# 3X AAV PRODUCTION YIELD USING TFDF® FOR CELL GROWTH INTENSIFICATION AND CLARIFICATION

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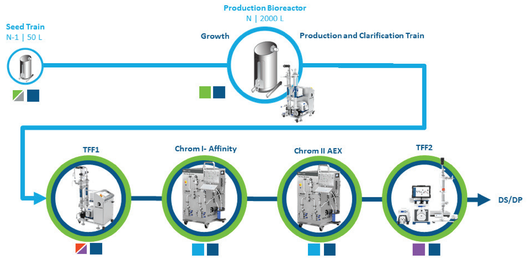
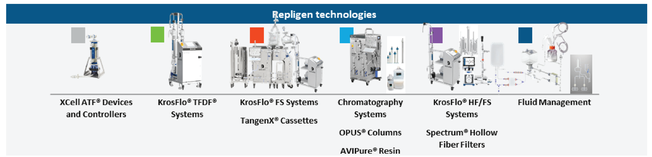
## Introduction

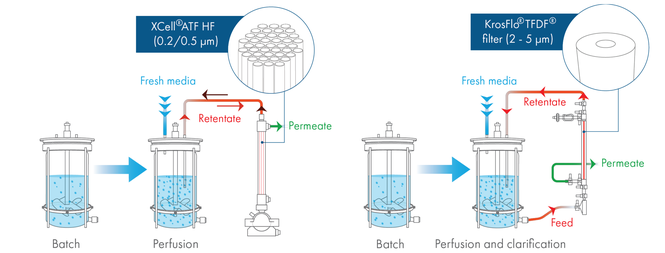
Gene therapy demand generated by high dosage and large patient populations require significance improvements to viral vector manufacturing. Two integrated perfusion platforms, XCell ATF® (alternating tangential flow) and the KrosFlo® TFDF® (tangential flow depth filtration) were evaluated for growth, production and clarification recovery. KrosFlo® TFDF® increased total yield of adeno-associated virus serotype 8 (AAV8) by 3 fold compared to a batch bioreactor process; lysate recovery yield was comparable to depth filtration. This case study illustrates the benefits of intensified and integrated processes for high-productivity and cost-effective viral vector manufacturing to meet the increasing global demand.

## AAV Viral Vector Manufacturing Workflow




## Evaluating perfusion platforms to intensify vector production

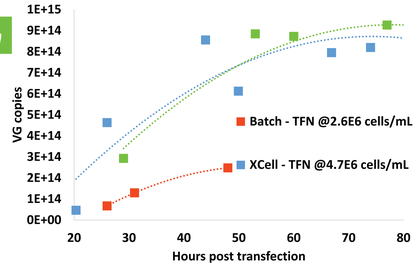


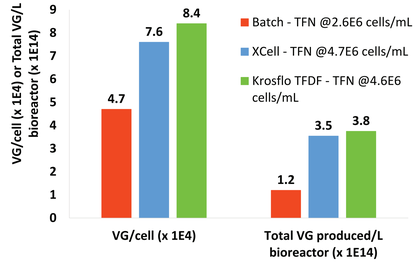

- Increase cell density
- Increase viral vector production
- Continuously harvest
- Integrated clarification simplifies

## Viral vector intensification helps meet manufacturing demand

| Process step | Batch process | Intensified process (XCell ATF® or KrosFlo® TFDF®) |
|---|---|---|
| Virus production | • Transfection at ~2E6 cells/mL (~48h after inoculation) | • Transfection at ~5E6 cells/mL (perfusion started 48 hours after inoculation at 1 vvd) |
| Lysate clarification | 2-stage filtration: DF-based process<br>• Glass Fiber filter (5 um nominal)<br>• 0.8/0.2 um (nominal) membrane filter | TFDF®-based perfusion process<br>• Integrated first stage clarification with TFDF® (2 - 5 um nominal)<br>• Multi-layer polypropylene depth filter (< 0.5 um nominal) |
| Inoculation | Seeding of HEK293T cells in BalanCD HEK293 media at ~6E5 cells/mL in glass vessel bioreactor (2 L working volume) | |

***AAV8 production***



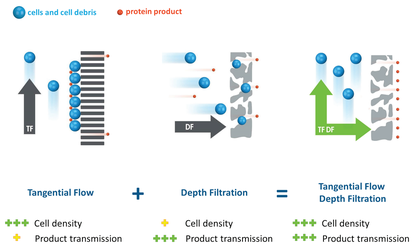

***Lysate clarification***

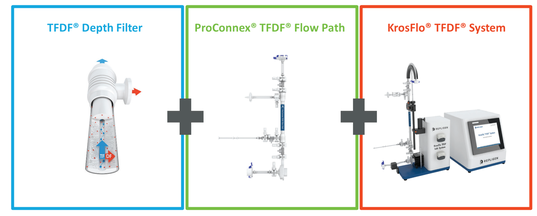
***> 3X virus production with XCell® and KrosFlo® TFDF® Technologies***

| Run | Filter | Load (L/m² @ 0.7bar) | Surface area (m²) @ 200 L scale | FLUX ( LMH) | Clarification step recovery yield (%) |
|---|---|---|---|---|---|
| Batch | Glass fiber (5 um) | 23 | 9 | 100 | 70 |
| | 0.8/0.2 um membrane | 30 | 7 | 100 | |
| KrosFlo® TFDF® (Perfusion and clarification) | Integrated TFDF® (2 - 5 um) | 300 | 0.6 | 450 | 70 |
| | Multi-layer polypropylene (< 0.5 um) | 53 | 4 | 100 | |

***Simplified and smaller footprint clarification process with integrated KrosFlo® TFDF® filter***

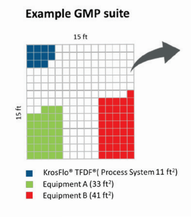
## Processes high cell densities with high product transmission

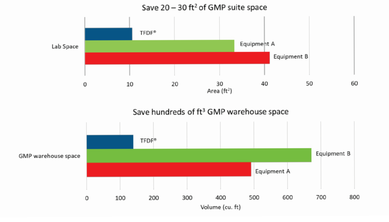



## TFDF® reduces GMP and storage space footprint by 3X fold

- >2X reduced primary depth footprint at 2000 L increases your facility capacity
- Overall cost saving associated with lower DF footprint in the GMP manufacturing and storage areas




***AAV8 cost of manufacturing***

- The cost assessment of manufacturing per year taking only into account the cost of major raw materials and consumables used in the process (cell culture media, plasmids, nuclease, filters, chromatography media)
- We see that for Hemophilia, the implementation of perfusion processes reduce the number of 2000 L from 74 to 25 batches, the cost savings per year can be almost $24M

| Indication and dosage | Patients /year | vg*/year | Cell culture mode | Cell cult. vol. /year (KL) | 2 KL batches/ year | # of 2KL bioreactors @ 25 batches/year | Consumable cost*/year (M$) | Cost* savings/ year (M$) |
|---|---|---|---|---|---|---|---|---|
| Hemophilia A (3 x $10^{13}$ vg/kg40kg/patient) | 5000 | 6 x $10^{18}$ | Batch | 148 | 74 | 3 | 72 | - |
| Duchenne muscular dystrophy (2 x 1014 vg/kg 40kg/patient) | 5000 | 8 x $10^{19}$ | Perfusion | 50 | 25 | 1 | 48 | 24 |
| | | | Batch | 980 | 490 | 20 | 482 | - |
| | | | Perfusion | 330 | 166 | 9 | 320 | 162 |

## Conclusion

- Krosflo® TFDF® provides one solution for upstream viral vector intensification and clarification
- High-productivity and cost-effective manufacturing processes help meet the global demand for viral vectors
- > 3-fold increased AAV8 production yield
- Improved process economics
- Simplified process enhances cell specific productivity and growth leading to higher vector yield



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