Developing an effective scale-down model for a suspension adapted HEK293T-derived lentiviral vector stable producer cell line

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Abstract

Lentiviral vectors (LVV) represent an important tool for vaccine development and other therapeutic modalities. However, inefficiencies in LVV manufacturing processes, such as the inability to achieve high cell densities with HEK293T cell lines in a fed batch process, have resulted in poor upstream yields. Optimisation of cell culture conditions is needed to improve upstream yields, which can be expedited by high-throughput screening (HTP). In this work, we describe the use of the 24 deep square well (24-DSW) microwell platform to develop a scale-down mimic of GSK's established stable suspension LVV production process model at 2 L bioreactor scale. We found that matched mixing time was an effective basis for scale-translation between the stirred tank reactor (STR) and microwells. The growth kinetics and LVV productivity profile in the microwell were reproducible and comparable to the 2 L bioreactor process model. In both vessels, a 6-fold increase in cell density was achieved at the harvest time point and high cell viability (i.e. $>$ 90 %) was also maintained throughout the entirety of the cultures. The 24-DSW model, therefore, is an effective scale-down model for larger-scale stirred-tank bioreactor culture and provides an important tool for rapid, high-throughput optimization of the LVV production process.

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Abstract

Lentiviral vectors (LVV) represent an important tool for vaccine development and other therapeutic modalities. However, inefficiencies in LVV manufacturing processes, such as the inability to achieve high cell densities with HEK293T cell lines in a fed batch process, have resulted in poor upstream yields. Optimisation of cell culture conditions is needed to improve upstream yields, which can be expedited by high-throughput screening (HTP). In this work, we describe the use of the 24 deep square well (24-DSW) microwell platform to develop a scale-down mimic of GSK's established stable suspension LVV production process model at

2 L bioreactor scale. We found that matched mixing time was an effective basis for scale-translation between the stirred tank reactor (STR) and microwells. The growth kinetics and LVV productivity profile in the microwell were reproducible and comparable to the 2 L bioreactor process model. In both vessels, a 6-fold increase in cell density was achieved at the harvest time point and high cell viability (i.e. > 90 %) was also maintained throughout the entirety of the cultures. The 24-DSW model, therefore, is an effective scale-down model for larger-scale stirred-tank bioreactor culture and provides an important tool for rapid, high-throughput optimization of the LVV production process.

Keywords

Lentiviral vector; gene therapy; mixing time; microtiter plate; stirred tank reactor

1 Introduction

Lentiviral vectors (LVV's) are one of the fastest growing vectors under development within the cell and gene therapy industry (McCarron, Donnelley, McIntyre, & Parsons, 2016). They offer many advantages including the ability to transduce dividing and non-dividing cells, offer high transduction efficiency, and low immunogenicity (Kotterman, Chalberg, & Schaffer, 2015). These favourable characteristics make them a promising tool for use in the treatment of various genetic and acquired diseases such as beta-thalassemia (Cavazzana-Calvo et al., 2010), Parkinson's disease (Palfi et al., 2014) as well as oncology (Levine, 2015). With the increasing use of LVV in translational research and clinical programs, robust and scalable production processes are of critical importance in view of implementing these novel therapies for routine use (Merten, Hebben, & Bovolenta, 2016). However, current LVV batch or fed-batch upstream processes are limited by the inability to achieve high cell densities with the HEK293T cell line, resulting in poor yields (Manceur et al., 2017). Optimisation of cell culture conditions is therefore necessary to improve upstream efficiency.

Initial optimisation of suspension cell culture is traditionally carried out at small scale (i.e. microlitre to millilitre scale) under conditions that aim to mimic the large-scale bioreactor environment (Kumar, Wittmann, & Heinzle, 2004), and where possible, at high-throughput. However, adopting such an approach for LVV production employing conventional scales (e.g. miniature stirred tank reactors (STRs) and shake flasks) is both costly and impractical. Microwell based systems offer a suitable alternative to obtain key process design data early and cost-effectively (Martina Micheletti & Lye, 2006).

Previous studies have demonstrated the use of 24-standard round well (24-SRW) microtiter plates for the suspension cultivation of mammalian cell lines such as hybridoma (Barrett, Wu, Zhang, Levy, & Lye, 2010; M. Micheletti et al., 2006) and Chinese Hamster Ovary (CHO) (Chaturvedi, Sun, O'Brien, Liu, & Brooks, 2014; Mora et al., 2018; Silk et al., 2010) for monoclonal antibody production. However, examples of their use for LVV production are few in literature. Guy *et al.* , 2013 describe cultivation of HEK293 cells in 24-SRW plates to establish operating conditions for a 0.5 L wave bioreactor culture and demonstrated that the 24-SRW is an effective scale-down model (Guy, McCloskey, Lye, Mitrophanous, & Mukhopadhyay, 2013). HEK293T stable cell line cultivation in 24-deep square well (24-DSW) plates has yet to be demonstrated in literature. Compared to the 24-SRW plate, the 24-DSW format is advantageous as it offers 3-6 times greater working volume. Studies have also shown that square shaped wells have a better mixing and oxygen transfer profile compared to round well plates (Wouter A. Duetz & Witholt, 2004). To adopt the microwell system as a scale-down tool for LVV production, cell culture performance such as growth kinetics and LVV productivity must be matched with bench to pilot scale bioreactor processes. If not, there is no guarantee that the optimised process conditions achieved using microwells will be maintained upon scale-up.

Scale translation in cell culture is notoriously challenging due to the number and complexity of the variables affecting the physical and biological process. Criteria such as gas-liquid mass transfer coefficient (k_La), power input per unit volume (P/V) and mixing time have been used in chemical processes and adopted in biochemical engineering for bioreactor scale translation for different cultures (Alvin W. Nienow, 2006). Matched k_La is often used for scaling cell cultures with high oxygen demand (Ferreira-Torres et al., 2005). The oxygen demand for mammalian cells (e.g. HEK293) is relatively low compared to bacterial cell lines

(e.g. E. coli) (Alvin W. Nienow, 2006), therefore matched k_La was not considered for the HEK293T stable producer cell line cultures. The use of matched P/V in this study was limited by the lack of accurate methods of determining P/V in microwells. Current methods only provide estimates of the power input using computational fluid dynamics (CFD) predications of the mean energy dissipation (Zhang, Lamping, Pickering, Lye, & Shamlou, 2008). As there are no direct methods to compare against, the CFD predictions are yet to be validated as accurate models of the actual P/V. In comparison, there are now more accurate and semi-automated methods of measuring mixing time such as the dual indicator system for mixing time (DISMT) technique. This method also enables mixing time to be characterised across reactors with different scales and geometries. Furthermore, Sani and Baganz, 2016 demonstrated matched mixing time as a suitable criterion for scaling between 0.5 L micro-bioreactor (MBR) and 5 L STR for a GS-CHO cell line (Sani & Baganz, 2016). However, scale translation for smaller scale vessels with different geometries is yet to be established. Therefore, in this work, we evaluate mixing time as a basis for HEK293T stable LVV producer cell line culture scale-translation between 24-DSW microtiter plates and bench-scale 2 L STR. First, we characterise mixing times in the 2 L STR and 24-DSW plates using the DISMT technique. Thereafter, growth kinetics, LVV productivity and cell metabolism of the HEK293T stable producer cell line are compared between the microwell and STR cultures at matched mixing time.

2 Materials and methods

2.1 Quantification of mixing time

The dual indicator system for mixing time (DISMT) technique was used to quantify mixing time in this work. The method used was the same as that reported by Li, Ducci, & Micheletti, 2019. The DISMT technique was first developed by Melton, Lipp, Spradling, & Paulson, 2002 and is based around pH dependent colour changes. As the name implies, two pH indicator solutions - thymol blue (Fisher Scientific, Loughborough, UK) and methyl red (Fisher Scientific, Loughborough, UK) were used for visualisation of pH change with the fluid appearing red in acidic conditions, yellow at neutral pH and blue in basic conditions. The pH of the working reagent was adjusted until it became neutral (i.e. yellow). After filling the vessel with the working reagent, a stoichiometric amount of 0.075M hydrochloric acid (HCL) (Sigma-Aldrich, St. Louis, Missouri, United States) was added to make the colour transition from yellow to red. Thereafter, an equal amount of 0.075M sodium hydroxide (NaOH) (Sigma-Aldrich, St. Louis, Missouri, United States) was added to neutralise the acid and return the colour to yellow. The mixing process was recorded using the iCube (NET GmbH, Germany) high speed camera upon the addition of base. Mixing can be visualised since only regions that are well mixed appear yellow. Each condition was repeated five times to reduce statistical error and the average value was used as the mixing time. A universal MATLAB (MathWorks, Massachusetts, United States) script was used to compute the mixing time. The image processing method was the same as that used by Li et al., 2019.

2.2 Cell line and media formulation

A GSK proprietary HEK293T suspension-adapted stable cell line was used for this study. This cell line has the green fluorescence protein (GFP) transgene as well as the LVV packaging constructs stably integrated in the host genome (Chen et al., 2020). When induced, this cell line produces LVV with an RNA genome encoding for GFP. For cell culture, the proprietary media formulation from GSK's 2 L bioreactor (Sartorius AG, Gottingen, Germany) process model was utilised. For the STR cultures, the media was also supplemented with 0.5 mL/L antifoam (Cytiva, Washington, United States) to allow direct sparging and prevent excessive foaming.

2.3 2 L stirred tank reactor cultures

All STR studies were conducted in 2 L UniVessel® SU single-use bioreactors (Sartorius AG, Gottingen, Germany) in conjunction with the Biostat® B universal benchtop controller (Sartorius AG, Gottingen, Germany). The STRs were run with GSK's platform process operating conditions with defined inoculation and induction cell densities as well as concentrations of induction agents (i.e. doxycycline and sodium butyrate). In addition, the STRs were also operated with a controlled stirrer speed, temperature, pH and pO_2.

Agitation was provided by two 3-blade segment impellers (diameter = 54 mm) generating a down-flow flow characteristic. Data acquisition and process control was managed by an MFCS/Win 2.0 system (Sartorius AG, Gottingen, Germany). Temperature was controlled using a heating/cooling jacket. The pH was controlled around the set-point through the addition of sodium carbonate base and CO_2 in the inlet air. The dissolved oxygen tension (DOT) was maintained at the set-point using a cascade of air, oxygen and nitrogen. In order to prime the DOT and pH patch sensors, the STRs were media filled a day prior to inoculation. Just before inoculation, the pH was measured using a blood gas analyser (BGA) (Siemens, Healthineers, Erlangen, Germany) and recalibrated if it deviated by more than 0.05 units from the target value. The STRs were inoculated using a diluted mid-exponential phase shake flask culture. Once the target induction cell density was reached, the cultures were induced to initiate the production of the LVV. Induction was carried out by the addition of Doxycycline (Sigma-Aldrich, St. Louis, Missouri, United States) and Sodium Butyrate (Sigma-Aldrich, St. Louis, Missouri, United States). The induction agents were diluted in complete culture medium prior to being added to the STR. The working volume was between 1.5 L to 1.8 L. The vector was harvested at a defined number of hours post-induction which has been determined as part of GSK's platform process. Vector harvests were performed by spinning down the cell culture in a bench top centrifuge (Eppendorf, Hamburg, Germany) at 1000g for 5 min at room temperature. The vector supernatant was aliquoted into cryovials (Corning Inc. New York, United States) and stored in a -80 °C freezer. The STR cultures were performed in triplicate.

2.4 Shaken microwell cultures

The microwell format used for this study was the 24 deep square well (24-DSW) microtiter plate (Adolf Kuhner AG, Switzerland) with a square cross section of side = 17 mm, height = 40 mm and maximum well volume = 11 mL. The microwells were inoculated using a diluted mid-exponential phase shake-flask culture. Once the target induction cell density was reached, the cultures were induced to initiate the production of LVV. Induction was carried out by the addition of Doxycycline (Sigma-Aldrich, St. Louis, Missouri, United States) and Sodium Butyrate (Sigma-Aldrich, St. Louis, Missouri, United States). Similar to the STR, the induction agents were diluted in complete culture medium prior to being added in the microwell. The plates were sealed with a 'sandwich lid system' (W. A. Duetz et al., 2000) in conjunction with a metal clamp (EnzyScreen BV, AL Leiden, Netherlands) to minimize evaporation over extended periods of culture (Figure 1). The plates were incubated in an orbital shaker (shaking diameter = 25 mm) (Adolf Kuhner AG, Switzerland) with shaking speed set at 250 rpm, temperature at 37°C and CO_2 at 5%. The well fill volume varied between 5 mL at the start of the culture and 2.5 mL at the harvest time point. The reduction in volume was due to sampling of cell culture each day for analysis. For sampling, 0.5 mL of cell culture was removed from triplicate wells. For this work, sacrificial well sampling was not considered since the 24-DSW plate is not limiting in terms of working volume, as is the case with the 24-SRW plate. In addition, sacrificial well sampling also leads to a reduction in experimental throughput. To validate the aforementioned well sampling approach, the cell culture performance using sacrificial well sampling was compared against the same well sampling method used in this work.

Figure 1

2.5 Cell density and viability measurements

Cell counts and viability measurements were performed immediately post-sampling using an automated cell counting and viability measurement system i.e. Vi-cell XR Cell Viability Analyser (Beckman Coulter, Brea, California, United States). Briefly, a 0.5 – 1 mL of cell suspension was aliquoted into a Vi-Cell cup (Beckman Coulter, Brea, California, United States) which was then run on the Vi-Cell system to determine cell counts and viability measurements by trypan blue exclusion.

2.6 Quantification of extracellular metabolites

Glucose and lactate were monitored daily in this study. Briefly, 200 µL of cell culture supernatant was aliquoted into a round bottom 96 well microtiter plate (Corning Inc. New York, United States) which was then run on the YSI 2950D metabolite analyser (YSI Inc., Yellow Springs, USA).

2.6 pH measurements

In addition to the online pH measurements from the STR, offline pH readings were also taken daily from the microwell and STR cultures using the RAPIDLab 348EX blood gas analyser (Siemens Healthineers, Erlangen, Germany). For the STR cultures, the pH was recalibrated if the online and offline pH value deviated by more than 0.05 pH units.

2.7 Quantification of lentiviral vector infectious titre

To quantify the infectious titre in terms of transducing units per ml (TU/ml), HEK293T cells were transduced with serial LV dilutions in the presence of polybrene (8 μg/ml) (MilliporeSigma, Burlington, Massachusetts, United States). Polybrene is a polycation agent which has shown to enhance transduction by retroviral vectors (Davis, Morgan, & Yarmush, 2002). The transduced cells were adherent HEK293T cells which were derived from a GSK in-house research cell bank. Cell counts were performed at the time of transduction. Following this, cells were cultured for 3 days at 37°C in a 5% (v/v) CO_2humidified incubator. The eGFP expression in the transduced cells was measured by flow cytometry using the Accuri c6 Plus system (BD Biosciences, Franklin Lakes, New Jersey, United States). The titre was determined using the formula: Transducing Units/mL = [(% of GFP positive cells) x (number of cells transduced) x (dilution factor/transduction volume)].

2.8 Quantification of lentiviral vector physical titre

The LV physical titre was measured using the Simple Plex HIV-1 Gag p24 ELISA kit (Protein Simple, San Jose, California) following manufacturer's instructions. The vector samples were diluted according to the estimated p24 concentration to ensure they were in the linear range of the assay. The first dilution was performed with 0.5% Triton X Lysis buffer (Sigma-Aldrich, St. Louis, Missouri, United States). To ensure lysis, the sample preparations were incubated for 1 hour at 37°C in a 5% (v/v) CO_2 humidified incubator. A second dilution was then performed with SD30 dilution buffer (Protein Simple, San Jose, California) prior to loading the samples on the cartridge. Samples was analysed in triplicate and read using the Ella microfluidic device (Protein Simple, San Jose, California). The p24 concentration was calculated relative to the factory-calibrated standard curve supplied with the kit. The LV specific infectivity was calculated as the ratio between infectious titer and physical titre.

2.9 Statistics

All statistical analyses were performed using Prism 8 (GraphPad, San Diego, CA). The Student's t-test was used to compare the difference in LVV productivity profile between the microwell and STR cultures. For the t-test, statistical significance was determined using a minimal confidence level of 95% ($p < 0.05$).

3 Results

3.1 Mixing time characterisation

Mixing time is an important parameter for mammalian cell culture processes. Inadequate mixing results in cells being exposed to different environments within the reactor, which can have adverse consequences on cell growth kinetics and productivity (Gogate, Beenackers, & Pandit, 2000; Alvin W. Nienow, 2006). Hence mixing should be optimised to produce a homogenous cell culture environment whilst avoiding any deleterious effects caused by potential hydrodynamic forces and bubble bursting. Short mixing times ($t_m <$ 10s) should provide the necessary homogeneity as well as ensuring a fast response to any changes in operating conditions such as pH and temperature (Amanullah, Buckland, & Nienow, 2004; Langheinrich & Nienow, 1999; Silk, 2014). For fed-batch culture, efficient mixing is particularly important to avoid formation of local nutrient gradients (Enfors et al., 2001; Alvin W. Nienow, 2006).

Mixing times in the 5 L STR and 24-DSW were determined using the DISMT method as described in section 2.1.

3.1.1 Mixing time in 2 L STR

Figure 2A shows the mixing time variation in the 2 L STR with varying stirrer speeds and two different working volumes, 1.5 L and 1.8 L, to account for changes upon addition of induction agents. As expected, the graph shows that mixing time was inversely proportional to the stirrer speed. It can also be seen that for the two fill volumes evaluated, the mixing times were comparable as demonstrated by the overlapping error bars. The mixing time ranged from 7 – 17 s for 1.5 L and 7 – 24 s for 1.8 L. At higher agitation rates (i.e. [?] 225 rpm), the mixing time values were less than 10 s, which is necessary to maintain a homogenous culture environment (Amanullah et al., 2004; Alvin W. Nienow, 2006; Silk, 2014). With respect to the 2 L STR LVV production process model, the platform operating stirrer speed fell in the range of higher agitation rates, hence, was sufficient to maintain the required homogeneity for optimal HEK293T growth and LVV productivity. In addition, the mixing time values from this work was comparable with those found in literature for other bench-scale bioreactors as summarised in Table 1. From the table, we observe that the variety of mixing time methods used by different groups all produced consistent datasets for a similar range of Reynolds numbers as evaluated in this study.

Table 1

3.1.2 Mixing time in 24-DSW plate

Figure 2B shows the mixing time variation in the 24-DSW plate with varying shaker speed and four different fill volumes, ranging from 2 – 5 mL. From the graph, it is evident that mixing time was inversely proportional to shaker speed in the lower end range of shaker speeds investigated i.e. between 175 to 250 rpm. Within this range, mixing time was also reduced for smaller fill volumes. At the lowest shaker speed tested, mixing time for V_L = 5 mL was approximately 4 times greater than that of 2 mL. For smaller fill volumes, mixing times are expected to be lower since there is a constant amount of energy being dissipated to a reduced volume, resulting in more turbulence and hence better circulation (Omar. Al Ramadhani, 2015). At shaker speeds greater than 250 rpm, the mixing process became very rapid with mixing times less than 10 s for all fill volumes evaluated.

For the same 24-DSW format, Li et al., 2020 reported an unexpected increase in mixing time in the higher speed range which was associated with a change in free surface dynamics in the well. The authors attribute this phenomenon to the very small shaking diameter (d_s) used in their study (i.e. 3 mm). A similar increase in mixing time at higher shaker speeds was not observed in this work, perhaps due to the higher shaking diameter of 25 mm being used.

The mixing time results from this work were also compared against the scaling law proposed by Rodriguez et al., 2014 for orbitally shaken reactors (OSR's) (Rodriguez, Anderlei, Micheletti, Yianneskis, & Ducci, 2014). A comparison between the two studies could be made since the same experimental technique and image processing methodology was used. In their study, Rodriguez *et al* ., 2014 demonstrated good correlation between two dimensionless parameters, mixing number ($N.t_m$) and Froude number ratio (Fr/Fr_c). From this, the power law relationship for scaling was derived as follows: $N.t_m = 100.7(Fr/Fr_c)^{-1.245} + 25$. This relationship was found to best fit data from a wide range of operating conditions including reactors of different sizes (d_i = 8 – 13 cm) and orbital shaking diameters (d_s = 15 – 50 mm). Li et al., 2020 assessed the applicability of the scaling law, however, the power law function over predicted the mixing numbers for the 24-DSW plate used in their study (Li, Ducci, & Micheletti, 2020). This was due to the very small orbital diameter used in which case the different fluid and mixing dynamics within the well meant that the Froude number ratio was not an effective scaling parameter. As the orbital diameter in this work was larger compared to Li et al., 2020, the applicability of the scaling law was re-assessed using the present data set. The mixing number curves for the 24-DSW plate from this study was plotted in Figure 3 and compared against the power law function. From the graph, all the mixing number curves showed good correlation with the power law function confirming its applicability as a scaling law for OSR's of different sizes and geometries. Furthermore, it also demonstrates confidence in the accuracy of the mixing time values, given that mixing time was selected as the basis for scale-translation in this work.

Figure 2

Figure 3

3.2 Scale translation of HEK293T stable producer cell line culture at matched mixing time

The scalability of cell culture performance such as growth kinetics and LVV productivity was investigated in 24-DSW microtiter plates (5 mL scale) and stirred tank reactors (2 L scale). As described earlier, mixing time was chosen as the basis for scale translation. Table 2 summarises the operating conditions selected in each of the two vessel formats for operation at matched mixing time of <10 s. The mixing time obtained in the STR under platform operating conditions was selected as the benchmark. For the 24-DSW plate, the mixing time was matched at shaking speeds [?] 250 rpm for all fill volumes examined. A larger fill volume is more advantageous since there is greater scope for analytics. It also means sacrificial well sampling can be avoided thus permitting a higher experimental throughput. Hence, 5 mL was selected as the initial microwell fill volume. This was reduced to 2.5 mL at the harvest time point as the culture was sampled from the same well each day. It should be noted that a constant mixing time is still achieved for this range of working volume at 250 rpm as supported by Figure 2B. We also demonstrated comparable cell culture profiles between wells with reducing volume (i.e. same well sampling) and the sacrificial well sampling control with 5 mL culture volume (see Figure S1 in the Appendix). Hence, the reduction in volume up to 2.5 mL does not have any impact on cell culture performance, thus, validating the sampling approach used in this study.

Table 2

3.2.1 Cell growth kinetics

The growth kinetics of HEK293T cell cultures in 24-DSW plates and 2 L STR are depicted in Figure 4. The fold change in viable cell density for both vessel systems were comparable for the entire duration of culture (Figure 4A). The first two days correspond to the growth phase where cells proliferate before being induced to initiate LVV production. Within 48 hours of culture, cells in both reactor formats reached the target induction cell density. The decrease in cell density at day 2 corresponds to the induction time point; here, the cell density decreased due to dilution of the culture upon addition of induction agents. After induction, the fold change in viable cell density remained comparable, with both cultures achieving a similar cell density at the harvest time point.

High cell viability values (i.e. > 90 %) were also maintained in both systems throughout the entirety of the culture (Figure 4B). A slight decrease in cell viability in generally observed after 24 hours post induction (i.e. day 3). Studies have linked this with the onset of viral release (Ansorge, Lanthier, Transfiguracion, Henry, & Kamen, 2011; Petiot, Ansorge, Rosa-Calatrava, & Kamen, 2017). As the LVV buds off from the plasma membrane of the producer cell, the integrity of the membrane is compromised which may lead to apoptosis and a decrease in cell viability. A similar trend was also observed in Figure 4B, however, it should be noted that the decrease in viability after day 3 was very marginal with values still above 90% at the end of the culture.

Similar glucose and lactate metabolite concentrations (Figure 4C and 4D respectively) were achieved in both platforms up until the induction time point (after day 2). Thereafter, glucose depletion and resulting lactate accumulation was greater in the 2 L STR compared to the microwell plate. This trend in metabolites post-induction could be explained by the contrasting pH profiles for both systems. Liste-Calleja*et al* ., 2015 have demonstrated that culture pH below 6.8 triggers lactate co-metabolism in HEK293T cells (Liste-Calleja et al., 2015). This cellular adaptation is used as a pH detoxification strategy by means of co-transporting extracellular protons together with lactate into the cytosol. For the STR, the pH was maintained above 6.8 through the controlled addition of sodium carbonate base and CO_2. However, no such control was available in the microwell system which was evident by the significant fluctuations in pH as depicted in Figure 5. From the graph, it can be seen that the microwell pH decreased immediately after induction (i.e. below 6.8) as the cellular metabolic activity increased. Around the same time point, a plateau in lactate accumulation for the 24-DSW plate was also observed (Figure 4D) possibly due to lactate co-metabolism as suggested by Liste-Calleja *et al* ., 2015. However, despite the disparity in pH control between the two platforms, the 24-DSW plate was still a good scale-down model for the 2 L STR with respect to cell growth and LVV

productivity.

Figure 4

Figure 5

3.2.2 LVV productivity

A comparison of the LVV productivity in the 24-DSW plate and 2 L STR is shown in Figure 6. Several methods were used to characterise the LVV productivity. The infectious titre method was used to measure the number of functional LVV particles produced whilst the physical titre method was used to estimate the total number of virus particles generated, whether infectious or not, by measuring p24 concentration. In Figure 6, both titres were normalised relative to the STR to facilitate easier comparison between both systems. Dividing the infectious titre with the physical titre gives an infectivity ratio in terms of the number of transducing units per pg of p24 protein (TU/pg p24). This ratio provides an indication of the quality of LVV particles produced. A higher ratio of TU per pg p24 signifies a higher infectivity since there are more transducing units in a given quantity of virus particles. Lastly, the cell specific productivity normalises LVV production relative to the cell density at induction. This way, any bias relating to differences in induction cell densities between conditions is removed thus providing a more accurate measure of comparison. According to Figure 6, all four measures of LVV productivity showed good similarity between both vessel formats. In addition, a t-test confirmed any differences between the two systems were statistically insignificant at 95 % confidence level. Overall, mixing time proved to be a good basis for scale translation and yielded comparable LVV productivity.

Figure 6

4 Discussion

In this work, we demonstrated that mixing time can be used as a scaling parameter for HEK293T stable producer cell line culture between 24-DSW plates (operated with a large shaking diameter, $d_s = 25$ mm) and 2 L STRs. Based on the published literature and previous works' results, for geometrically similar reactors, a constant power per unit volume is generally the more preferred scaling option compared to mixing time. This is due to a potentially prohibitive power input requirement for large scale bioreactors in order to achieve short mixing times (Diaz & Acevedo, 1999; Schmidt, 2005; Yang et al., 2007). Larger fluid volumes result in longer flow paths for bulk circulation, hence higher fluid velocities are required to achieve similar mixing times (A. W. Nienow, 1998; Omar. Al Ramadhani, 2015). As the reactor scale increases, more power input is required if higher agitation rates are used in order to achieve higher velocities (A. W. Nienow, 1998). For scale-translation at small scale, the power input requirement is not limiting as successfully demonstrated by Sani and Baganz, 2016 who used mixing time as a basis to scale between 0.5 L micro-bioreactor (MBR) and 5 L STR for a GS-CHO cell line (Sani & Baganz, 2016).

In this study scale translation was required between small scale vessels having very different geometries and flow patterns (i.e. shaken microwells to bench-scale STRs) and matched mixing time was considered as a possible scaling method. In addition, the use of P/V was limited by the lack of accurate methods to measure the power input in microwell plates. Current methods only estimate the P/V using CFD predictions of the mean energy dissipation as demonstrated by Zhang *et al* , 2008 (Zhang et al., 2008). Barrett *et al* ., 2010 used the P/V estimates from Zhang *et al* ., 2008 as the basis for scaling a hybridoma cell culture process from a shaken 24-SRW system (800 µL) to a 250 mL shake flask culture (Barrett et al., 2010; Zhang et al., 2008). The data was also indicative of results obtained in the 5 L STR albeit at unmatched P/V. The STR could not be matched at constant P/V due to an unfeasible operating stirrer speed in excess of 300 rpm, at which point excessive foaming and vortexes formed in the vessel. The P/V estimates followed a counter-intuitive trend whereby microwell power consumption decreased with increasing shaking speeds. As there are currently no direct methods of measuring P/V in microwells, the accuracy of the CFD predictions are yet to be validated. Hence, it cannot be certain whether scaling in Barret *et al* ., 2010's study was actually achieved at constant P/V. On the other hand recent developments in imaging techniques and the availability

of high speed cameras and automated image processing methods allow accurate mixing time measurements in very different configurations.

The 24-DSW model for LVV production developed in this work offers great scale-down potential for carrying out initial high-throughput screening and optimisation studies. Compared with other scale-down systems used in early stage bioprocess development, this 24-DSW model offers several benefits in terms of cost, flexibility and ease of use. Micro-bioreactors (MBR) are frequently used in upstream processing for cell culture process development work. Applikon's micro-Matrix (Applikon Biotechnology B.V, Delft, Netherlands) is a MBR based on the 24-DSW platform where in each well is individually controlled for pH, DO and temperature (Wiegmann, Martinez, & Baganz, 2020). The micro-Matrix's measurement and control capabilities offer a better insight into the bioprocess compared to the 24-DSW model from this study. The incorporation of pH control also means that similar metabolite profiles to the STRs could be also be achieved with the micro-Matrix. However, the investment and operating costs are significantly higher than the more simplistic 24-DSW model. In terms of ease of use and flexibility, the 24-DSW model is also advantageous compared to MBR platforms, as these require time-consuming set-up with steps such as sterilisation and probe calibration. Furthermore, MBRs are often limited in terms of the number of vessels/wells that can be used for a particular run. In contrast, the metal clamp system (EnzyScreen BV, AL Leiden, Netherlands) holding the 24-DSW system accommodates up to four plates giving a total of 96 wells. Hence, the 24-DSW model offers a higher degree of parallelisation making it more suitable for high-throughput screening work. Other microwell based models have also been developed using the 24-SRW plate as the model system (Barrett et al., 2010; Guy et al., 2013; Silk et al., 2010). In comparison to the 24-SRW plate, this 24-DSW scale-down model offers over 6-fold greater working volume which increases scope for analytics. In addition, it also offers a higher experimental throughput since the larger working volume means several wells do not have to be sacrificed for a single condition.

5 Conclusion

This work has successfully demonstrated the use of mixing time as a suitable criterion for scale translation between a microwell model and bench scale STRs. The DISMT technique was used to characterise mixing time in both vessels. The mixing data showed good correlation with the power law function proposed by Rodriguez et al., 2014, confirming its applicability as a scaling law for OSR's of different sizes and geometries. At matched mixing time, comparable HEK293T stable lentiviral vector producer cell line growth kinetics and LVV productivity profiles were achieved in 24-DSW plate and 2 L STR cultures. However, lack of pH control in the microwells may have contributed to differences in glucose and lactate concentrations post-induction. Overall, the microwell approach offers many advantages in terms of reduction in scale of operation (by over 10-fold compared to 50 mL shake flask, and 300-fold compared to 2 L STR), reduced cost and increased experimental throughput. Given its many advantages, as well as proven scalability with STR's, the 24-DSW scale down model provides an ideal platform for rapid upstream process development of LVV production.

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TABLE CAPTIONS

Table 1: Comparison of mixing times for small/bench scale bioreactors from studies published in literature.

Table 2: Summary of operating parameters for 24-DSW plate and 2 L stirred tank reactor at matched mixing time.

TABLES

Table 1:

Reactor configuration	**Number and type of impeller**	**Method of mixing time study**	**I**
5 L STR (Sartorius, Germany)	1 x 3-blade segment	Iodine decolourisation	7
3 L STR (Applikon, Netherlands)	1 x Rushton turbine	Iodine decolourisation	3
5 L STR (Sartorius, Germany)	1 x Marine	Iodine decolourisation	6
0.5 L MBR (HEL-BioXplore, United Kingdom)	1 x Marine	pH tracer	4
2 L STR (Sartorius, Germany)	*2 x 3-blade segment*	*DISMT*	3

[+]Reynolds numbers have been rounded to the nearest thousand

Table 2:

Reactor	**24-DSW Plate**	**2 L STR (Sartorius)**
Shaker/stirrer speed	250 rpm	GSK platform stirrer speed
Mixing time	7.1 ± 0.1 s	9.5 ± 1.5 s
Aeration system	Headspace	L-type sparger and/or headspace
Working volume	2.5 – 5 mL	1.5 – 1.8 L
pH/DO/Temperature control	n/a[+]	Yes

[+]Parameter control cannot be performed for the 24-DSW plate cultures

FIGURE CAPTIONS

Figure 1: Set-up of 24-DSW scale-down model for LVV production.

Figure 2: Liquid phase mixing times for 2 L STR (A) and 24-DSW plate (B) measured using the dual indicator system for mixing time (DISMT) technique. For the 2 L STR, mixing time was characterised at stirrer speeds between 100 – 300 rpm and two different bioreactor working volumes, V_L = 1.5 L and V_L = 1.8 L. For the 24-DSW plate, mixing time was characterised at shaking speeds between 175 – 300 rpm (d_s = 25 mm) and four different fill volumes between 2 – 5 mL. Error bars represent one standard deviation about the mean (n=5).

Figure 3: Comparison between data shown in present study with the mixing time model reported in Rodriguez *et al* ., 2014. The model is based on the Froude number ratio (Fr/Fr_c) and is used for scaling between OSR's of different sizes and geometries.

Figure 4: Cell culture profile for cultivation of a HEK293T stable producer cell line in 24-DSW plate with 5 mL starting volume and 2 L STR at matched mixing time, t_m of < 10 s. (A) Viable cell density (B) Cell viability (C) Glucose concentration (D) Lactate concentration. Error bars represent one standard deviation about the mean (n = 3); Note: For day 0 and day 2 time points in Figure 4 C and 4 D, n = 2 is reported as the data for one vessel at each time point was considered to be erroneous i.e. the measurements from the metabolite analyser were below the lower limit detection range suggesting the target metabolites were not detected in the sample.

Figure 5: pH profile for cultivation of a HEK293T stable producer cell line in the 24-DSW plate. Error bars represent one standard deviation about the mean (n = 3).

Figure 6: LVV productivity profile in 24-DSW plate with 5 mL starting volume and 2 L STR at matched mixing time,t_m of ˜ 9 s. The infectious titre and physical titre values were normalised relative to the 2 L STR. Error bars represent one standard deviation about the mean (n = 3). Statistical comparisons were done using t-test where 'ns' means not significant at 95% confidence level.

Figure S1: A comparison of the viable cell density profile of HEK293T stable producer cell line culture in 24-DSW plates using same well sampling and sacrificial well sampling methods.