On-orbit strategies to improve scale of layer-by-layer manufacturing of protein-based artificial retinas

Hope A. Sylva,[1] Krishna K. Dixit,[1] Daniel B. Sylva,[1] Jordan A. Greco,[1,2] Robert R. Birge,[1,2] and Nicole L. Wagner[1,2]

[1]*LambdaVision Inc., 400 Farmington Avenue, Farmington, CT 06032, United States.*

[2]*Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, CT 06269, United States.*

Introduction

LambdaVision has developed a unique protein-based artificial retina technology to treat patients that are blinded by retinal degenerative diseases[1-2]. Our commercialization strategy is to target the orphan indication of retinitis pigmentosa (RP) first (1.5M patients worldwide) and then to follow on to the larger market of age-related macular degeneration (AMD, 30M patients worldwide). Importantly, for the work done onboard the International Space Station, targeting RP first allows for a reasonably low demand for pilot-scale good manufacturing practice (GMP) production of the implants, and a small Phase I clinical trial.

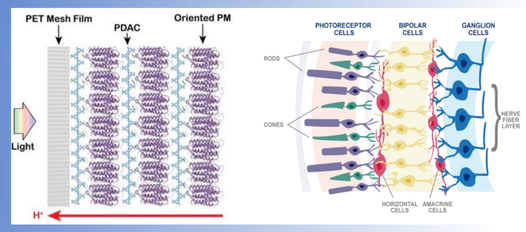


Figure 1. LambdaVision's biomimetic artificial retina serves as a replacement for photoreceptor cells in damaged retinas.

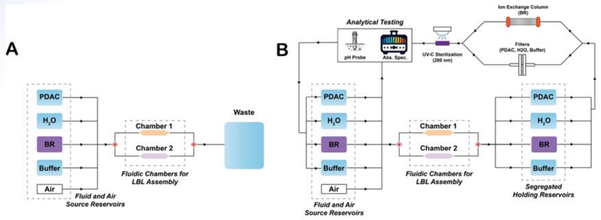
Emerging commercial markets of interest include in-space manufacturing, bioengineering, regenerative medicine, and advanced materials production. LambdaVision's protein-based artificial retina is one example of a promising drug product that may benefit from production in low Earth orbit. Through efforts by LambdaVision and its implementation partner, Space Tango, recent research flights to the ISS have demonstrated the potential of producing LambdaVision's protein-based artificial retinas in a microgravity environment. We have now turned our attention to methods to minimize fluid waste during the manufacturing process, an issue that is problematic for production of pharmaceuticals and implants on the International Space Station (ISS).

Figure 2. LambdaVision has flown 9 payloads to the ISS, including manufacturing payloads on SpaceX Crew-4, Crew-5, and CRS-26.

Figure 3. (A) LambdaVision and Space Tango developed a miniaturized and automated layer-by-layer manufacturing device for artificial retina production in LEO. **(B)** The sealed device is housed in a Space Tango CubeLab™, which contains bags for manufacturing solutions along with fluid handling hardware, sensors, and cameras.

We are developing a closed-loop method of recycling the solutions used to produce artificial retinas that involves filtering of waste solutions, analysis of solutions *in situ*, and sterilization of fluids. This will significantly reduce the cost of sending materials to orbit. These methods can be extended to other pharmaceuticals as well.

Concept of Operation



LBL Manufacturing Approach A (No Recycling) | **LBL Manufacturing Approach B (Bulk Recycling)**

Figure 4. (A) Fluidic diagrams of the current LBL manufacturing device, as well as a **(B)** conceptual approach for implementing solution recycling during production. Approach B will be the scheme used to integrate the recycling modules in our Phase II SBIR work.

Methodology

1. **Trace Protein Quantification**
   Well-established absorption spectroscopy methods for the precise quantification and detection of BR within filtered solutions were improved. A spectrophotometer measured absorbance at two distinct wavelengths, 280 nm and 568 nm. The BR concentration was estimated from the A_{568} measurement using the Beer-Lambert equation, and the purity ratio was determined using A_{280}/A_{568}.

2. **Trace Polymer Detection & Quantification**
   A new assay was needed to detect the polymer, Poly(diallyldimethylammonium chloride) (PDAC), used in implant manufacturing, as it is transparent across the UV and visible spectrum. We adapted the methods from Levakov et al.[3] to detect and quantify trace quantities of the polymer in water using Fast Green FCF dye. The dye exhibits a concentration-dependent decrease in absorbance at three different wavelengths (204 nm, 420 nm, and 624 nm) when it interacts with PDAC.

3. **Polymer Separation from Protein Solutions**
   A standard sterile hydrophilic polyethersulfone (PES) filter was used to filter BR from solution. Centrifugal concentration and spectroscopy was utilized to determine the presence of residual BR in the filtered solution.

   Similarly, we tested filters for their ability to retain or elute PDAC. Two filters were tested: a hydrophilic PES filter and a hydrophobic polytetrafluoroethylene (PTFE) filter.

4. **UV-C sterilization of fluids**
   LambdaVision has performed proof-of-concept experiments with a UV-C sterilization device. This flow-through system is specifically designed for materials with relatively low UV transmittance (e.g., BR) that require high UV-C doses for bioburden reduction. A light bank of 280 nm LEDs is used to treat a sample that flows through a chamber, and the flow rate can be adjusted to optimize the dose.

5. **Design of a miniaturized colorimeter to monitor protein solution concentration**
   An absorption spectrophotometer was implemented to analyze protein concentration and purity by measuring absorbance at specific wavelengths (280 nm and 568 nm), utilizing a modified Open Colorimeter. The calibration curve for BR was generated, showing an extinction coefficient close to expected value. A UV colorimeter is being developed for further testing.

Results

Repeated measurements were used to determine the limit-of-detection (LOD) and limit of quantitation (LOQ) of the trace BR quantification assay. The LOD was determined to be 0.0023 mg/mL and the LOQ was calculated to be 0.0069 mg/mL. We found that centrifugation and resuspension in a minimal volume raises the concentration above the LOQ for all tested concentrations. Reliable quantitation of BR down to 0.00069 mg/mL with the 10x concentration factor is sufficient for LambdaVision's current needs.

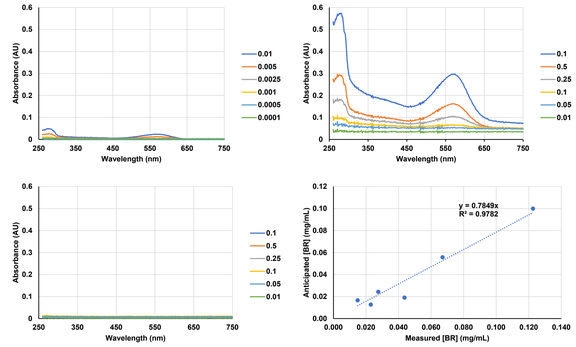


Figure 5. The concentration of BR can be reliably determined down to minute quantities. Trace BR quantification was used to demonstrate the effectiveness of filtration to remove BR from a sample.

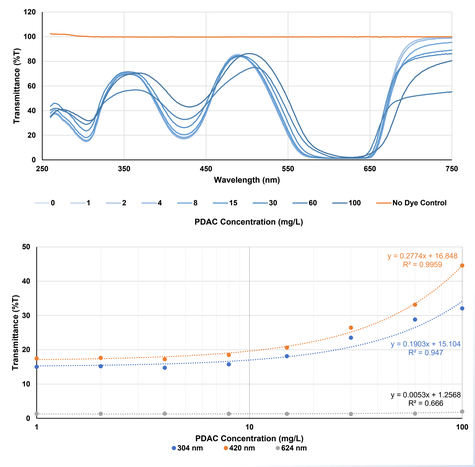


Figure 6. PDAC concentrations can be determined by measuring shifts in the transmittance spectrum of Fast Green FCF dye.

A hydrophilic PES filter removes some, but not all of the polymer from a solution. The PDAC molecules are smaller than the pore size of the filter, so retention is likely driven by interactions between the charged polycation with the hydrophilic surface of the membrane. Multi-pass filtering may be required for more complete removal of the polymer from a solution. Smaller pore size filters may also result in greater PDAC retention. The hydrophobic filter removes very little polymer from solutions and may be suitable for sterile filtering or removal of BR from the PDAC solutions.

Results

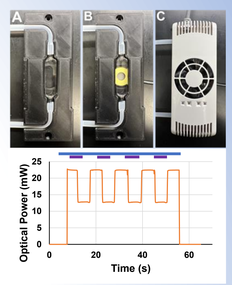


Figure 7. (A) UV-C solution irradiation using a flow-through quartz glass cuvette. **(B)** Power sensor positioned under the cuvette. **(C)** Lamp positioned above the cuvette. **(D)** Optical power output vs. time as the UV-lamp is turned on and BR is repeatedly injected into the irradiation chamber. Note: Blue bar above plot indicates when lamp was powered on. Purple bars above plot indicate when chamber was filled with BR.

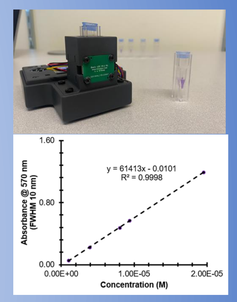


Figure 8. (Top) The IO Rodeo Open Colorimeter with a 570 nm bandpass filter and sample cuvettes of BR. **(Bottom)** The absorbance at 570 nm was plotted against concentration to establish a calibration curve with excellent linearity. The linear regression equation was used to estimate the molar extinction coefficient, resulting in a value of 61,413 $M^{-1}cm^{-1}$, which is only 3% different from the accepted value of 63,000 $M^{-1}cm^{-1}$.

Conclusion

- A method for centrifugal concentration was developed to detect trace quantities of BR in solution.
- A dye-based assay was developed to quantify the concentration of PDAC in solution.
- BR can be reliably filtered, and initial progress towards filtering PDAC has been made.
- A fluidic system to sterilize solutions with controlled UV-C dosages has been developed. The UV-C device can monitor the absorbed UV-C dose as well as protein degradation in real time.
- A miniature colorimeter was effectively used to estimate protein concentration with excellent linearity.
- Future work will consolidate these subsystems to develop a closed-loop system to fabricate artificial retinas with minimal waste for economic production in LEO.

Acknowledgements

References

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3. Levakov, I.; Maor, I.; Barak, C.; Kirshenbaum, Y.; Rytwo, G., Colorimetric Quantification for Residual Poly-Dadmac in Water Treatment. *Water* 2023, *15*, 3352.

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