

Optimizing High-Density Human iPSC Expansion

and Process Efficiency Using Next-Gen Bio-Processor

Introduction

Stämm Bio has developed a novel bubble-free bioreactor that has demonstrated significant advantages in space-time yields in highdensity mammalian cell culture applications in suspension (CHO, JURKAT) and adherent (HEK, MSC, iPSC) cultures.

As cell therapies targeting adherent cell types advance through clinical trials, the transition from traditional and labor-intensive manual culture systems (e.g., T-flasks, Cell Factories) to robust, scalable bioprocess platforms becomes critical (1, 2).

Conventional culture systems face severe scalability constraints, high footprint requirements, manual labor intensity, and increased contamination risks (3). Stämm has eliminated most of them through the unique design and automation of it’s BFBs (bubble-free bioreactors).

This application note demonstrates the operational advantages and performance capabilities of the BFB (bubble-free bioreactor) model Aries with a 6 mL volume run in a commercial incubator with the HEK293T cell line as a model adherent cell of human kidney lineage.

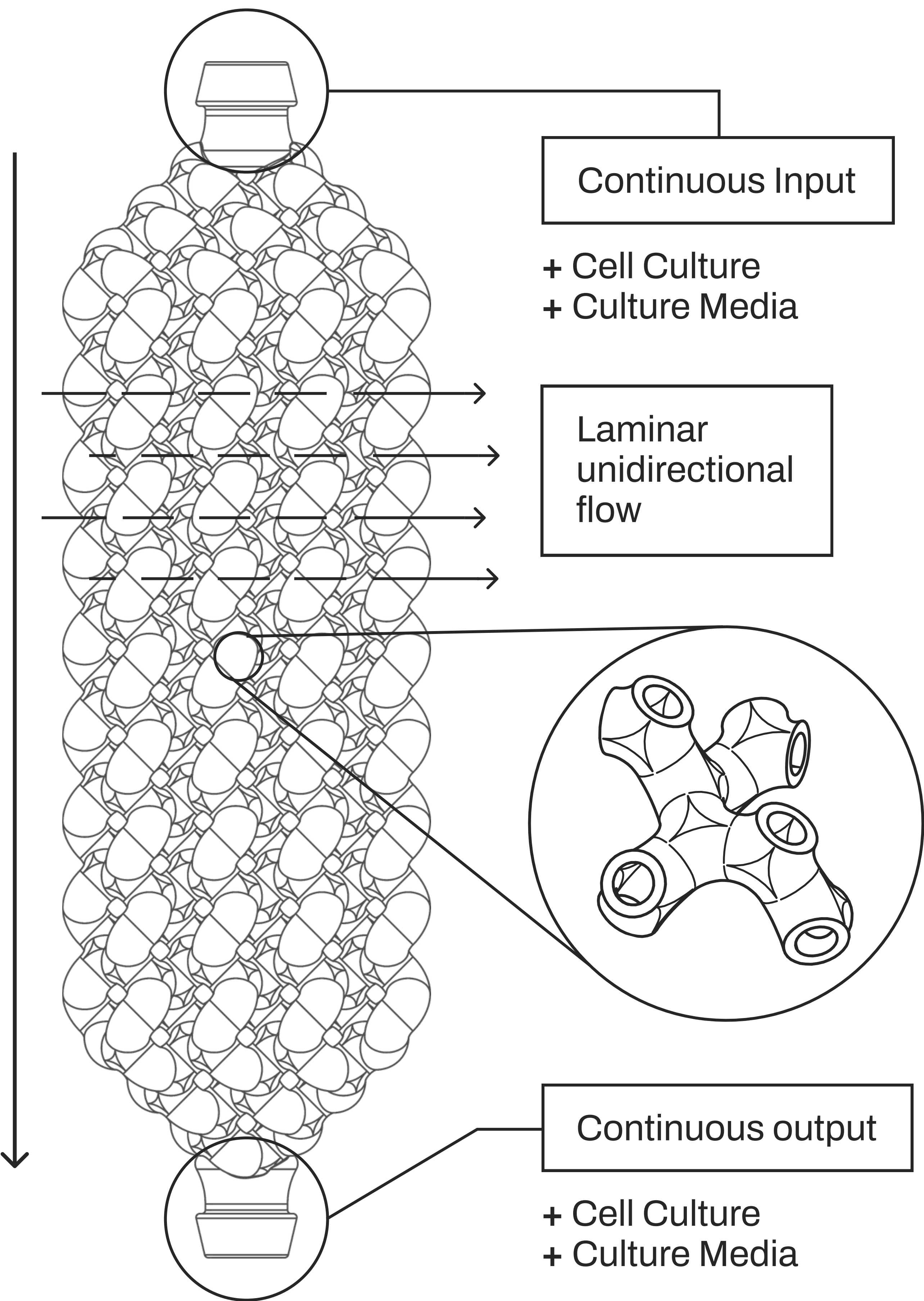


Image 1. Bubble-free Bioreactor model Aries 6 mL.

Material and Methods

HEK293T cells (ATCC) were cultured and maintained in DMEM (Thermo Fisher Scientific) media throughout the cell culture process. Initial cultures after thawing from stocks were done on T25 flasks with the same media, followed by seeding in the BFB. The details of the materials used are provided in Table 1.

Table 1. Materials used for adherent HEK293T cell culture

Componentes	Descriptions
Cell Line	HEK293T
Media	DMEM
Wash Solution	PBS1X
Dissociation Reagent	Trypsin-EDTA(Gibco®)
Quenching agent	Media + 10% FBS
Hardware	Incubator setup; Volume -6 mL; Available surface area 120 cm ²

Cells were cultured in controlled environment at 37°C with 5% CO2 in an incubator. Automated sampling was performed at 24 hrs interval to assess media metabolites, pH, and dissolved oxygen (DO2) using the FLEX2™ (Nova Biomedical).

On day 6, cells were harvested using trypsin via semi-automated fluid dispensing, cell washing, and collection.

Automated cell extraction from the BFB does not require TFF. Subsequent assessment of cell viability and concentration was performed with Trypan Blue staining.

Results and Discussions

Stämm's BFB supported robust cell expansion, maintaining growth and reaching a density of 1.01×10^6 cells/cm² while keeping 98% of cells viable throughout the process. After 6 days of culture, the BFB generated 9×10^5 cells/cm², similar to the control set (Figure 1).

However, the BFB consumed 0.8 mL media/cm² as compared to 1.9 mL media consumed by the control (Figure 2). Thus, the same expansion of HEK was obtained in the BFB with only 40% of media usage.

Another valuable attribute of Stämm's BFB is the uniform distribution of nutrients and gases, along with uniform growth of cells throughout the bioreactor. Figure 3 depicts the distribution of HEK cells after 3 days of expansion. Five out of six zones show almost similar quantities of HEK cells.

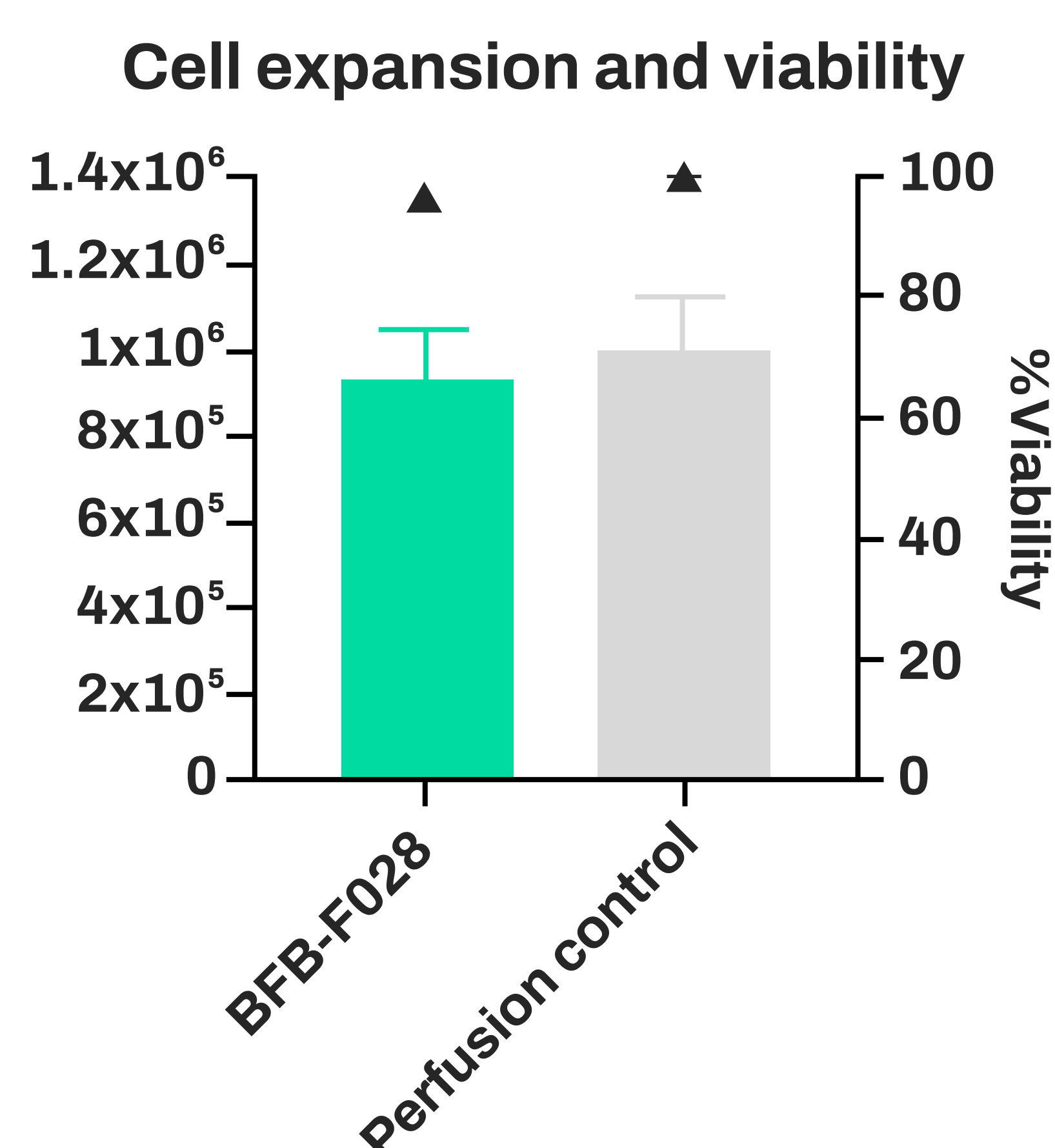


Figure 1. HEK cell density and viability after 6 days. Cells in Stämm's BFB reached a mean cell density of 0.9×10^5 cells/cm², whereas the control reached 0.92×10^5 cells/cm². The media perfusion rate of the BFB was 3 vessel volumes per day (VVD). In the control condition, cells were cultured in a P6 multiwell plate with daily media changes. Triangles over the graph indicate cell viability for the respective culture conditions. Bar heights represent the mean, with standard deviation (SD) as error bars. n=3.

Conclusions

Addressing bottlenecks in scalable, automated, and functionally closed manufacturing systems for adherent cells, Stämm's BFB provides an effective solution with excellent cell viability and functional cellular health.

Within 6 days, similar to the reference, cell expansion can be achieved. Besides, the high surface-to-volume ratio (20 cm²/mL) of the cell culture cartridges saves 60% of media when compared to traditional expansion methods.

References

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Culture media consumption per surface area

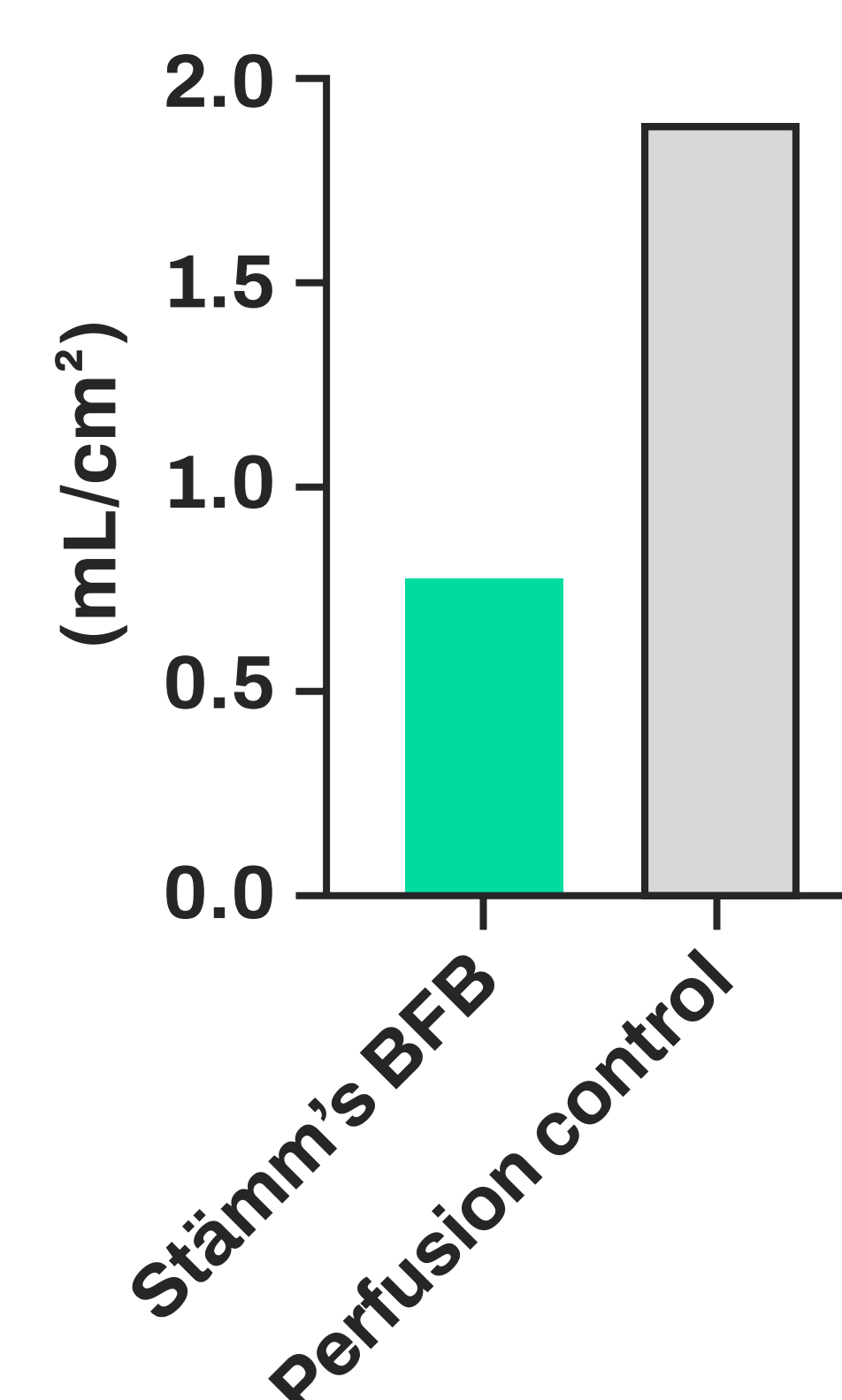


Figure 2. Culture media consumption per available surface. Bar graphs depict total media usage during 6 days of perfusion culture, normalized to the surface area available for cell growth. The graph compares Stämm's BFB against a control set using a 6-well plate.

Figure 3: Distribution of cells across the device

Figure 3A

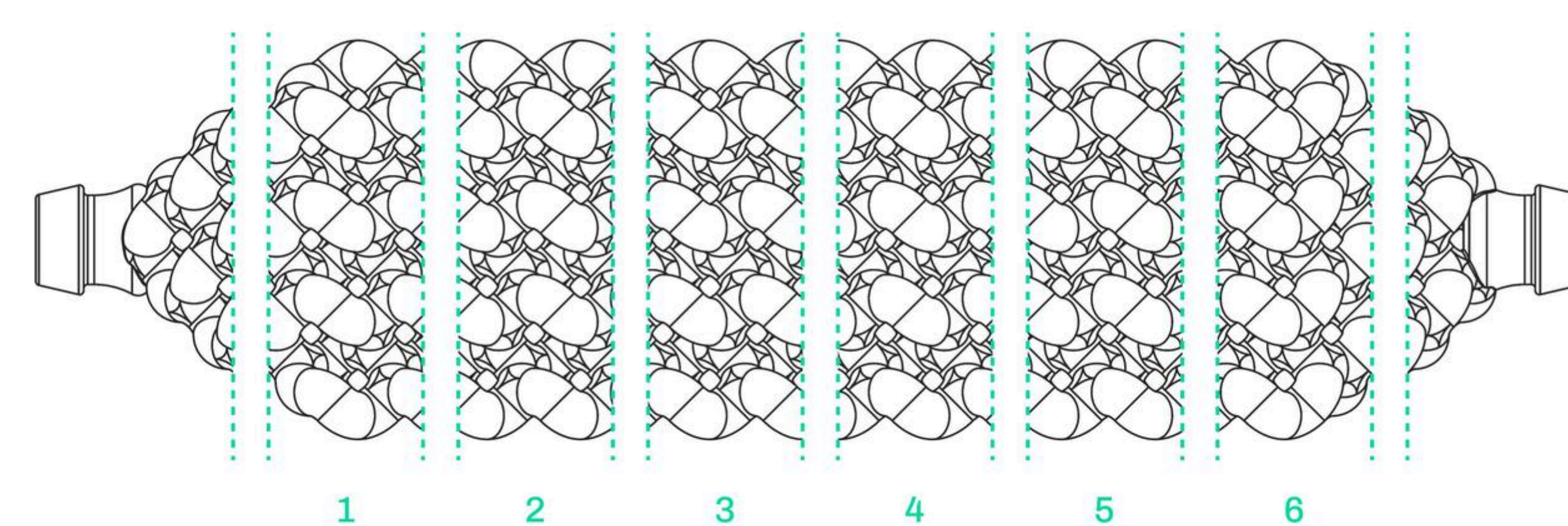


Figure 3B

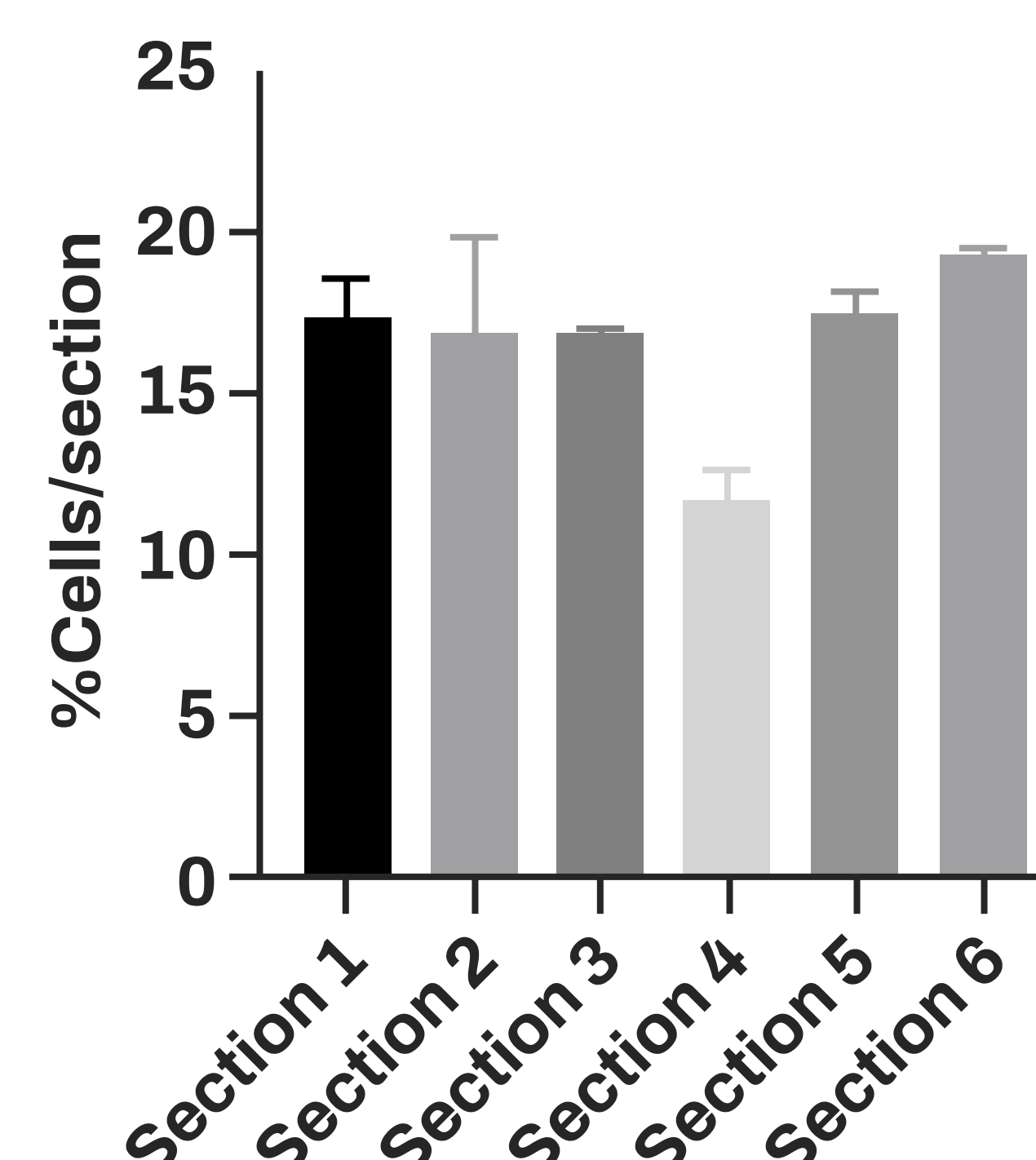


Figure 3. Cell distribution through the BFB device. **(3A)** The top schematic depicts the physical device divided into different sections. The BFB was cut into 6 parts after 3 days of culture. Cells in each section were stained with Calcein Red and counted from images obtained with a fluorescence microscope. **(3B)** Bar graphs represent the mean percentage of cells counted in each section, with standard deviation (SD) plotted as error bars.

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