recent years, cell cultivation for food applications, such as cultured meat, has emerged as a potential alternative to conventional livestock farming [1]. This offers the opportunity to

substantially reduce greenhouse gas emissions, land and water use, and ethical concerns associated with animal slaughter, although the scenarios depend considerably on process assumptions and energy supply [1–3]. Despite rapid progress in cell line development and tissue engineering strategies, cultured

Abbreviations: β_L , Liquid-side mass transfer coefficient (m h^{-1}); ABS, Acrylonitrile butadiene styrene; ACC, Accession number (DSMZ cell line identifier); BMFTR, Federal Ministry of Research, Technology and Space; C(t), Dissolved oxygen concentration at time t (mg L^{-1}); C*, Oxygen saturation concentration (mg L^{-1}); C2C12, Mouse myoblast cell line; CAD, Computer-aided design; CO_2 , Carbon dioxide; CPE, Copolyester; DMEM, Dulbecco's Modified Eagle Medium; DO, Dissolved oxygen; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; FBS/FCS, Fetal bovine serum / fetal calf serum; FDM, Fused deposition modeling; $k_L a$, Volumetric oxygen mass transfer coefficient (min^{-1}); LB, Luria-Bertani medium; M, Pressure-normalized oxygen mass transfer coefficient ($\text{g m}^{-2}\text{bar}^{-1}\text{h}^{-1}$); Na_2SO_3 , Sodium sulfite; NaCl, Sodium chloride; NT, No-treatment control; OD_{600} , Optical density at 600 nm; OTR, Oxygen transfer rate ($\text{mg L}^{-1}\text{h}^{-1}$); P/S, Penicillin/streptomycin; PA, Polyamide; PETG, Polyethylene terephthalate glycol-modified; PLA, Polylactic acid; PTFE, Polytetrafluoroethylene; PVDF, Polyvinylidene fluoride; rpm, Revolutions per minute (min^{-1}); STL, Stereolithography file format; t, Time (min); v/v, Volume per volume; w/v, Weight per volume; w/w, Weight per weight.

Laurenz Köhne and Pia Elisabeth Lorenz contributed equally to this work.

For review purposes, the .stl file of the 3D-model has been attached to the submission. We furthermore intend to upload the file to the Technical University of Munich (TUM) file repository, or another repository upon request pending evaluation of this manuscript.

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Practical application

This technical report presents a 3D-printable, membrane-based aeration module characterized under given process conditions that enables controlled oxygen transfer without the need for direct sparging. Its primary application lies in small-scale process development and scale-down studies for shear-sensitive mammalian cell cultures, including emerging fields such as cultured meat, cell-based therapeutics, and advanced tissue engineering. The externally mounted, modular design enables straightforward integration into existing benchtop bioreactors, perfusion systems, and reference cultivation setups without major hardware modifications. Because the module is 3D-printed and supported by editable CAD files, it can be rapidly adapted to different reactor geometries, flow regimes, and oxygen demands, thus supporting agile prototyping. By decoupling oxygen supply from hydrodynamic stress, the system allows more representative investigation of large-scale oxygenation concepts at small scale, improving process understanding, scalability, and robustness while reducing development time and experimental risk.

meat remains an early-stage technology with several unresolved technical challenges that must be addressed before industrial implementation becomes feasible [4]. Among these challenges, the construction and design of suitable bioreactor systems for the expansion and differentiation of animal cells represent a central bottleneck [5]. Perfusion systems are particularly attractive due to their ability to support high cell densities, prolonged culture durations, and controlled microenvironments. However, the successful implementation of such systems requires precise control over mass transfer, with oxygen supply being one of the most critical parameters [6]. Conventional aeration strategies, including sparger-assisted gas introduction, are well established in bioprocesses but are often ill-suited for shear-sensitive systems [7]. Gas sparging introduces bubbles that generate local shear forces, promote foam formation, and increase the risk of cell damage through bubble rupture and gas–liquid interfaces [6]. As such, there is a clear need for defined, validated, and transferable aeration concepts that enable gentle yet efficient oxygen delivery.

Membrane-based aeration represents a promising alternative approach, enabling bubble-free oxygen transfer via diffusion across semi-permeable hydrophobic membranes [8]. By physically separating the gas and liquid phases, membrane aeration minimizes shear stress and allows for more controlled oxygen supply, making it particularly suitable for shear-sensitive (bio)systems [9]. When implemented as an external module operated in a side stream, membrane aeration further enables the independent optimization of oxygen transfer, flow rates, and mixing conditions, an important feature for perfusion-based and modular bioreactor concepts [10, 11]. In parallel with advances in bioprocess design, the increasing diversification of biotechnology applications, including cell-based food production, calls for flexible and rapidly adaptable hardware solutions [12, 13]. Additive manufacturing technologies such as fused deposition modeling (FDM) provide a cost-effective means to fabricate customized reactor components with complex geometries, short iteration cycles, and high configurability [14]. Importantly, 3D-printing

enables the sharing, modification, and scaling of design files, supporting reproducibility and transferability across laboratories.

In this technical note, we present the development and characterization of a small-scale, yet scalable, modular aeration system designed specifically for shear-sensitive (bio)systems, with potential applications in tissue engineering and cultured meat research. By providing a fully characterized and configurable aeration solution, including CAD files for adaptation, we aim to support standardized laboratory-scale testing and facilitate the transfer of oxygen supply strategies toward larger-scale perfusion bioreactor systems. This work contributes to closing an important gap between early-stage process prototyping and scalable cultured meat bioprocess development.

2 | Materials and Methods

2.1 | Chemicals and Media

Sodium chloride (NaCl $\geq 99\%$, Ph. Eur., USP, Art. No. P029.3) and Sodium sulfite (Na_2SO_3 $\geq 98\%$, p.a., ACS, wasserfrei, Art. No. P033.1) were attained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Dulbecco's Modified Eagle Medium (DMEM, Art. No. 31966047), fetal calf serum (FCS, Art. No. A5256701), Trypan Blue solution (0.4 %, Art. No. 15250061), TrypLE Express Enzyme (1 $\times$, Art. No. 12604039) and Penicillin/streptomycin (10,000 U mL^{-1} , Art. No. 15140122) were attained from Thermo Fisher Scientific (Waltham, MA, USA).

2.2 | Origin and Maintenance of Cell Lines

Mouse C2C12 skeletal myoblasts (ACC 565) were purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). Cells were shipped 10/2024 and used at passages 12–15 in all experiments. The employed cell line was authenticated for the described experiments and has not been previously reported as misidentified or contaminated. In addition, routine checks indicated that the cell line was free of mycoplasma contamination for the described experiments.

2.3 | Sterilization of 3D-Printed Filaments

Five commonly used FDM-printing materials were selected, namely, acrylonitrile butadiene styrene (ABS; 3DJake, Germany), copolyester (CPE; Art. No. HG100, Fillamentum, Czech Republic), polyamide (PA; Flashforge, China), polyethylene terephthalate glycol-modified (PETG; RAL 9017, Spectrum Filaments, Poland), and polylactic acid (PLA; AmazonBasics, USA). For sterility tests, a test geometry consisting of a $1 \times 1 \times 1$ cm cube with a central 1-mm recess was designed in SolidWorks (Dassault Systèmes, Vélizy-Villacoublay, France) and printed using an FDM printer (Prusa i3; Prusa Research, Czech Republic). The respective printing and bed temperatures for each filament are shown in Table S1. Following fabrication, the printed cubes were subjected to six sterilization procedures: (1) 70% (v/v) ethanol, (2) 70% (v/v) isopropanol, (3) dry-heat treatment at 120°C for 8 h, (4) dry-heat treatment at 160°C for 2 h, (5) dry-heat treatment at

180°C for 30 min, and (6) steam autoclaving at 121°C for 20 min at 2 bar with three vacuum pulses. To assess sterilization efficacy, the sterilized cubes were transferred under sterile conditions into a flask containing 25 mL LB medium. Three independent replicates were performed for each condition. Samples were incubated for 24 h at 37°C and 180 rpm, after which the optical density at 600 nm (OD_{600}) of the medium was measured. In addition, 200 μ L of each culture was plated onto LB agar and incubated for a further 16 h at 37°C. Colonies appearing on the agar plates were counted the following day to evaluate microbial contamination.

2.4 | Material Compatibility With Mammalian Cells

To evaluate the biocompatibility of the selected filaments for mammalian cell culture, each material was sterilized using the most effective method identified in the preceding experiments. The sterilized cubes were then incubated for 2 days in culture medium (DMEM supplemented with 10 % FBS and 1 % P/S, 37°C, 5% CO_2 , 95% humidity). After incubation, the conditioned medium was used to generate growth curves with C2C12 myoblasts, while the cubes themselves remained in the wells to allow direct contact between cells and material. C2C12 cells were seeded at a density of 3000 cells cm^{-2} in six-well plates in triplicate for each material, alongside a no-treatment control (NT), which is C2C12 growing without any materials. Cell proliferation was monitored every 24 h over 4 days. At each time point, cells were stained with trypan blue, and both viable and nonviable cells were recorded by a cell counter (Countess, Thermo Fisher Scientific, Waltham, MA, USA); viable cell numbers were used for data analysis and growth curve generation.

2.5 | Design, Manufacturing, and Setup of the Aeration Module

The 3D-architecture containing a stackable membrane module adapted from Merkel et al. [15]. The modular membrane-based aeration units were designed as compact and functional prototypes enabling gas-liquid oxygen transfer without phase mixing. A parametric 3D-model of the gas transfer module was created in Autodesk Inventor (Autodesk Inc., San Francisco, USA). We used commercially available membranes similar to those reported by Merkel et al., matching in diameter and partially in material and nominal pore size. The module design was therefore oriented around these membranes to facilitate handling and 3D printing. The final designs were exported as .stl files and imported into Ultimaker Cura (Ultimaker B.V., Utrecht, Netherlands) for slicing. Printing parameters are stated in the [Supporting Information](#) (Table S1). Prototypes were fabricated with the FDM printer, equipped with a 0.4 mm nozzle. The filament “niceABS red” was used for easier post-processing. To reduce FDM layer lines and improve sealing surfaces, ABS parts were mechanically smoothed and then chemically post-treated with acetone. Depending on part geometry, components were either briefly immersed (10–15 s) or selectively treated with an acetone-soaked brush, followed by 24 h evaporation to remove solvent residues.

Membranes (Zhejiang ALWSCI Technologies Co., Ltd., Shaoxin, China) were bonded to dedicated mounts (Figure 2) using a two-component epoxy resin mixed 1:1 (w/w). After a 15 min pre-curing time to increase viscosity, the resin was applied to the mounting surface using a 10 μ L pipette to minimize coverage of the active membrane area. The membrane was positioned and sealed at the edges with an additional resin layer and cured for 24 h. A second reinforcing layer was subsequently applied and cured under identical conditions. The leak-tightness of individual chambers and interfaces was evaluated using a custom test rig. The module was connected via hoses to a 50 mL Luer-Lock syringe (Sarstedt AG & Co. KG, Nümbrecht, Germany) at the inlet and to a hose leading to a water reservoir at the outlet, which could be closed with a clamp. Each chamber was filled with water, the outlet was closed, and manual pressure was applied by the syringe while monitoring for external leaks. After testing, the module was disassembled to verify the absence of cross-leakage between chambers.

2.6 | Oxygen Measurements

Oxygen levels were monitored using electrochemical DO-sensors (Mettler-Toledo, Columbus, USA). A two-point calibration, with nitrogen and 2 bar compressed air, was performed before each measurement run. Unless otherwise stated, all oxygen measurements were conducted with 400 mL of a 0.9 % saline solution (w/v) as reference medium. Oxygen transfer rates (OTRs) were measured using a dynamic gassing-out experiment based on the general dynamic method described by Van’t Riet et al. [16], and analogous to the implementation reported by Merkel et al. [15]. To reset the oxygenated system to an oxygen-free solution, 1 mL of 200 mM Na_2SO_3 solution was injected into the medium following each measurement. Subsequently, a constant air flow of 300 mL min^{-1} was introduced into the system. The media flow rate through the aeration module was set between 10 and 150 mL min^{-1} . DO signals and temperature were recorded using eve software (INFORS HT, Bottmingen, Switzerland).

Calculation of oxygen measurements:

The dissolved oxygen (DO) data were evaluated using the linearized form of the oxygen transfer equation. In the absence of biological oxygen consumption, the change in DO concentration $C(t)$ ($mg\ L^{-1}$) over time is described by:

$$\frac{dC(t)}{dt} = k_L a \cdot (c_{O_{2,L}}^* - c_{O_{2,L}}(t)) \quad (1)$$

where $k_L a$ is the volumetric mass transfer coefficient (min^{-1}) and C^* is the saturation concentration of oxygen in the liquid phase ($mg\ L^{-1}$). Integration yields:

$$\ln(c_{O_{2,L}}^* - c_{O_{2,L}}(t)) = -k_L a \cdot t \quad (2)$$

with C denoting the DO concentration at the time point t (min).

For each experiment, the DO concentration $C(t)$ was calculated from the DO sensor signal and plotted as $\ln(c_{O_{2,L}}^* - (c)_{O_{2,L}})$ versus time t (min). Additionally, the temperature and salinity were taken into consideration, as they can affect the concen-

tration of DO [17]. Linear regression was performed over the time interval exhibiting the highest oxygen increase, that is, the region showing exponential response. The slope of the regression line corresponds to $-k_L a$, yielding $k_L a$ per min, which was subsequently converted to h^{-1} .

In the next step, the volumetric OTR was calculated from the determined $k_L a$ -value according to:

$$OTR = k_L a \cdot c_{O_2,L}^* \quad (3)$$

OTR is expressed in $\text{mg L}^{-1} \text{h}^{-1}$. The total oxygen input rate for the aeration module was obtained by multiplying the volumetric OTR by the liquid volume $V(L)$ inside the module. To directly compare different membrane materials and geometries, the liquid-side mass transfer coefficient β_L was calculated as a measure of oxygen transfer efficiency per unit membrane area:

$$\beta_L = \frac{OTR_{\text{Total}}}{A \cdot c_{O_2,L}^*} \quad (4)$$

where A is the effective membrane surface area (m^2). The resulting β_L values (m h^{-1}) reflect the combined effects of membrane material and structure. No additional correction was applied for membrane porosity or pore size, as these structural characteristics are inherently captured in the experimentally determined β_L . Furthermore, mass transfer normalized by the oxygen partial pressure difference (Δp_{O_2}) was calculated to enable a more detailed comparison of membrane materials. The pressure-normalized oxygen mass transfer rate $\dot{M}_{O_2}$ was defined as:

$$\dot{M}_{O_2} = \frac{OTR_{\text{Total}}}{A \cdot \Delta p_{O_2}} \quad (5)$$

where Δp_{O_2} is the oxygen partial pressure difference across the membrane (bar). $\dot{M}_{O_2}$ is reported in $\text{g m}^{-2} \text{bar}^{-1} \text{h}^{-1}$, providing a pressure-normalized measure of membrane oxygen transfer performance.

3 | Results and Discussion

3.1 | Material Selection

The sterilization tests revealed material-dependent differences in the suitability of the evaluated methods (Figure 1). Neither ethanol nor isopropanol treatment resulted in effective sterilization for any of the tested filaments (ABS, CPE, PETG, PA, PLA), as microbial contamination was detected in all cases. Dry-heat sterilization at 120°C caused deformation of ABS and CPE, whereas PA, PETG, and PLA remained structurally intact and sterile. At 160°C , ABS, CPE, and PLA showed deformation, while PA and PETG tolerated the treatment without structural changes. At 180°C , PETG again remained stable and sterile, whereas PLA deformed, and ABS and CPE were not tested due to prior deformation. Autoclaving at 121°C and 2 bar successfully sterilized PA, PETG, and PLA without structural alterations. In contrast, ABS and CPE deformed during autoclaving, making this method unsuitable for these materials. Overall, PA demonstrated the highest thermal robustness, tolerating all tested dry-heat and

autoclave conditions, while PETG was compatible with all tested methods except solvent-based sterilization.

The biocompatibility of the selected 3D-printing filaments was evaluated by monitoring the proliferation and viability of C2C12 myoblasts over 4 days. All materials were previously sterilized using the most effective method identified (ABS, CPE: Dry-heat at 120°C ; PA, PETG, PLA: Steam autoclaving) and tested as conditioned medium and in direct contact with the cells. As shown in Figure 1, the NT showed continuous cell proliferation from approximately 3×10^4 cells cm^{-2} after 47.5 h to over 2.5×10^5 cells cm^{-2} after 96 h. Among the materials tested, PLA and PETG showed growth rates comparable to the positive control, with final cell counts of approximately 2.6×10^5 cells cm^{-2} , indicating very good biocompatibility. ABS and CPE also showed cell proliferation, but with slightly lower final cell counts (2×10^5 cells cm^{-2}), possibly indicating minor material toxicity or delayed cell adhesion. PA, on the other hand, had the lowest cell count after 96 h (1.4×10^5 cells cm^{-2}), suggesting reduced biocompatibility compared to the other filaments. The results suggest that PLA and PETG are particularly well suited for the culture of C2C12 cells, while PA appears less suitable for applications involving direct cell contact. Differences in cell proliferation could be due to material properties such as surface chemistry, roughness, or residual chemicals from the printing process. The low cell count on PA could also be due to insufficient cell adhesion or the release of cell-inhibiting substances. Overall, the data confirm the suitability of PLA and PETG for further use in applications involving direct cell contact. However, differences in filament formulation related to manufacturer-specific additives and color pigments may result in compositional variability and thereby affect cell-material interactions.

3.2 | Design Considerations and Preparation of the Aeration Module for the Reference Setup

For the reference setup, the aeration module size was set to an inner diameter of 45 mm with an outer diameter of 62 mm and a total height of 40 mm when stacked together. The membrane holder (supporting structure) depth is 2 mm. All corresponding dimensions are provided in the Supporting Information together with the 3D render of the module (Figures S1–S4). This design resulted in a mechanically stable and reliably printable component (Figure 2B). Initial prototypes were fabricated with ABS. The aeration module was subsequently evaluated for leak-tightness. No leakage was detected between the liquid and gas chambers or at the sealing interfaces. However, under elevated pressure, water permeation through the porous 3D-printed walls was observed. This issue was mitigated by acetone-based surface smoothing of the ABS parts as previously described. The post-processing step removed the roughness of the surface originating from support structures and effectively sealed the printed structures. The iterative development of the aeration units demonstrated that leak-tightness, printability, and handling are closely linked to the underlying design principles. The circular geometry minimized potential dead zones within the liquid flow and promoted homogeneous distribution across the chamber. Direct bonding of the membranes significantly reduced the risk of leakage, which is particularly relevant for sterile biotechnological applications. The internal flow paths were arranged to promote uniform

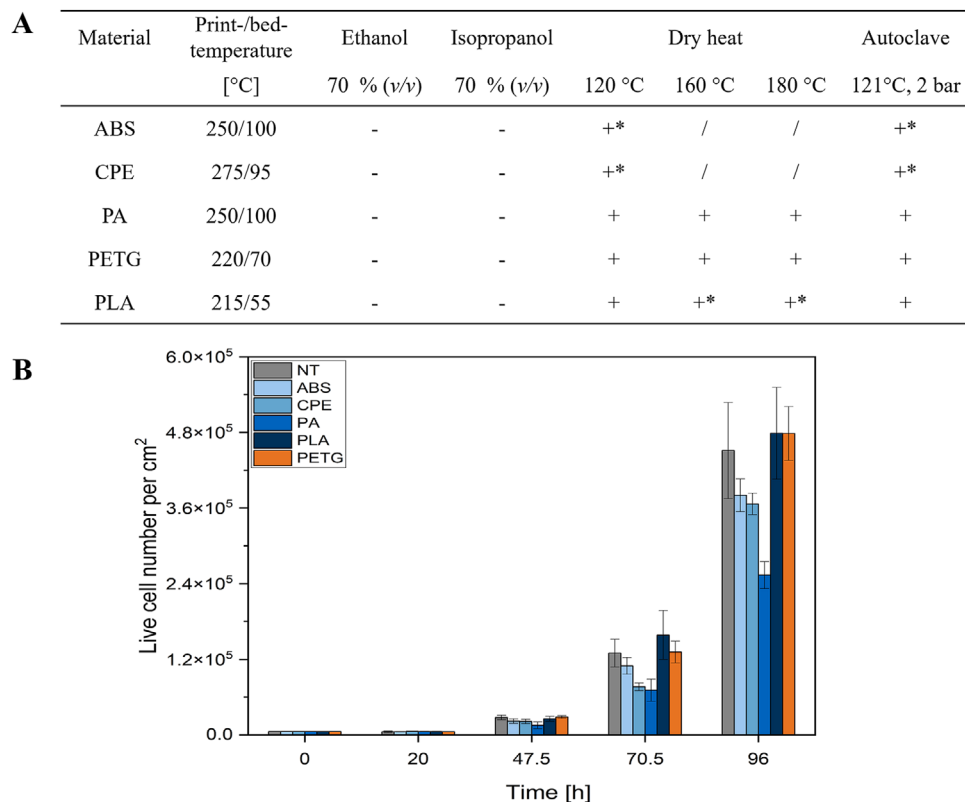


FIGURE 1 | (A) Comparison of different sterilization methods for various 3D-printed materials.—contamination, + sterile, *deformation of material, / not tested because of deformation. (B) Biocompatibility tests of 3D-printed and sterilized materials for C2C12 cells. All experiments were performed in biological triplicate.

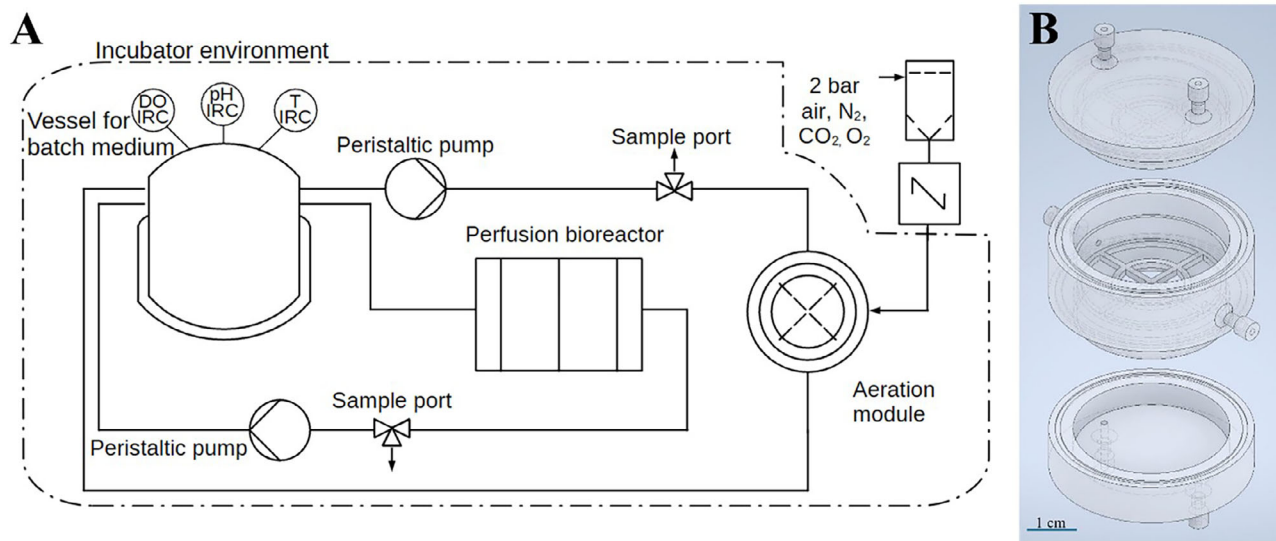


FIGURE 2 | Exemplary process layout and render picture of the aeration system. (A) Flow chart of the fully planned bioreactor setup. (B) Render a picture of the oxygen transfer module.

contact between the medium and the membrane surface, thereby reducing the likelihood of local oxygen gradients. The modular membrane architecture enables straightforward scaling and adaptation to different reactor volumes. The choice of smooth, chemical-resistant, and autoclavable materials supports sterility

and reduces contamination risk; however, several possible filaments could not be used because the aeration units deformed after autoclaving, leading to the loss of sealing integrity. Therefore, and for easier post-printing preparations, ABS was selected as reference material, ensuring sufficient mechanical robustness

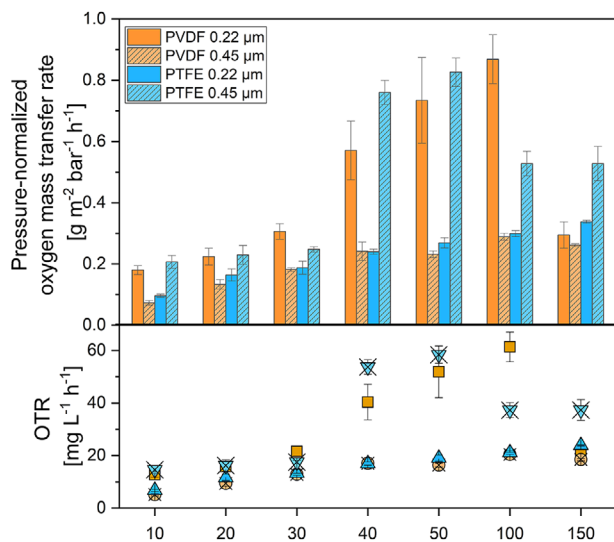


FIGURE 3 | Pressure-normalized oxygen mass transfer rate and OTRs for the different membranes (PTFE and PVDF, each with pore sizes of 0.22 and 0.45 μm) with various media flow rates (10–150 mL min^{-1}).

to withstand pressure differentials and enable reliable operation under process conditions. The overall design also facilitated user-friendly assembly and disassembly. The development of the aeration module was guided by a systematic design approach that integrated functionality, reliability, and practical feasibility. These considerations formed the basis for the subsequent evaluation of oxygen transfer performance.

While the exemplary process layout is shown in Figure 2, photographs of the complete laboratory setup are provided in Figure S5. It shows the full setup, including all relevant components, as well as a magnified view of the module.

3.3 | Oxygen Measurements

The OTR and the normalized oxygen mass transfer rate ($\dot{M}$) were determined to identify suitable operating conditions for the membrane-based aeration module. OTR and $\dot{M}$ were evaluated for different membrane materials and pore sizes, as well as for various medium flow rates, while the gas flow rate was kept constant, as shown in Figure 3.

Across all membranes, the OTR initially increased with flow rate due to a decrease in the diffusion boundary layer and enhanced convection, but plateaued or declined beyond a critical flow because of insufficient contact time. Only PTFE membranes reached the bubble point pressure; therefore, it was a necessity to reduce the gas flow rate to the lowest medium flow rate. A direct quantitative comparison of absolute OTR values between PTFE and PVDF membranes is limited by the calibration procedure of the DO sensor. For each membrane, the sensor signal was calibrated by defining the maximum DO concentration achieved during the calibration as 100% relative DO. As PVDF membranes yielded higher absolute DO concentrations than PTFE membranes, the same relative DO level (e.g., 100%) corresponds to different absolute oxygen concentrations for the two membrane types. Among all configurations, the PVDF membrane with a

pore size of 0.22 μm provided the highest OTR values, reaching approximately 61.4 $\text{mg L}^{-1} \text{h}^{-1}$. This membrane outperformed the other variants at medium flow rates of 50 and 100 mL min^{-1} , whereas an increase to 150 mL min^{-1} caused a marked decrease in OTR, consistent with reduced residence time at the membrane surface. The PVDF membrane with 0.45 μm pores exhibited lower OTR values under all conditions, indicating less favorable structural properties for gas transfer. PTFE membranes showed an inverse pore-size effect. The 0.45 μm PTFE membrane achieved higher OTR values with 58 $\text{mg L}^{-1} \text{h}^{-1}$ compared to the 0.22 μm variant with only 23 $\text{mg L}^{-1} \text{h}^{-1}$, and displayed the maximum at intermediate flow rates of 40–50 mL min^{-1} followed by a decline at 100 mL min^{-1} . Therefore, the difference between the pore sizes of the PTFE membrane shows roughly a difference of 152% at the same flow rate of 50 mL min^{-1} . Analysis of the normalized oxygen mass transfer rate $\dot{M}$ confirmed the trends observed for OTR. The highest transfer efficiency was obtained for the PVDF membrane with 0.22 μm pores at a medium flow rate of 100 mL min^{-1} , with $\dot{M}$ reaching 0.87 $\text{g m}^{-2} \text{bar}^{-1} \text{h}^{-1}$. At high flow rates, PVDF showed a more pronounced decrease in $\dot{M}$ than PTFE, suggesting membrane-specific differences in boundary layer behavior and diffusion mechanism.

Overall, the most suitable configuration in the reference setup identified in this study was the PVDF membrane with 0.22 μm pore size operated at a medium flow rate of 50 mL min^{-1} . Although the mass transfer for 100 mL min^{-1} was higher, when the doubled flow rate was taken into account, the increase was only 18.3%. The setting of 50 mL min^{-1} combines high oxygen transfer performance with stable operation and straightforward handling. Compared with literature, the OTR values obtained here appear moderate, which can be partly explained by the downstream positioning of the DO sensor and the non-optimized flow characteristics of the present module. Optimizing the inner flow structures presents an opportunity for further improvement of contact time. The area-normalized mass transfer coefficient, therefore, provides a more appropriate basis for comparison, but it also shows deviation from reported reference values.

4 | Concluding Remarks

In this work, a modular, membrane-based aeration module was designed using additive manufacturing and systematically characterized with respect to its oxygen transfer performance. The external, membrane-stacking design enables bubble-free oxygen transfer while decoupling aeration from mixing, thereby minimizing hydrodynamic stress. Experimental results demonstrate that oxygen transfer efficiency depends strongly on membrane material, pore size, and liquid flow regime, and confirm effective and controllable oxygenation under laboratory-relevant conditions suitable for shear-sensitive systems. Beyond its immediate performance, the primary contribution of this work lies in providing a fully characterized 3D-printable aeration solution for bioprocess prototyping. The modular design and availability of editable CAD files enable rapid adaptation to different reactor configurations and experimental requirements. As such, the aeration module is well-suited for use in small-scale perfusion bioreactor systems and laboratory scale-up evaluations, where a fixed, validated, and reproducible oxygen transfer setup is essential. This approach supports systematic process development in

tissue engineering and cultured meat research and facilitates the transfer of oxygen supply strategies from laboratory models towards larger-scale bioprocesses.

Author Contributions

Laurenz Köhne: writing – original draft, methodology, investigation, formal analysis, data curation, conceptualization. **Pia Lorenz:** writing – original draft, methodology, investigation, formal analysis, data curation. **Marie-Luise Schlieker:** writing – original draft, methodology, investigation, formal analysis, data curation. **Chantal Treinen:** writing – review and editing, formal analysis, conceptualization, supervision. **Marius Henkel:** writing – original draft, writing – review and editing, methodology, formal analysis, conceptualization, supervision, project administration, funding acquisition.

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Conflicts of Interest

The authors have declared no conflicts of interest.

Data Availability Statement

The data that support the findings of this study (characterization of oxygen transfer) are available from the corresponding author upon reasonable request. Additional information on the FDM manufacturing protocol, renders of the aeration module divided into three components, as well as the 3D-model (.stl file), are provided as supporting information.

Use of Generative AI and AI-Assisted Technologies in the Writing Process

The authors used ChatGPT (OpenAI), DeepL Translator (DeepL SE, Köln, DE) and Grammarly AI writing assistant (Grammarly, Inc., San Francisco, USA) to assist with language editing and stylistic improvements. The authors reviewed and edited all content and take full responsibility for the final manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File: elsc70073-sup-0001-SuppMat.docx.