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Detachment factors for enhanced carrier to carrier transfer of CHO cell lines on macroporous microcarriers

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Abstract

In this publication different detachment factors were tested for enhancing carrier to carrier transfer for scale-up of macroporous microcarrier based bioprocesses. Two Chinese hamster ovary cell lines, CHO-K1 and a genetically engineered CHO-K1 derived cell line (CHO-MPS), producing recombinant human Arylsulfatase B, were examined. The cells were grown on Cytoline 1 microcarriers (Amersham Biosciences, Uppsala, Sweden) in protein-free and chemically defined medium respectively. Fully colonised microcarriers were used at passage ratios of approximately 1:10 for carrier to carrier transfer experiments. To accelerate the colonisation of the non-colonised, freshly added microcarriers the detachment reagents trypsin, papain, Accutase™ (PAA, Linz, Austria), heparin and dextransulphate were used. Both cell lines showed good results with trypsin, Accutase and dextransulphate (Amersham Biosciences, Uppsala, Sweden), while papain failed to enhance carrier to carrier transfer in comparison to the non-treated reference. The maximum growth rate of cells on microcarriers with 2% dextransulphate in the medium was 0.25 ± 0.02 d^{-1} and 0.27 ± 0.03 d^{-1} for the CHO-MPS and CHO-K1, respectively. The CHO-K1 grew best after detachment with trypsin (μ = 0.36 ± 0.03 d^{-1}). This indicates, that one of the key parameters for carrier to carrier transfer is the uniform distribution of cells on the individual carriers during the initial phase. When this distribution can be improved, growth rate increases, resulting in a faster and more stable process.

Introduction

Industrial biopharmaceutical production processes using animal cells have a number of specific safety and technical requirements, such as defined media devoid of substances from animal origin, standardisation, high product yield, high product concentration, scale - up potential, etc.

Microcarrier based bioprocesses fulfill a number of these requirements (van der Velden-de Groot 1995). Especially for fermentations with anchorage dependent cells, such as Vero or MDCK, microcarrier based systems are required. Vaccine fermentation is mostly performed in these systems. Cells grown on carriers, particularly on macroporous carriers, condition their microenvironment, so complex media supplements can be avoided or at least reduced (Merten et al. 1994; Xiao et al. 1999; Jayme and Smith 2000). A majority of animal cell based recombinant protein production processes employ homogeneous, low cell density culture systems, mostly batch processes. To increase fermentation yield, either fed-batch or continuous perfusion technologies using cell retention systems are used. Perfusion systems such as microcarrier based fluidised bed fermentations can be run for months (Goldman et al. 1998; Dürrschmid et al. 2001). The advantage of such long, stable processes is the uniformity and thus the standardisation of the

product. High perfusion rates reduce product residence time in the bioreactor (Kong et al. 1999b), and can be coupled to continuous down-stream processing procedures (Facius and Lohster 1999). Residence time can be of importance as the sialylation of recombinant glycoproteins produced in stirred tank batch culture may decline significantly during the stationary and death phase of batch culture (Goldman et al. 1998).

Typically fluidised bed processes can be run at perfusion rates of 7 to 12 times settled bed volume per day, increasing the productivity up to 5-fold compared to processes using microcarriers in stirred tank bioreactors (Kong et al. 1999a). Besides productivity the scale - up capability is one of the most important factors for process design. Providing sufficient inoculum to fluidised bed bioreactors of industrial scale can be a limiting scale-up factor. For an industrial 100 L fluidised bed bioreactor the required initial cell number is approximately $2 \cdot 10^{11}$ cells equalling a 200 L suspension bioreactor. Optimally, a production stage fermenter, either fluidised bed or stirred tank reactor, should be inoculated directly from a small volume. This can be achieved by starting fermentation with a small carrier volume and then by a stepwise increase of the microcarrier volume, seeding freshly added carriers through carrier to carrier transfer (Hu et al. 2000). Ohlson et al. (1994) reported of spontaneous carrier to carrier transfer on Cytopore 1 macroporous microcarriers in spinner flasks and a 5 L stirred tank with Chinese hamster ovary cells. The important factor for colonisation itself is cell distribution. If cell distribution can be controlled, carrier to carrier transfer can be standardised. Different methods are available to achieve this goal. Commonly, proteolytic enzymes such as papain or trypsin, are used. Animal derived trypsin has been widely replaced by recombinant enzymes of bacterial origin. In general the drawback of proteolytic enzymes is the negative effect on the cells and difficulties in standardisation (Ohlson et al. 1994). An alternative may be the use of substances which mimic biological structures. Especially dextran derivatives exhibit a number of interesting features for example sulphonate, carboxylate, amino acid sulphamide and amide groups on dextran backbones are able to mimic the hydroxyl, carboxylate, sulphamate and sulphate groups on the polysaccharide chains of heparin, which have been demonstrated to be essential for its anticoagulant activity (Jozefowicz and Jozefonvicz 1997; Maiga-Revel et al. 1997; Logeart-Avramoglou and Jozefonvicz 1999). Barabino et al. (1999) reported the inhibition of adhesion of sickle red blood cells on human umbilical vein cells through anionic polysaccharides. Especially high molecular weight dextransulphate (MW 500.000) has the ability to decrease the adherence capacity of animal and insect cells (Dee et al. 1997; Zanghi et al. 2000).

In this study we tested proteolytic enzymes, heparin and dextransulphate as detachment factors for two CHO cell lines from macroporous microcarrier to enhance a carrier to carrier transfer.

Materials and methods

Cell line and media

The cell lines used were CHO-K1 and a genetically engineered CHO-MPS cell line producing recombinant human Arylsulfatase B. The standard medium for both cell lines was a 1:1 mixture of Dulbecco's Modified Eagle medium with Ham's F12 medium (Biochrom, Berlin, Germany), supplemented with 6 mM L-glutamine (Sigma, St. Louis MO, USA), and protein-free-additives (Polymun Scientific, Vienna, Austria) at a pH of 7.1. For CHO-K1 cells soy-peptone (Quest, Teterboro NJ, USA) was additionally used and for inoculum in spinner flasks, 0.05% Pluronic F68 (Invitrogen, Carlsbad CA, USA).

The inoculum for the microcarriers was cultivated in 250 mL spinner systems (Integra Biosciences, Wallisellen, Switzerland) with an agitation speed of 55 rpm and at 37 °C. Dextransulphate T500 with a substitution grade of 16–18% sulphur (Amersham Biosciences, Uppsala, Sweden) autoclaved as 15% w/w solution, was further used as detachment factor.

Cell counting

Cell number of the supernatant was determined by the trypan blue dye exclusion method using a hemocytometer and following standard protocol (0.2% trypan blue (Sigma, St. Louis MO, USA)). For cell density determinations of cells on the microcarriers nuclei were counted after cell lysis using a Coulter counter (Coulter Electronics LTD, Luton, UK), with a minimum cut-off set at 2.67 μm to discriminate whole cells from nuclei and debris.

In the case of dextransulphate additions to the medium cell counts could not be performed with the coulter counter as dextransulphate interacted with the Triton solution (Coulter Electronics LTD, Luton,

UK). Therefore cell density was determined with the hemocytometer after trypsination and thoroughly vortexing. This method is less accurate than the coulter counter based determinations.

The growth rate was calculated by linear regression of the log of cell densities versus time of the logarithmic phase of the microcarrier culture

Cell cultivation

Roller bottle experiments using macroporous microcarriers, Cytoline 1™ (Amersham Biosciences, Uppsala, Sweden), were prepared and colonised according to manufacturers recommended procedures. Standard colonisation was performed in 490 cm^2 roller bottles (Corning, Wiesbaden, Germany) with 3 mL microcarrier in 50 mL medium. Bottles were agitated for 5 min every 30 min for 24 h by a GFL 3017 (GFL, Burgwedel, Germany) orbital shaker at 43 rpm in an upright position. After 24 h the bottles were agitated by using a roller system (Wheaton, Millville NJ, USA) at 2 rpm. Medium exchanges and sampling were performed after 24 h, 72 h and every following 24 h. Fully colonised carriers were used as inoculum for the scale-up experiments.

To simulate conditions in the fluidised bed, we established a second method to test proliferation under elevated shear force on macroporous microcarrier using roller bottles in an upright position which are agitated by a GFL (GFL, Burgwedel, Germany) 3017 orbital shaker at 43 rpm. The cell densities achieved with this method were approximately equal to the fluidised bed reactor (CytopilotMini)(data not shown).

Scale - up experiments with the enzyme producing recombinant CHO-MPS were done with colonised carriers obtained from a fluidised bed bioreactor after 43 days of fermentation (data not shown). The reactor was a 2 L CytopilotMini (Amersham Biosciences, Uppsala, Sweden) with 300 mL Cytoline 1 microcarrier. Inoculation was performed with basal medium supplemented with soy-peptone. The perfusion was started with peptone-free medium after achieving 10^7 cells·ml^{-1} carrier.

Detachment experiments

Fully colonised microcarriers were obtained from a fluidised bed fermentation. 2 mL carrier, $1 \cdot 10^8$ cells·mL^{-1} carrier, were transferred to 490 cm^2 roller bottles. The cells were treated with different reagents of animal and non-animal origin. The tests were performed with 61.25 mg·L^{-1} papain-solution (Cat. No. L2223, used pure) (Biochrom, Berlin, Germany), Accutase-solution (Cat. No. L11-007, used pure) (PAA, Linz, Austria), dextransulphate (1% w/v and 2% w/v end concentration, used as 15% stock solution in high quality water) (Amersham Biosciences, Uppsala, Sweden) and heparin (1 mg·mL^{-1} and 2 mg·mL^{-1} end concentration, dissolved in PBS)(Sigma, St. Louis MO, USA). The cells were treated with 2 mL and 5 mL of the enzyme solutions for five min and then filled up to 20 mL with fresh soy-peptone containing medium. Dextransulphate stock solution was added directly to the standard medium; 20 mL were used for each test. 5 mL of the two heparin solutions were directly incubated with the carriers and filled up to 20 mL with medium, as above. As reference we used standard medium without any further supplementation. Cell density and viability measurements were performed as described above. The percentage of cells in suspension was calculated using the total cell number in suspension compared to the total cell numbers on the microcarriers.

Cell counting with the hemocytometer was performed after 6 h for all agents and additionally after 24 h for all non enzymes. All experiments were performed in parallel and as replicates.

Carrier recolonisation experiments

The medium of the fluidised bed fermentation was spiked with 1% dextransulphate to detach the CHO-MPS cells from the microcarrier on day 51 of a perfusion fermentation (data not shown). After 4 h of incubation the supernatant containing the detached cells was removed via the sampling valve, transferred to roller bottles with 3 mL fresh microcarriers and subsequently the standard colonisation strategy, according to manufacturers recommended procedure, was performed. After 24 h and 48 h 20 mL of medium were replaced by fresh standard medium. The first full medium exchange was performed after 6 days and then every day. Sampling was done in the same time pattern. As a reference, carrier were inoculated with cells obtained from a spinner culture. The test was performed in parallel experiments in two replicates.

Scale up - carrier to carrier transfer experiments

Approximately 300 μL fully colonised microcarriers

(CHO-MPS obtained from the reactor and CHO-K1 from roller bottles) were transferred to 3 mL fresh carriers. The transfer passage ratios vary due to the scale of the experiment as the number of carriers used can not be controlled efficiently under sterile conditions. The ratios were determined indirectly by dividing the cell density on the carriers prior to the carrier to carrier transfer by that directly after transfer. The substances used for this experiment were Accutase, Papain, trypsin-solution (0.1% trypsin + 0.02% EDTA in PBS) (Invitrogen, Carlsbad CA, USA), 1% and 2% dextransulphate and a non-treated reference as described for the detachment experiments. Heparin was not used, because it had not shown the expected effect.

In the case of enzyme addition after 5 min of treatment the medium was filled up to 25 mL. In the case of non-treated standard and dextransulphate containing medium the initial volume was 25 mL. After 24 h the cells in the supernatant were counted and then additional 25 mL of fresh medium were added. A complete medium exchange was performed on day four and each day thereafter. All tests were performed in parallel and as replicates.

Confocal microscopy

After sampling, carriers were washed with PBS and stained with a 0.01% acridinorange - solution (Sigma, St. Louis MO, USA) (Hohenwarter 1990). The microcarriers were examined with a laser confocal microscope (Biorad, Vienna, Austria).

Statistics

Statistics were performed with Statgraphics 3.0 (Statistical Graphics Corp., Solingen, Germany) or with MS-Excel 8.0 (Microsoft, Vienna, Austria) at a confidence level of 95%.

Results and discussion

Detachment experiments

The test shows (see Figure 1), that enzymatic detachment is faster but not necessarily more efficient than detachment achieved with substances mimicking biological effects. Using heparin 0.6% of cells had detached after 6 h and 0.7% after 24 h. Merten et al. (1997) also tested heparin as detachment reagent and found that for microcarrier cultures (Cytodex 1; Amersham Biosciences, Sweden) the concentration of used substance must be much higher than for polystyrene surfaces. In our experiment heparin was not efficient to detach cells from macroporous microcarrier. Dextransulphate 2% detached 1.9% and 8% of the cells at 6 h and 24 h respectively and dextransulphate 1% detached 1.6% and 7% of the total amount of cells attached on the microcarriers. 2 ml Accutase detached 1.7% and 5 mL 2.9%, while papain released 5.0% and 5.8% of cells. Viability of detached cells ranged from 70% to 90% for treated cells and 63% after 6 h and 23.5% after 24 h in the non-treated reference experiment, while the cell density of the reference slightly decreased resulting in 0.5% and 0.4% of detached cells in suspension. The low viability in the reference experiment was as expected, because dead or dying cells detached from the carriers, while most of the viable cells remained on the carrier. We did not use trypsin as a reference in this test series as the initial aim was to test non-animal derived substances as detachment factors. However, due to the wide spread use, we added trypsin as reference in the scale-up experiments.

Carrier test with suspension cells from fermentation

The test with roller bottles was established to analyse the capability of microcarriers to support cell proliferation and attachment. Due to the slow motion of the roller bottles the cells are less affected by shear stress. Therefore the maximal cell density measured on microcarriers was much higher than in a fluidised bed reactor, as Dürrschmid et al. (2001) reported for CHO-MPS. Under less shear force conditions the cells grew in multilayer on the surface of the carrier (Figure 3). In a fluidised bed the movement of the carrier through the bed and carrier collisions inhibited this growth.

The test showed that CHO-MPS cells, which were detached with 1% dextransulphate in standard medium, can reattach normally to Cytoline 1 microcarriers (Figure 2). Cells obtained from a spinner culture using the standard inoculation protocol, repopulated microcarriers with nearly the same rate as cells obtained from the reactor. The growth rate in the logarithmic phase of the reference was 0.32 ± 0.008 d^{-1}, while the cells detached with dextransulphate showed a specific growth rate of 0.31 ± 0.009 d^{-1}. The observed difference in the duration of the lag

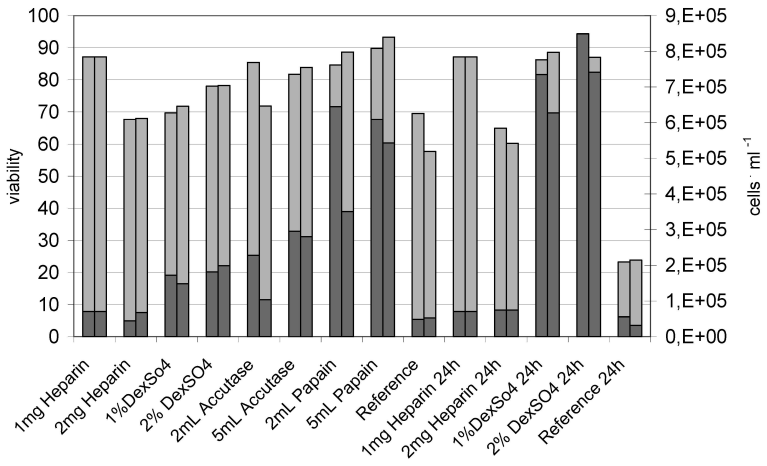

Figure 1. detachment experiments of CHO-MPS from Cytoline 1 microcarriers; □ viability, ■ cell density in supernatant.Note to Figure 1: As reference, the standard medium without any further supplementation was used.

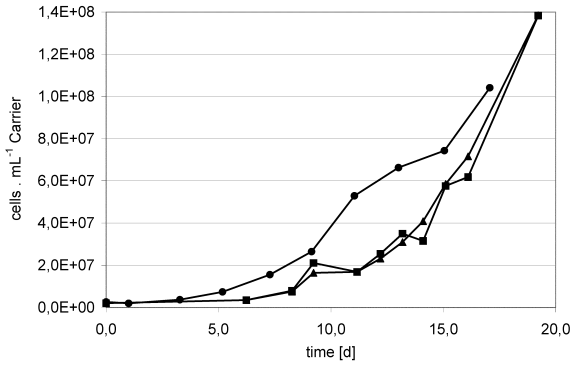

Figure 2. Carrier experiments with dextransulphate treated suspension cells of a stable fluidised bed bioprocess with CHO-MPS cells; ● reference, inoculated with cells from spinner flasks; ■ and ▲ two tests with the cells from the bioreactor.

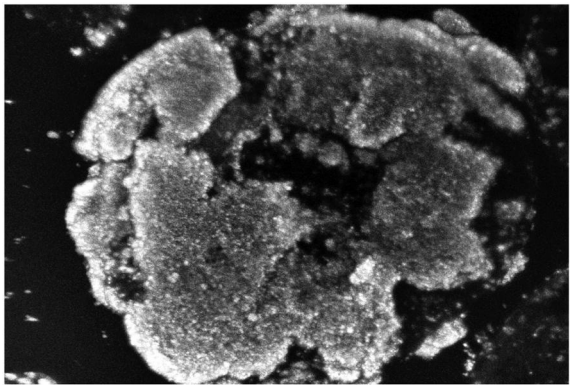

Figure 3. Photograph of an overgrown microcarrier with acridinorange stained CHO-MPS cells.

phase (one day for the non-treated reference and approximately 4 days for the dextransulphate experiment) can be explained by the continued biological effect of the dextransulphate.

Scale up - carrier to carrier transfer experiments

The scale - up experiments confirm the detachment tests (Figure 6) although the different detachment protocols did exhibit marked differences in growth rates. As a measurement of carrier to carrier transfer the regression of the exponential growth rate was performed and used as indicator for the scale-up efficiency. The highest cell densities achieved were not taken into account, because the passage ratios cannot be held constant (see above). The shortest lag-phase of the CHO-MPS cell line was obtained by colonising the microcarriers with a cell suspension while performing a carrier to carrier transfer increased the lag-phases in the peptone-free medium (Figure 4). With CHO-K1 in soy-peptone containing medium on the other hand (Figure 5) only trypsinisation and 2% dextransulphate treatment exhibited a lag phase. The observed lag-phases can be explained by two facts. First the MPS clone was cultivated in peptone-free chemically defined medium and therefore the cells were more sensitive to stress. This can lead to a more pronounced lag-phase compared to the CHO-K1 clone. Secondly, the most distinct lag-phases for both clones were observed for trypsin and dextransulphate treatment and for the CHO-K1 the Accutase experiment, which provided best growth afterwards. This indicates either that the detachment process was effec-

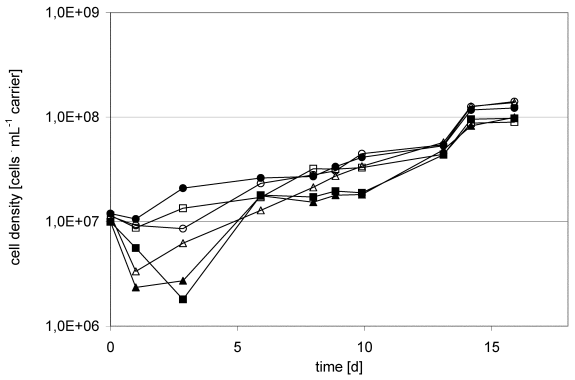

Figure 4. Carrier to carrier transfer of CHO-MPS cells using Cytoline 1 in roller bottles. Four different detachment factors were tested for enhancing the proliferation of cells after carrier to carrier transfer; ▲ dextransulphate 2%, △ trypsin, ○ Accutase, ■ dextransulphate 1%, ● reference; without further treatment, □ papain.

tive and therefore lead to the lag-phases, or that the substances had a growth inhibiting effect. For the enzymes this effect may be attributed to the continued activity in the cells themselves (Merten 2000), and in the case of dextransulphate the lag phase may be due to retarded re-colonisation.

The growth rate of cells on microcarriers inoculated with cells in suspension is significantly higher than the growth rate achieved on microcarriers inoculated through carrier to carrier transfer (Table 1). One of the most important parameters for optimal colonisation is the initial distribution of cells on the microcarriers. The best distribution can be achieved with cell suspensions containing single cells or small aggregates. To achieve optimal colonisation of freshly added carriers, adherence and aggregate formation must be controlled. In the case of cell lines exhibiting strong adherence, carrier to carrier transfer will be insufficient to achieve adequate carrier colonisation. In the case of low adherence but elevated aggregate formation, carrier colonisation will also be impaired as the aggregates will not readily reattach to vacant carriers and are subsequently lost to the harvest stream. A carrier to carrier transfer without detachment agent is possible, but resulting growth rates may be reduced. Therefore the use of detachment factors is beneficial. Enzymes help to shed more cells into suspension, but require an inactivation step in protein-free media. Therefore the use of dextransulphate can result in a less complicated procedure, as it can easily be removed by perfusion. In addition dextransulphate is cheap and of non-animal origin.

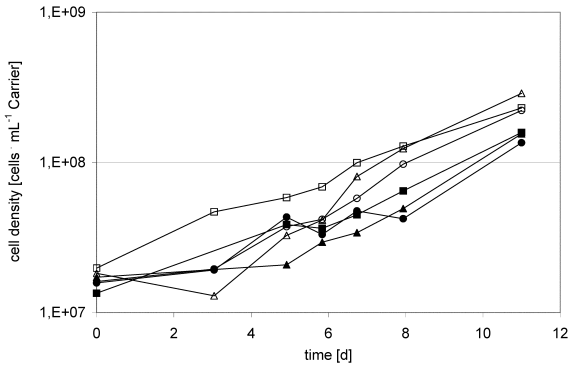

Figure 5. Carrier to carrier transfer of CHO-K1 cells using Cytoline 1 in roller bottles. Four different detachment factors were tested for enhancing the proliferation of cells after carrier to carrier transfer; △ trypsin, ○ Accutase, ▲ dextransulphate 2%, ■ dextransulphate 1%, □ papain, ● reference, without further treatment. For experimental detail, see Materials and Methods.

The time period proteolytic enzymes will be active in the medium is limited by enzyme inactivation or enzyme inhibition and in the case of too high enzyme concentrations, cells may be damaged and will not readily reattach. Substances mimicking biological functions such as dextransulphate retain their activity for longer periods of time. With such reagents the cells start to detach or can not firmly reattach. The cells are therefore more transferable as long as dextransulphate is in the medium resulting in an enhanced carrier to carrier transfer. In this case, the medium has to be exchanged to remove the detachment substances. To reduce the dextransulphate to a non functional concentration of about 0.1% will take 4 reactor volumes. As the macroporous microcarriers can also retain non-adhered or not fully adhered cells in the macroporous cavities (Voigt and Zintl 1999), the medium can be exchanged to the normal fermentation medium without substantial cell loss, thereby reenhancing attachment. The possibility of reattachment of dextransulphate treated cells was tested in a simple carrier test with dextransulphate detached cells from the reactor. These cells performed comparably with a conventionally grown inoculum. As Hu et al. (2000) reported, cell transfer depends on the inoculated cell density and the scale up ratio, the higher the cell density in seed microcarriers and the lower scale up ratio, the faster the cells move from seed microcarriers to vacant carriers. In addition with the control of the attachment and thus the distribution of cells on fresh microcarriers the carrier to carrier transfer can be carried out as required.

The choice of detachment factors may be depen-

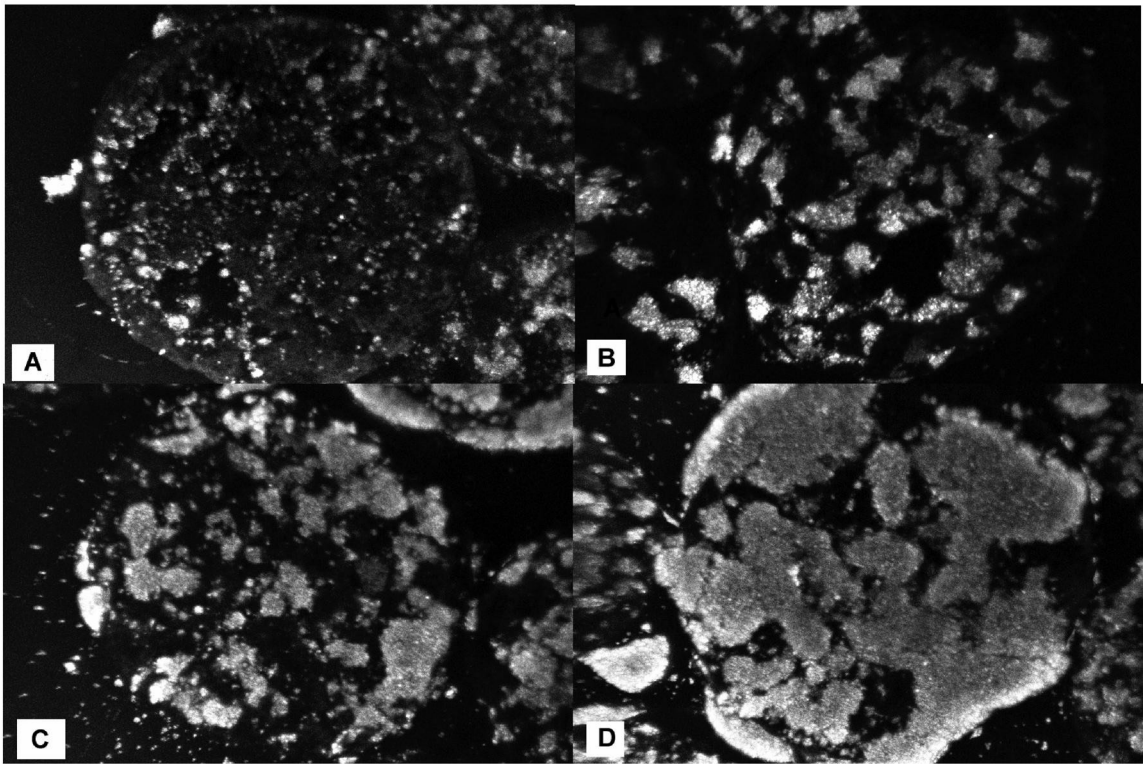

Figure 6. Photographs of Acridinorange stained CHO-MPS cells, in different stages after carrier to carrier transfer, cultivated on macroporous microcarriers; cells were detached from previous carriers with dextransulphate 1%. A: Day 3; B: Day 6; C: Day 9; D: day 15.

dent on the cell line. In our case, of the tested protocols only trypsinisation of CHO-K1 exhibited a higher growth rate during the carrier test. Therefore it was superior to the inoculation with suspension cells, although the trypsinisation procedure induced a distinct lag-phase. This result could either have been caused by the different cell history or a growth enhancing effect of the trypsin itself, as observed by

Table 1. Growth rates of tested CHO cell lines using different detachment protocols for carrier to carrier transfer.

cell line	substance	growth rate [d^{-1}]	passage ratio
CHO-K1	trypsin-solution	0.36 ± 0.026	7.9 and 6.6
CHO-K1	standard protocol	0.32 ± 0,0075	suspension cells
CHO-K1	Accutase-solution	0.31 ± 0.017	8.8 and 7.6
CHO-K1	2% dextransulphate	0.27 ± 0.029	9.2 and 6.5
CHO-K1	1% dextransulphate	0.22 ± 0.013	10.5 and 9.1
CHO-K1	papain-solution	0.21 ± 0.025	7.7 and 5.8
CHO-K1	reference	0.20 ± 0.033	9.7 and 7.7
CHO-MPS	standard protocol	0.37 ± 0.014	suspension cells
CHO-MPS	2% dextransulphate	0.25 ± 0.016	16.7 and 11.4
CHO-MPS	trypsin-solution	0.24 ± 0.0065	8.1 and 6.4
CHO-MPS	Accutase-solution	0.21 ± 0.019	10.8 and 5.6
CHO-MPS	1% dextransulphate	0.19 ± 0.017	11.0 and 6.7
CHO-MPS	papain-solution	0.15 ± 0.023	8.0 and 6.6
CHO-MPS	reference	0.15 ± 0.013	7.2 and 6. 7

Note to Table 1: The substances used for different detachment protocols are ranked by the achieved growth rates after carrier to carrier transfer. The growth rates were calculated via linear regression of the logarithm of the cell densities of the replicates of the tested CHO cell lines. The passage ratios of the replicates are both indicated in the table and were calculated indirectly by dividing the cell density on the carriers prior the carrier to carrier transfer and directly afterwards.

other groups (Amano et al. 1996). Under the given experimental conditions, trypsin showed the highest growth rate in the exponential phase followed by Accutase, dextransulphate 2% and 1%, papain and the non-treated reference.

In the case of the recombinant CHO-MPS the standard protocol using suspension cells turned out to be the optimal procedure. This was in accordance to our expectations. 2% dextransulphate and trypsin led to nearly the same growth rate during the exponential phase, followed by Accutase and 1% dextransulphate. Again papain treatment and the non-treated reference showed the slowest cell proliferation during exponential phase.

These results were not in accordance with our predictions based upon the detachment experiments. Papain, which had exhibited the best detachment performance, had nearly no effect on the carrier to carrier transfer in the scale - up experiments. This result indicates that the cells detached with papain under serum-free conditions were not able to re-attach (Merten 2000). In contrast dextransulphate with its sustained activity enables carrier to carrier transfer of cells for a larger period of time. Even at high passage ratios of 16.7 and 11.4 enhanced growth rates compared to the non-treated control were observed.

The calculated ideal passage ratio for a CHO-MPS fermentation with 2% dextransulphate in the medium during the up-scale step with $1.2 \cdot 10^8$ cells$\cdot$mL^{-1} carrier is 1:10. This calculation is based on the assumption that 8% of cells detach during this treatment and $1 \cdot 10^6$ cells$\cdot$ml^{-1} carrier are needed for inoculation of fresh carriers. For a 400 L fluidised bed reactor this would lead to an initial bed volume of 40 L with $4 \cdot 10^{10}$ cells needed for inoculation. On day 11 post inoculation the carrier should be fully colonised, thus carrier to carrier transfer could be initiated. 17 days later the newly added microcarriers should also be fully colonised and a stable fermentation phase should be reached. Starting with $4 \cdot 10^{10}$ cells approximately $5 \cdot 10^{13}$ cells could be achieved after 28 days. In comparison to a batch fermentation this would require a 50 m^3 reactor. Without any treatment the scale-up of a fluidised bed fermentation would be more difficult. The ideal scale-up ratio has to be 1:2.5 (0.5% of cells in suspension), thus a two step scale-up would be needed. An initial bed volume of 64 L and $6 \cdot 4 \cdot 10^{10}$ cells would enable the first scale-up to 160 L after 11 days. The final 1:2.5 microcarrier addition to 400 L bed volume could then be performed after 28 days. The production cell density would be reached after another 28 days. In summary this strategy would need approximately 67 days in comparison to the 28 days calculated for the fluidised bed fermentation using dextransulphate as a detachment agent. Such a fluidised bed process could be performed in one vessel, fresh carrier could be directly transferred after the first colonisation. With perfusion rates of approximately 7 bed volumes per day, a process would deliver 2800 L a day, with only one reactor.

For the MPS clone there are no differences in the specific production rate before and after a carrier to carrier transfer (unpublished data). Though, we did not test the glycosylation pattern, differences in the behaviour of the product could not be observed (unpublished data). This indicates, that there is no significant difference in the cell states of old and fresh colonised microcarriers.

Conclusions

Simple and robust inoculation strategies are essential for optimised scale-up of industrial microcarrier based bioprocesses. Carrier to carrier transfer as inoculation mode is an attractive alternative to suspension cell based inoculum strategies for either fluidised bed or stirred tank reactor fermentations. Our experiments have shown, that the careful selection of detachment factors to enhance carrier to carrier transfer can improve homogeneous colonisation of carriers and improved subsequent growth rates. Non-enzymatic detachment factors especially dextransulphate, as compared to proteolytic enzymes, have the advantage of extended gentle activity and therefore enhanced carrier to carrier transfer, controlled removal by perfusion and the substitution of animal derived enzymes and can therefore be considered a promising strategy for large scale biopharmaceutical productions.

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