Setting up transfection-based recombinant AAV production in the iCELLis™ single use fixed-bed bioreactor

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Introduction

In July 2013 the Journal of Gene Medicine counted a total of 1902 gene therapy clinical trials: 5.1% of these are using AAV as vectors to deliver the therapeutic gene. To meet the future demands of this growing market, we are currently establishing a technology platform for industrial scale production of rAAV.

Biological starting materials are a key feature when establishing a **technology platform** to manufacture high-quality vectors based on rAAV. When using a transfection-based approach, the plasmids have a strong influence on certain characteristics of the vectors. Adverse events like (non-)homologous recombination can lead to potentially harmful by-products, such as replication-competent AAV or vectors with encapsidated resistance gene sequences. Such impurities represent a problem regarding safety of the vector preparations and have to be minimized as subsequent downstream processing will not lead to their depletion. Further aspects that have to be considered in the plasmid design are flexibility for exchange of specific expression cassettes as well as the capability to achieve high vector titers.

In many cases, especially in early clinical development, rAAV is still produced on anchorage-dependent cells. Hence for large scale production cell factories or roller bottles are often the first choice. Recently, ATMI has introduced the **Integrity™ iCELLis™** technology **(Figure 2)** to offer a solution for the drawbacks of these conventional 2D culture vessels. The heart of this single use bioreactor system is a fixed-bed comprising carriers made of medical grade polyester microfibers (PET). The system is available for process development and production purposes. The fixed-beds are manufactured in two different compaction rates and in three bed heights. Linear scalability is achieved through variation of the diameter of the fixed-bed just like in column chromatography. During operation, the medium flows through the fixed-bed and falls off the top like a waterfall allowing for efficient oxygenation and CO_2 stripping. Since the cells are attached to the carriers, the system can easily be set up in perfusion mode offering the possibility for high volumetric cell density. The implementation of controller units enables the regulation of relevant process parameters. Here we present data for transfection-based production of rAAV-2 in HEK-293T cells using the iCELLis laboratory scale bioreactor. Cell growth, metabolic data and virus titers are compared to benchmark values of conventional cell culture vessels.

Advanced AAV two plasmid system

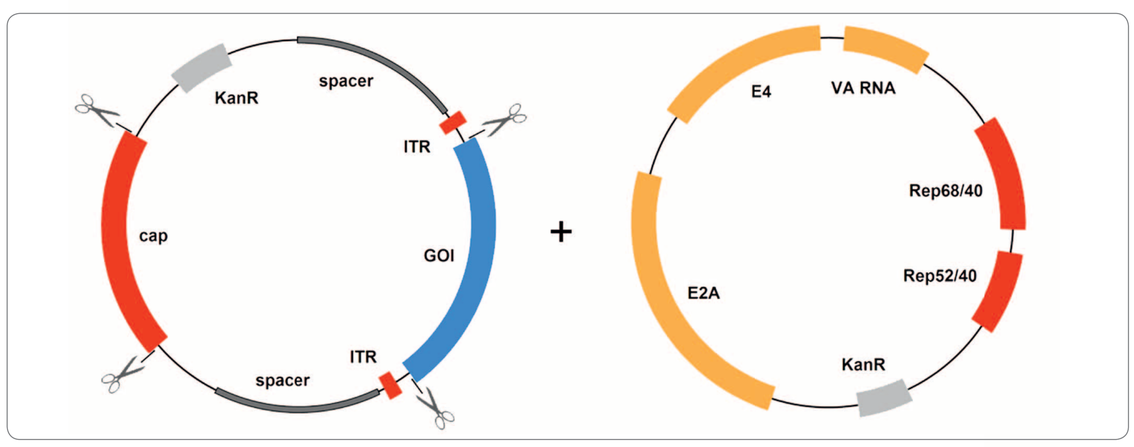

Figure 1: Design of the helper-virus free AAV two plasmid system

Table 1: Characteristics of the AAV two plasmid system

Criterion	Feature
Quality	No overlap of vector ITR and AAV *rep* and *cap* sequences
	Trans-split packaging design: separation of *rep* and *cap* gene
	Deletion of an artificial Rep-binding site (RBS) within the plasmid backbone
	Usage of Kanamycin instead of Ampicillin resistance gene
	Spacer adjacent to ITR sequences leading to an oversized plasmid backbone
Flexibility	Unique restriction sites for cloning of GOI and *cap* sequences
Quantity	Elimination of Rep78 synthesis reduces toxicity
	Maintaining complete intrinsic AAV gene regulation

The two plasmid system **(Figure 1)** is designed to reduce encapsidation of AAV-derived (e.g. replication-competent AAV) and other unwanted (e.g. resistance gene) impurities. It allows for fast and easy switch of vector and capsid to meet customer needs and contains features enabling high production yields **(Table 1).**

Recombinant AAV production in the iCELLis™ single use fixed-bed bioreactor

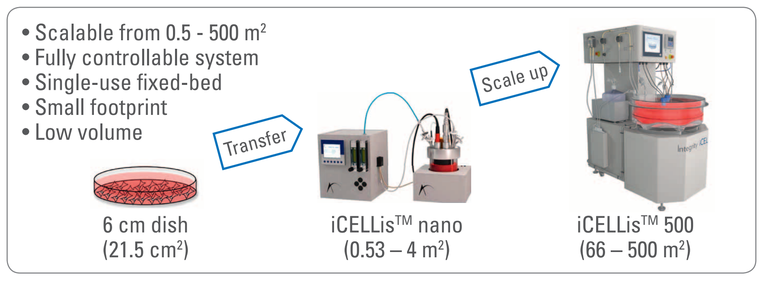

Figure 2: Features of the ATMI Integrity™ iCELLis™ technology

In order to evaluate the potential of the iCELLis technology, a transfection-based model process for the production of rAAV-2 established in conventional 2D culture vessels was transferred to the iCELLis nano system. For practical reasons 6 cm cell culture dishes were chosen as the reference system. The experimental setup is summarized in **Table 2.** Cells were seeded at 1E+05 cells/cm² and cultivated for 4 days. Transfection was performed 24 h after inoculation. 72 h post transfection virus particles were harvested by three consecutive cycles of freezing and thawing (note: ATMI considers this method of cell disruption as suboptimal. When using iCELLis bioreactors, development of chemical lysis is recommended). **Figure 2** and **Table 3** show the results of the experiment. Metabolites and cell concentration were monitored daily. Transfection efficiency was assessed by GFP fluorescence.

Table 2: Experimental setup

Parameter	6 cm dish	iCELLis nano
Area	21 cm²	5,300 cm²
Cell line	HEK-293T	
Medium	DMEM + FCS + GlutaMax	
Seeding density	1E+05 viable cells/cm²	
Seeding volume	3 mL	800 mL
Transfection	PEI, 4 plasmid system	

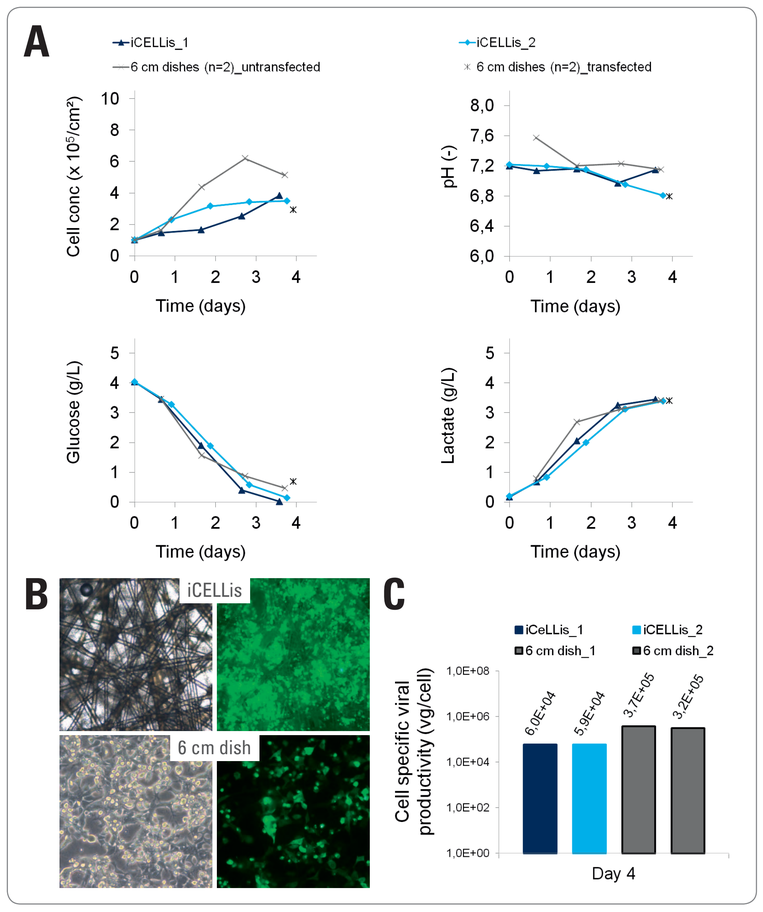

Figure 2: Proof of Principle (A) Total cell concentration and metabolic data; (B) Transfection efficiency 72 hours post transfection (GFP flourescence); (C) Cell specific productivity (based on qPCR data)

Table 3: Comparison of virus titers from different culture vessels

	iCELLis#1	CF10#2	6 cm dish#3
Vector genomes/cm²	2.2E+10	4.7E+10	1.0E+11
Vector genomes/mL	1.3E+11	2.7E+11	6.3E+11

#1 Mean value of iCELLis run 1 and 2
#2 Mean value of 4 CF10 runs
#3 Mean value 6 cm dish 1 and 2 (transfected)

In an additional experiment untransfected cells were cultivated for 13 days in the iCELLis nano system to determine maximum cell concentration. To maintain cell growth the culture medium was exchanged depending on metabolite concentration. Additionally, a significantly lower seeding density of 1E+04 cells/cm² was chosen to simulate a seed train for large scale production. Growth curve and metabolic data are summarized in **Figure 4.**

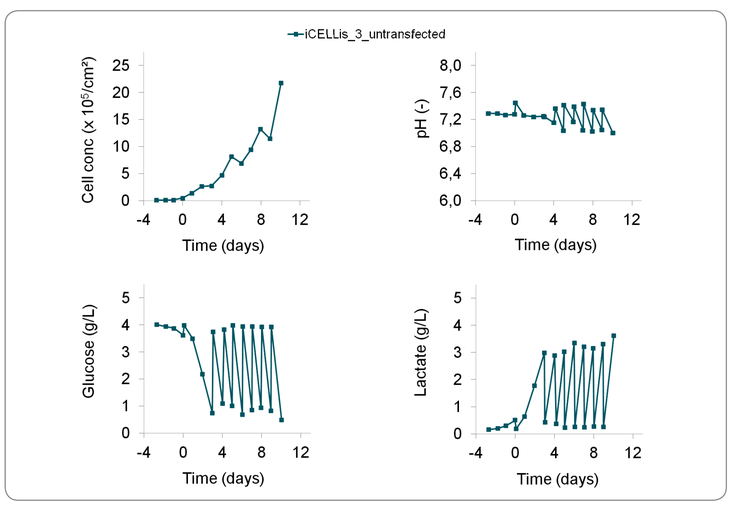

Figure 4: Growth curve and metabolic data

Summary and Outlook

Advanced AAV two plasmid system

An advanced two plasmid system is currently under development displaying a panel of beneficial features **(Table 1).** Firstly, the safety profile of the AAV vectors is improved to meet the growing regulatory demands on vector quality. Secondly, the plasmid design is offering flexibility as a prerequisite for versatile applications of recombinant viruses with different vector and capsid sequences. Lastly, generation of high vector titers is anticipated, since a four plasmid system already containing most of the features enables competitive production yields.

Recombinant AAV production in the iCELLis nano bioreactor

Total cell counts and metabolic data (glucose and lactate concentration, pH) of both bioreactor runs and the reference dishes were very comparable indicating that cell metabolism was unaffected by the choice of the culture vessel **(Figure 3 A).** Expectedly, cell concentration of untransfected cells was higher. According to fluorescence microscopy, transfection efficiency was on a high level in both systems **(Figure 3 B).** Mean cell specific viral productivity was approximately fivefold higher in the reference dishes than in the bioreactor **(Figure 3 C).** However, when compared to results from production runs in ten-layer cell factories, productivity in the iCELLis nano was only two times lower **(Table 3).**

Overall, this first set of data is very promising, especially when considering that the underlying process was optimized for 2D culture vessels. The controlled iCELLis bioreactor system offers many possibilities for process development. For instance, the results of the growth experiment **(Figure 4)** show that lower seeding densities could facilitate the seed train for large scale production. Moreover, the fact that the cells could be grown to more than 2E+06 cells/cm² by simple medium exchange indicates the potential for process optimization and, therefore, possible improvement of productivity.