Rotary Bioreactor for Recombinant Protein Production

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Abstract: The Rotary Cell Culture System (RCCS) developed at NASA has been used primarily for tissue engineering applications. Because it creates a low shear, high mass transport environment simulating microgravity, we tested the ability of the RCCS to enhance the production of recombinant proteins compared to a stirred bioreactor. A human cell line was transfected with LacZ or glycodelin and cultured in the RCCS and a spinner flask. The RCCS culture produced a 7-fold greater yield of beta galactosidase and 3-fold greater yield of glycodelin than the spinner flask suggesting that the low shear conditions in the RCCS promoted increased protein production.

Key words: cell culture, microgravity, recombinant protein, post-translational modify-cation,

1. INTRODUCTION

The original purpose of the Rotary Cell Culture System (RCCS) was to carry cell into space to study the effects of microgravity. The design incorporates a horizontally rotating, cylindrical culture vessel with a coaxial tubular oxygenator (Figure 1). The cells experience a microgravity-like environment with extremely low fluid shear stress (1). In other culture systems, cells are suspended by agitation or sparging which creates high shear and results in cellular damage. We hypothesized that transfected cells would produce higher levels of recombinant protein in the low shear conditions of the RCCS. To test this hypothesis, we transfected LacZ and glycodelin, a human glycoprotein gene, into a human cell line and compared protein production in the RCCS and a spinner flask.

R. Smith (ed.), Cell Technology for Cell Products, 567–569.

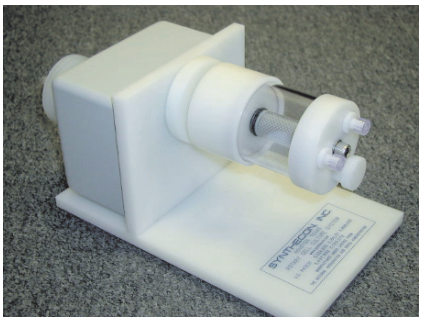

Figure 1. The Rotary Cell Culture System.

2. METHODS

cDNA's for LacZ and glycodelin were cloned into an expression vector, pcDNA/Myc-HIS (Invitrogen) and transfected into K562 cells. After selection with blasticidin, clones were screened for protein production. Transfected cells from a single clone were grown in flasks and then transferred to a spinner flask and an RCCS and cultured in RPMI 1640 media with 10% FCS. The media was changed every 48 hours for 7 days. At the end of the culture cells were harvested and analyzed for beta galactosidase and the media was analyzed for glycodelin. The biological activity of glycodelin was measured by the ability to inhibit IL-2 secretion from activated Jurkat cells.

3. RESULTS

Beta-galactosidase extracted from transfected K562 cells cultured in the RCCS was increased 7-fold compared to the spinner flask (Figure 2).

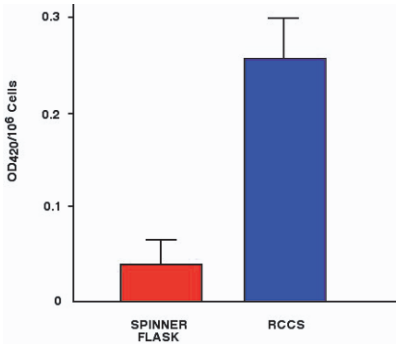

Figure 2. Comparison of beta-galactosidase production in a Rotary bioreactor (RCCS) and a spinner flask. K562 cells transfected with LacZ were initially seeded in the RCCS and a spinner flask. Media was changed at 48 hour intervals. After 7 days the cells were harvested and assayed for beta-galactosidase activity. N=3 Glycodelin secreted into the media was increased 4.2-fold in the RCCS compared to the spinner flask (Figure 3).

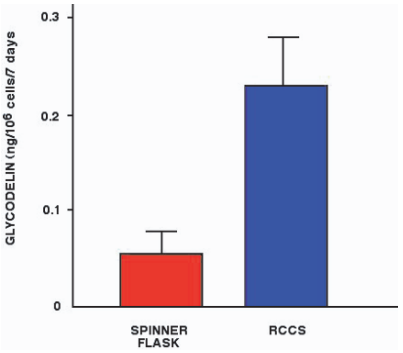

Figure 3. [Production of recombinant glycodelin in K562 cells cultured in a spinner flask and a Rotary Cell Culture System (RCCS). K562 cells transfected with glycodelin cDNA were seeded in an RCCS and a spinner flask. Media was changed at 48 hour intervals. After 7 days, the media was harvested and glycodelin isolated and quantitated. N=3].

4. CONCLUSIONS

The low shear stress environment of the RCCS facilitates higher levels of recombinant protein production compared to a spinner flask. The biological activity of the recombinant protein appears to be unaffected by the type of bioreactor in which it is produced.

Since the RCCS is capable of culturing virtually any cell type at high density, the choice of cell lines for recombinant protein production is greatly expanded. In cases where a recombinant protein might require species-specific or even cell-specific post-translational modifications, the RCCS offers the opportunity to use a cell line which may not be adaptable to high shear stress bioreactors.

REFERENCES

Hammond TG, Hammond JM. Optimized suspension culture: the rotating-wall vessel. Am. J. Physiol. Renal Physiol. 281: F12-F25, 2001