ADVANCING THE ROBUST MANUFACTURE OF T-CELL THERAPIES THROUGH THE APPLICATION OF STIRRED TANK BIOREACTORS

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Introduction

With the rise of T-Cell immunotherapies cell therapy developers have encountered the difficulty of producing consistent, quality products. Approved therapies Yescarta™ and Yespro™ as late stage clinical products BB2121 and UCART19 have been critiqued for potential obstacles to patient access due to high sticker prices and variable manufacturing outcomes. Manufacturing processes need to be developed to allow for increased control over each unit step to improve the uniformity of the cell journey over the life of the product. Bioreactor culture systems provide a pathway for a higher level of process control over cell expansion.

With the high cost of developing and manufacturing cell therapies, manufacturing techniques that allow for rapid translation to the clinic and reduce operating costs need to be investigated. Stirred-tank bioreactors have been heavily adopted by the biologics industry and have well defined characteristics that facilitate the scale-up from small scale clinical work to full scale clinical and commercial manufacture. Establishing modelling of the fluid dynamics present within these bioreactors may also reduce the reagent costs of cell culture with more efficient mass transfer.

Figure 1. Growth curves from two donors are compared independently to their control cultures (Ns). The largest sample to sample increase in growth occurs between day five and seven. Bioreactor cultures performed markedly better in two of the three donors, but more development is necessary to improve consistency across various donor types.

Figure 2. The change in key metabolite concentrations over the course of the thirteen day culture (Ns). The results of the small scale study showed that the shear stress of a stirred tank bioreactor does not inhibit cell proliferation and may provide additional efficiencies in mass transfer. The experimental conditions outperformed the static controls and were able to achieve cell densities comparable to continuously perfused systems using a rudimentary mock batch-fed process. The results indicate that high concentrations of lactate prevented further expansion of the cultures.

Figure 3. Activated T-Cells expanded in DiaSorin Bioreone bioreactors showed the capacity for significant expansion across four experiments and two donors. In all cases the cultures yielded greater than 1 x 10^10 cells prior to eight days of total culture time. The four experiments show consistent growth profiles within donors which indicates that the Bioreone is able to create a culture environment that is highly reproducible lot to lot.

Figure 4. Comparing a single donor across the BIOne stirred tank system and the rocking bioreactor system indicates that the BIOne enables increased cell expansion toward the later stage of the cell culture. The average increase in expansion was 1.22 population doublings. This difference in expansion shown in this experiment could correspond to a 24-48 hour reduction in total culture length depending on thelargest yield. Culture is typically the limiting factor in production speed and reducing the length of time required to achieve a therapeutic yield will result in faster product release.

Figure 5. In addition to higher total yields, the BIOne was shown to expand cells more efficiently than the rocking bioreactor. 2.99 x 10^9 cells were produced per milliliter of media consumed in the stirred-tank bioreactor which is a 58% increase over the 1.88 x 10^9 cells produced per milliliter in the rocking bioreactor. Accounting for the high cost of media components in standard T-Cell media, more efficient cell expansion would help reduce the cost of the resulting therapy.

Conclusion

T-Cell immunotherapies have proven efficacy as viable medications for previously untreatable diseases. However, manufacturing options for these transformative therapies threaten patient access due to inconsistent manufacturing, long processing times, and high cost. Stirred-tank bioreactors present an option for the expansion of primary human T-Cells that has previously not been pursued due to concerns about shear stress. Small scale studies have shown that improved mixing in stirred-tank bioreactors does not increase cell death or limit expansion potential. Translating the learnings from those small scale studies to the autologous manufacturing scale has shown that the BIOne stirred-tank bioreactor produces a consistent culture environment and high cell yields. The BIOne significantly outperformed rocking bioreactor systems in both total yield and production efficiency. These improvements could impact patient access by reducing the manufacturing timeline and reducing the cost of both reagents and labor. The high yield of the BIOne was only possible by integrating it with the lovo spinning membrane system to allow for media exchange; nutrient and metabolite analyses throughout the culture reveals that increasing the exchange rates would further prevent lactate accumulation and potentially further increase yields.

References


Autologous Scale Expansion of Primary T-Cells

Figure 6. Assuming the system functions as a true continuous stirred tank reactor, spectrally removed is modeled as an exponential decay function to determine the true media exchange rate at a set perfusion rate.

Figure 7. Nutrient and metabolite analysis shows that media exchange rates were not sufficient to prevent lactate accumulation and cell death late in the culture. Lactate concentrations and LDH, a measure of cell lysis, have a linear coefficient of determination of 0.72.