

Article

Efficient Manufacturing of Adherent Cells: Mesenchymal Stem Cells and Microcarriers in Alginate Tubes

Jack Rauch ¹, Luis G. Villa-Diaz ^{2,3}  and Hendrik Viljoen ^{1,*}¹ CellGro Technologies, LLC., Lincoln, NE 68516, USA; jack@cellgrotechnologies.com² Department of Biological Sciences, Oakland University, Rochester Hills, MI 48309, USA; luisvilladiaz@oakland.edu³ The Department of Bioengineering, Oakland University, Rochester Hills, MI 48309, USA

* Correspondence: viljoen@cellgrotechnologies.com

Abstract

A method is described for the culture of adherent cells to high densities in the protective environment of alginate tubes. Microcarriers (MCs) are co-extruded with 0.8 million cells/mL in submillimeter alginate tubes to create internal surfaces of $\sim 100 \text{ cm}^2/\text{mL}$. At 30% (v/v) MCs in the lumen, the final cell density was 79.5 million cells/mL. The cell viability was 94% and a 100-fold expansion was attained. The extrusion process is automated, which makes the extrusion process straightforward. Culturing cells in alginate tubes addresses three problems which plague their production in stirred tank reactors: (1) cells are shielded from shear stress, (2) uncontrolled cluster growth is prevented because cluster size is limited to the inner tube diameter, and (3) the micro-environment within the tubes affects cell fate and this micro-environment can be replicated independently of reactor size. Thus, this method allows scaling up to become significantly easier.

Keywords: adherent cells; culturing; alginate tubes

1. Introduction

Mesenchymal stem cells (MSCs) are an important building block in tissue engineering and endogenous wound repair, and significant work has been done to develop more efficient ways to culture them. Producing cells with well-defined characteristics at affordable costs and in quantities which meet clinical demands remains a challenge [1]. MSCs produce growth factors, cytokines and exosomes which make them of interest not only for structural uses, but also for producing valuable molecules. The characteristics and properties [2] of MSCs are affected by the culturing conditions, and this sensitivity emphasizes the importance of cell manufacturing practices to produce repeatable outcomes.

Godara et al. [3] reviewed bioreactors for MSCs' manufacturing. Spinner flasks, rotating wall reactors, wave reactors, packed bed reactors, hollow fiber reactors and parallel plates are platforms that provide surfaces for cell attachment. Harvesting cells from hollow fiber reactors and packed bed reactors is difficult and these reactors are mostly used for producing exosomes and other biologics [4]. Stirred tank reactors are most commonly used for culturing adherent cells. The reactors are seeded with 1–2% MCs with pre-attached cells and produce 0.2–0.7 million cells per mL^3 . The MCs have the propensity to form clusters. A main reason for the low seed density is to limit the cluster size—which can form necrotic centers if grown unchecked. Impeller speeds are adjusted to break up clusters and slough off cells in an effort to control the size of clusters, but this practice increases the shear stress on cells. The effect of shear depends on the cell type, but phenotypic changes,



Academic Editors: Francesca Raganati and Alessandra Procentese

Received: 17 September 2026

Revised: 21 October 2026

Accepted: 22 October 2026

Published: 27 October 2026

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reduced growth rates and cell death are attributed to shear stress [5,6]. Fluid motion in a stirred tank reactor depends on the impeller speed, reactor dimensions and fluid properties, and it is very difficult to obtain a quantitative picture of velocities and stresses on the millimeter-to-micron scale—the scale on which cell fate is determined. To reproduce the flow conditions on a larger scale is therefore extremely difficult and experimentation and empiricism are the avenues mostly used to solve scaling problems. The difficulty of scaling up the reactors to produce sufficient material for clinical applications is seen as a major bottleneck [3,7,8]. In stirred tank reactors a compromise is sought between minimizing shear and limiting cluster size. The main drawback to large-scale manufacturing of MSCs is surface area. Fixed-bed reactors, packed with microcarriers [9], offer large surface areas, but other challenges remain. Cell maintenance requires that media are percolated through the packed bed, which may still leave pockets of cells deficient in nutrients and oxygen. Harvesting is also more challenging, since the releasing agent (e.g., accutase) must access all regions in the reactor. Hollow fiber reactors also have large surface areas, and they provide a more orderly way to supply nutrients than fixed-bed reactors, but face the same challenges for cell harvesting. The manufacturing of MSCs on microcarriers which are suspended in stirred tank reactors is attractive from a nutrient-supply point of view, but the concern is that microcarriers can form clusters with subsequent formation of necrotic centers. The aggregation is oftentimes countered by increased stirring, but this approach is counter-productive from a hydrodynamic shear point of view.

The ideal solution would be to eliminate all shear on cells and to limit clusters to a size that would prevent necrosis. Culturing cells in a sheltered environment with effective nutrient and metabolite transport will bring us much closer to the ideal solution. An example of such an environment is hollow alginate tubes. The culturing of suspension cells in alginate tubes has been reported [10–20]. There have been attempts to culture adherent cells in alginate tubes, but with limited success. Wang [21] described a method to modify the alginate walls to help the attachment of MSCs. In this method, arginyl-aspartic acid (RGD) modifies the alginate surface to create attachment points for cells; however, it is a time-consuming process and cells only attach to the inner tube wall. Wang [21] found that the cells grew slowly, forming only single layers on the inner tube walls and not in the lumen of the tubes which is the large surface area inside the alginate tubes. To our knowledge, the encapsulation of MCs in alginate tubes has not been reported. Suspension cells are usually encapsulated in the alginate tubes with a tri-axial extruder [22,23]. Rauch and Viljoen [24] developed a tri-axial extruder that can encapsulate both MCs and adherent cells in alginate tubes without clogging. The choice of microcarriers is usually based on properties of the coating material like the affinity for cell adherence and promotion of cell growth and the carrier size. Typical carrier materials like poly-styrene offer ample binding opportunities for coatings. For example, Synthemax II is a proprietary peptide acrylate polymer that binds to poly-styrene and it was also the substrate used for the D1 cells' expansion. Coatings can also capture and stabilize growth factors, which extend the expansion horizon of cells. Synthesized sulfonated polymer coatings [25] that stabilized basic fibroblast growth factors resulted in a 40% increase in growth rate of hMSCs compared to tissue culture poly-styrene substrates. Cell harvesting requires the detachment of cells from carriers, and dissolvable microcarriers become an attractive option. Extrusion of microcarriers in alginate tubes also requires careful consideration of the particle size. By experimentation it was determined that microcarriers with an average diameter of less than 200 μm do not cause clogging problems, provided their volume fraction is less than 50%.

In this method, cell harvesting is straightforward, as the tubes are easily dissolved, and then the protocol for stirred tank reactors is followed. An additional advancement of this method over current extruder methodologies is the replacement of syringe pumps

with peristaltic pumps and pulse dampeners to eliminate the constraint that only limited volumes can be extruded. Cell encapsulation can be performed with different hydrogels. Carrageenan and alginate were considered for this study, and alginate was selected because it produced more consistent tube dimensions with our extruder.

Stankiewicz and Moulijn [26] offered an interesting overview of process intensification, and made a case for creative, non-traditional solutions to improve a process. Here we describe a process to co-extrude MSCs and MCs in alginate tubes, culture the cells to confluency and harvest them. It marks a creative departure from the traditional method of adherent cell manufacture.

2. Materials and Methods

2.1. Materials

Mouse bone marrow MSCs D1 cells (CRL-12424) were purchased from ATCC. Cell culture reagents include Trypsin-EDTA 0.25% (Gibco, #25,200,114), Accutase (Invitrogen, #00455556), Dulbecco's Modified Eagle Medium (Gibco, #11,965,126), Fetal Bovine Serum (Gibco, #A5256701), Antibiotic–Antimycotic (Gibco, #15240062), Geneticin Selective Antibiotic (G-418) (Gibco, #10,131,035), 100 μ m Cell Strainer (CELLTREAT, #229485), sodium alginate (Wako Chemicals, #194–13,321), Methylcellulose Stock Solution (R&D Systems, #HSC001), CGT Harvesting Solution (CellGro Technologies), Corning Low Concentration Synthmax II Microcarriers (Corning, #3781), CGT Automated Cell Encapsulation System (ACE-1, CellGro Technologies), Calcium chloride hexahydrate (Thermo Scientific Chemicals, #AC389262500), and Trypan Blue Stain (0.4%) (Invitrogen, #T01282).

2.2. Expanding D1 Cells in Preparation for Extrusion

D1 cells were seeded at 10^4 cells per cm^2 in 25 cm^2 flasks. The D1 cells were maintained as per the instructions provided by the ATCC. Cells were cultured in DMEM supplemented with 10% FBS and 1% Antibiotic–Antimycotic. The medium was changed every other day. The cells were incubated at 37 °C and with 5% CO_2 . Confluent cells were treated with 0.25% Trypsin-EDTA at 37 °C for 5 min and dissociated into single cells for passaging every 3–4 days.

2.3. Cell and Microcarrier Encapsulation

Cells and MCs were encapsulated in alginate tubes using the automated ACE-1 system [23] (CellGro Technologies, Lincoln, NE, USA). Cells were not pre-attached to MCs. Referring to Figure 1, a brief description of the encapsulation process is as follows. A core solution that contains single cells at a concentration of 0.8 million/mL and MCs at volume percentages of 22 and 30 in growth medium is constitutively modified with methylcellulose to increase its viscosity. The suspension is pumped through the central line of the extruder with a peristaltic pump. To maintain even flow rates, a pulse dampener is used. A 2% *w/v* sodium alginate solution is pumped through a second line that forms an annulus around the cell suspension. To maintain a stable laminar structure of the combined flows, the cell suspension must be of equal or greater viscosity than the alginate solution, hence the modification of the growth medium [27,28] with methylcellulose. A 100 mM CaCl_2 solution flows around the annulus and cross-links the alginate, to form a thin shell around the cell suspension. The dimensions of the tubes are in the order of 700–800 μ m OD and 550–700 μ m ID.

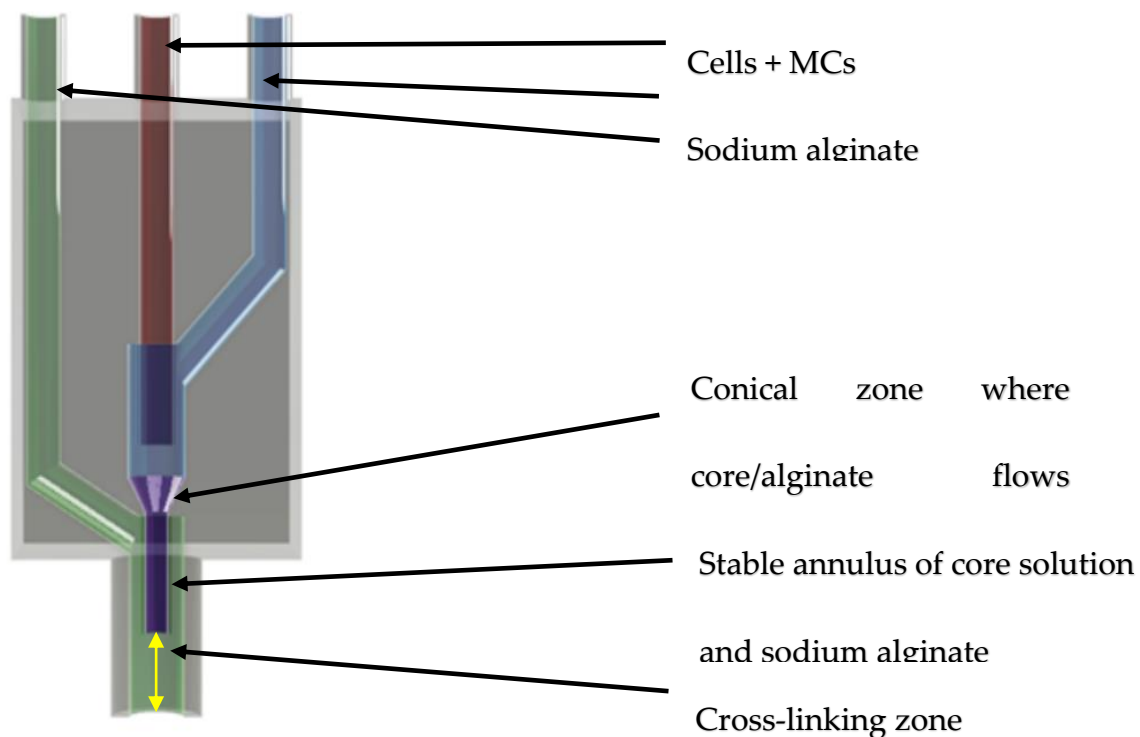


Figure 1. Cross-sectional schematic of a single port extruder. The core fluid (red) is a cell and MC suspension that flows in the center channel and the sodium alginate solution (blue) enters an annulus to flow concentrically around the core fluid. The CaCl_2 solution (green) enters a second annulus to flow concentrically around the alginate solution and cross-link it. The yellow arrow marks the zone where cross-linking occurs.

2.4. Cell Expansion

Alginate tubes with a lumen volume of 50 μL containing 22% (30%) MCs and 0.8 million cells/mL were cultured in 5 mL of complete medium in a 25 cm^2 flask. The tube length varied between 13 and 21 cm, based on the inner diameter. Each flask contained one tube. Medium was changed daily. Glucose concentration of fresh medium was 20.5 mM. Cells were incubated at 37 $^\circ\text{C}$ and with 5% CO_2 to control the temperature and pH.

2.5. Harvesting

D1 cells in alginate tubes were harvested on days 3, 5, 7, and 10. At least two tubes were harvested at each time. A schematic of the harvesting process is shown in Figure 2. First the media were removed, then the alginate tubes were treated with 10 mL of CGT Harvesting Solution and incubated at room temperature for 5–10 min. The CGT Harvesting Solution was carefully removed without disturbing the settled cells that adhered to microcarriers. To dissociate the cells from the microcarriers, they were treated with 5 mL of Accutase and incubated at 37 $^\circ\text{C}$ for 5 min. The D1 cells were removed and mechanically disrupted with a pipette. This was repeated twice. The D1 cell solution was passed through a 100 μm Cell Strainer into a 50 mL conical tube. The flask and strainer were rinsed with 5 mL of media. The D1 cell solution was centrifuged at 300 g for 5 min. The supernatant was removed after pelleting. D1 cell pellets were resuspended in respective media. A 30 μL sample of each cell solution was diluted with 30 μL of Trypan Blue, and the total cells and percentage of viable cells from each tube were calculated by a Thermo Fisher Countess 3 Cell Counter Assay Platform using Trypan Blue exclusion ($n \geq 2$).

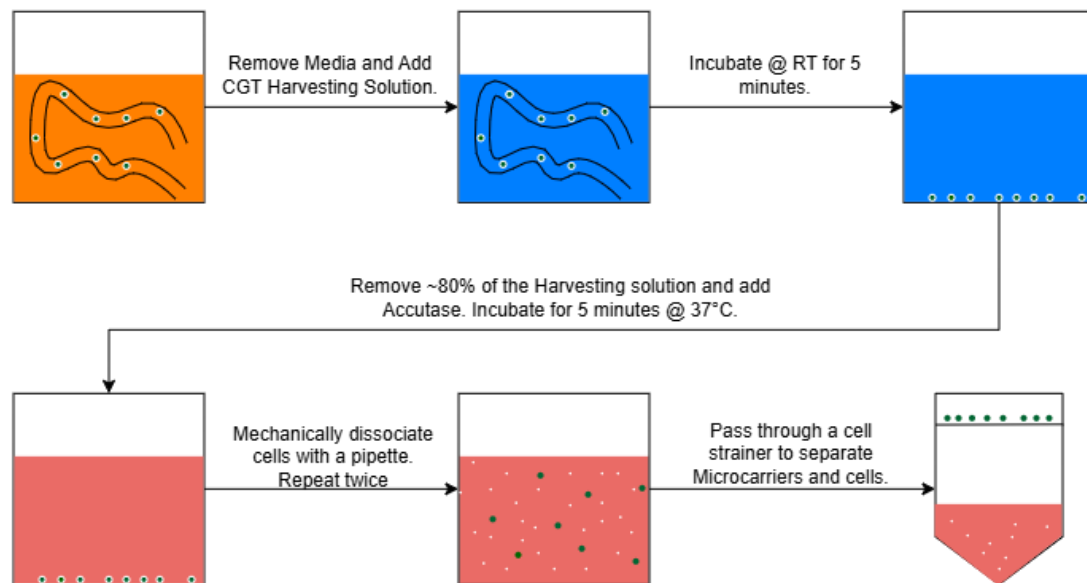


Figure 2. A schematic of the harvesting process.

3. Results

Figure 3 shows representative micrographs of D1 mesenchymal stem cells and MCs encapsulated in alginate tubes.

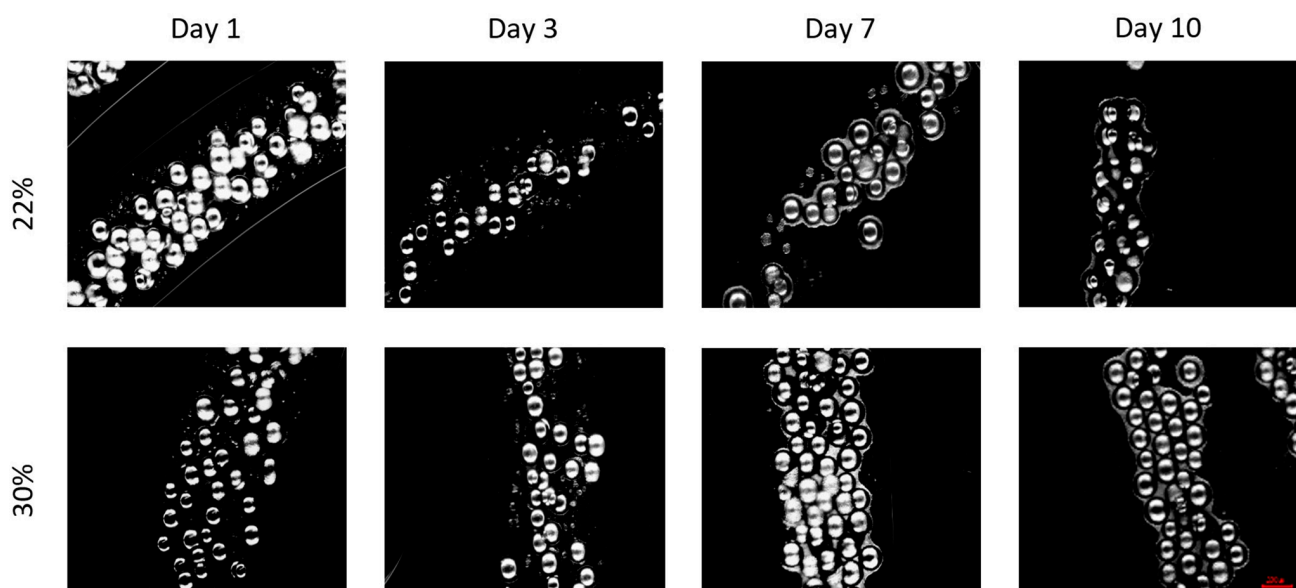


Figure 3. Representative micrographs of D1 cells growing on MCs in alginate tubes on days 1, 3, 7, and 10; (top) 22% and (bottom) 30% of MCs. Scale bar = 400 μm .

Cells first grow in layers around the MCs, then bridging occurs between adjacent MCs. In the case of the 30% vol., interstitial spaces appeared to be filled in.

Plots of cell densities in the lumen as functions of time are shown in Figure 4. For both volume fractions of MCs, the initial cell density is 0.8 million cells/mL. The growth rates were the same for the first four days (Figure 4a); however, after day 4 a faster growth rate was noted for the cells at 30% MCs. Over the first five days the doubling time was less than 24 h, it increased to 30 h after a week, and near confluency the doubling time was 40 h. The slowdown is attributed to contact inhibition after cells reached confluency and fewer attachment sites on the MCs can be found. On the other hand, the higher growth rates after

day 5 for 30% MCs might be due to the larger number of binding sites. The harvested cell densities of the 22% and 30% microcarrier concentrations were 60 and 79.5 million cells/mL respectively—that translates into 75-fold and 98-fold expansions. The cell viabilities were 90% and 94% for the 22% and 30% MC concentrations.

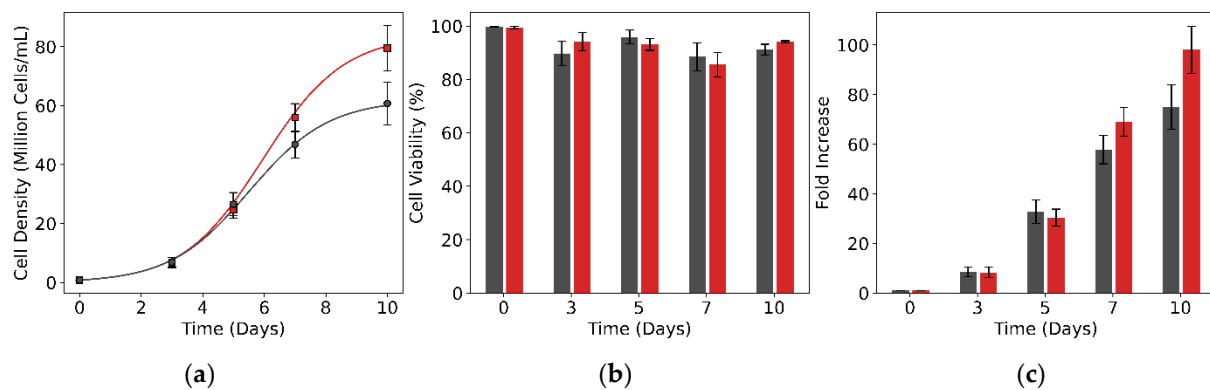


Figure 4. (a) Cell density, (b) cell viability, and (c) fold increase measured on days 0, 3, 5, 7, and 10 for 22% (black) and 30% (red) MC concentrations. Black is used for 22% and red is used for 30% MC concentrations. The experimental data show the mean $\pm$ one standard deviation.

The focus of this study was to demonstrate the feasibility to co-extrude microcarriers and MSCs in alginate tubes and culture the cells in a stress-free environment, and a complete characterization of stemness and tri-lineage differentiation is planned for a future study. However, it is instructive to compare micrographs of MSCs before extrusion (Figure 5a) and MSCs harvested on day 10 (from tubes at 30% *v/v* MCs) and plated at 100,000 cells in 25 cm² flasks (Figure 5b).

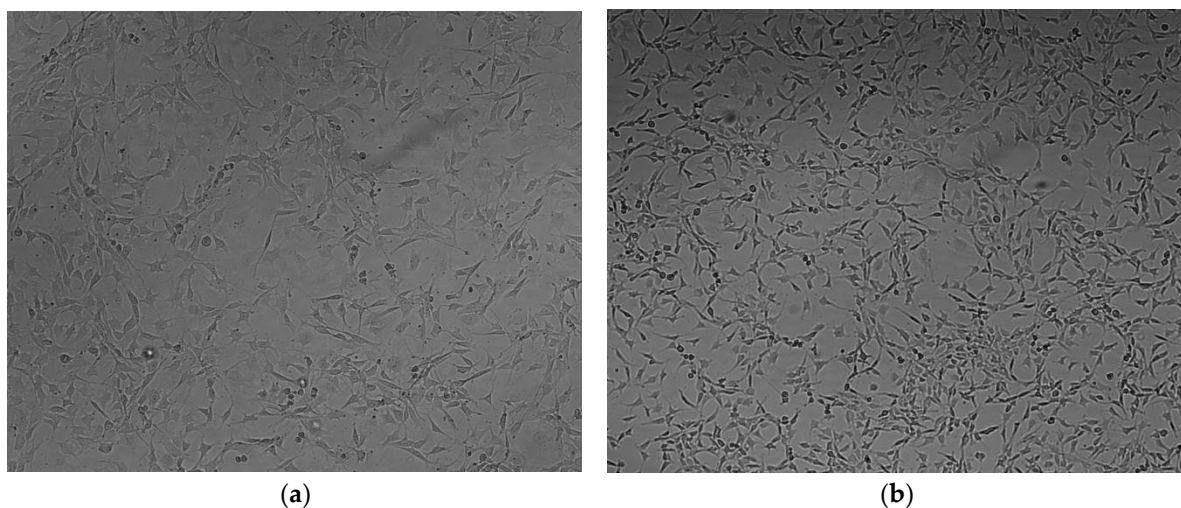


Figure 5. (a) Micrograph of D1 cells before extrusion. (b) Micrograph of D1 cells which were harvested from alginate tubes on day 10 and then plated. Scale bar: 100 μ m.

Cells from Figure 5 exhibit normal spindle morphology, having 2+ attachment points in both before and after extrusion. D1 cells harvested from 30% volume fraction microcarriers on day 10 were plated at 100,000 cells in 25 cm² flasks. This process was repeated two times for a total of three passages after expansion in the tubes. For each passage the cells were passaged on day 3. The doubling time for each passage was 22.3, 21.3, and 22.2 h. This is consistent with doubling times between 20 and 24 h for these cells before. The doubling

time of post-harvested cells is 22 ± 0.5 h (repeated three times), which is consistent with doubling times of 20–24 h for cells before extrusion.

In Figure 6 a single MC is shown, with the layer of cells surrounding it. There were sections of the tubes where cells appeared as a congruous mass, and the interstitial spaces have been filled in, but it was not the case everywhere. Based on these results, we develop an estimate of how many cells can be produced per mL of the lumen. There are approximately 85,500 MCs in 1 mL volume at 22% carrier load. Since the yield at harvesting was 60 million cells/mL, it implies that the average number of cells per MC is 700 cells. The cells are packed around a MC in a layer of average thickness L_c . The density of the layer is denoted ρ_c . The thickness of the layer estimated from the micrograph in Figure 6 was calculated as follows: $L_c = (238 \mu\text{m} - 175 \mu\text{m})/2 \approx 30 \mu\text{m}$. Thus, the cell density of the layer was

$$\rho_c = 700 \times \frac{6}{\pi \left(\left((D_p + 2L_c)^3 - D_p^3 \right) \right)} = 185 \text{ million cells/mL} \quad (1)$$

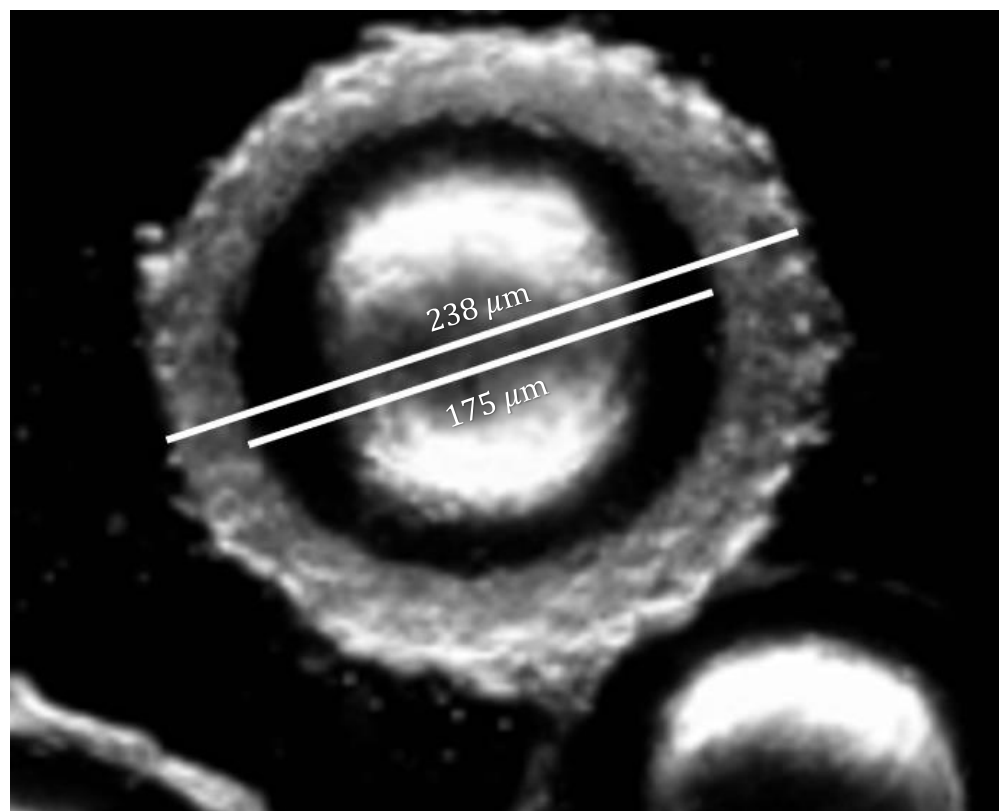


Figure 6. Micrograph of a single carrier with a cell layer around it; the MC density is 22%. Picture was taken on day 10. The thickness of the cell layer is approximately 30 μm .

Note that the cell density in the layer is higher than the cell density in the lumen, because it discounts the volume of the MCs and the interstitial spaces which are not filled by cells.

Using Equation (1), one can derive a general expression for the maximum cell density in the lumen as a function of microcarrier fraction C_f and average carrier size D_p :

$$C_{max} = C_f \rho_c \frac{\left((D_p + 2L_c)^3 - D_p^3 \right)}{D_p^3} \quad (2)$$

C_{max} is a theoretical maximum cell density that can only be reached if all void spaces between MCs have been filled by cells. Equation (2) can now be used to calculate the cell density when 30% MCs are used;

$$C_{max} = 0.3 \times 185 \frac{((0.175 + 0.06)^3 - 0.175^3)}{0.175^3} = 81.8 \text{ million cells/mL.} \quad (3)$$

The experimental cell density at confluency (30% MCs) was 79.5 million cells/mL (Figure 4a). The experimental result was close to the theoretical value. When 30% MCs were used, there were 116,600 particles in one mL of the lumen and the average number of cells per carrier was calculated to be 682 cells. This number is less than the theoretical estimate of 700 cells. It is reasonable to expect that at higher carrier concentrations, not all surfaces are accessible and steric hindrances can lower the cell number per carrier. One expects that the cells per carrier will decrease further, in higher MC concentrations. The point can be made, albeit heuristic, that there exists a local optimum of MC concentration to maximize cell density.

4. Discussion and Future Work

In this paper a small volume of alginate tubes was extruded with the objective to determine the final cell densities at 22% and 30% MC concentrations. The extrusion process worked flawlessly and the cell densities, as shown in Figure 3, had average experimental values of 60 and 79.5 million cells/mL. Significant process intensification is possible and future work will focus on expanding D1 cells and human MSCs in larger volumes of alginate tubes. Our 50 mL single-use bioreactor houses up to 10 mL alginate tubes, equivalent to 6 mL lumen volume. Based on current results, we expect that the 50 mL reactor could produce 450–500 million cells, which is equivalent to cell densities of 8–10 million/mL (reactor volume). This will result in a process intensification of more than ten times in comparison to stirred tank reactors. Typical doses of hMSCs are 1–4 million cells/kg body weight [29]. Although hMSCs have not been used in this study, the cell densities of 60–80 million/mL provide an indication of the number of cells which can be produced at larger scale. Multi-nozzle extruders [23] are available to produce larger volumes of tubes. The larger volume of tubes will be processed in a bioreactor that is specifically designed for this task. CellGro Technologies has developed a single-use bioreactor with a 50 mL working volume. It can house up to 6 mL (lumen) of alginate tubes and it operates in an incubator. The future work, referenced earlier in regard to more in-depth cell characterization, will demonstrate the culturing of MSCs in this reactor. To put it in perspective, culturing of MSCs on microcarriers in stirred tank reactors produces between 0.2 and 1 million cells per mL of reactor volume. If 4 mL tubes are cultured in our bioreactor to confluency, then one expects to harvest 240–300 million cells (20% and 30% microcarrier fraction). Thence 4.8–6 million cells/mL of reactor volume is a more-than-ten-fold process intensification.

The results reported in Figure 4 were obtained with MCs with an average size of 170 μm . The question is how will smaller MCs, at similar volume fractions, affect the final cell density? Consider MCs of uniform size 130 μm . Assuming that the cell layer thickness L_c and layer density ρ_C remain the same, then the final density of 30% MCs of size 130 μm is $C_{max} = 113$ million cells/mL.

In theory, the use of MCs smaller than 170 μm is expected to lead to higher cell densities in the lumen. However, one should keep in mind that the inverse of the carrier radius determines the curvature, and cell attachment is reduced when curvature becomes large [4]. A theoretical upper bound on cell density at 30% MC, based on all interstitial spaces filled to a density ρ_C , is 129.5 million cells/mL. To clarify the difference between ρ_C and C_{max} , the cell density of 113 million cells/mL is based on a layer of thickness $L_c \approx 30 \mu\text{m}$

around each carrier with some unfilled interstitial spaces, and the density of 129.5 million cells/mL is the case where all the interstitial spaces are filled with cells.

At such high cell densities, it becomes a concern whether cells located near the center of a tube will receive sufficient oxygen and nutrients. Denote the oxygen and glucose concentrations in the media surrounding the tube as C_{ox} and C_g (let glucose be an example representative of all nutrient species). The minimum nutrient and oxygen concentrations exist at the center of the tube. If any one of the concentrations at the tube center drops below a critical value C_{crit} (these critical concentrations exist for all the essential nutrients), necrosis could set in. Working backwards from the C_{crit} values for a cell density at confluency, one can calculate the minimum concentrations in the medium outside the tubes. Since C_{crit} depends on the tube diameter and wall thickness, the reactor is controlled to meet the demands at confluency for specified tube volumes and dimensions.

The concentrations of oxygen and the other nutrients are at minimum at the center of the tube. Equation (4) was derived [20] for the purpose to determine if the nutrient and oxygen concentrations in the reactor are sufficient to meet the demands. The criterion relates C_{crit} to tube dimensions (inner and outer tube diameters $D_{Ti,o}$) to the concentration outside the tube C_g and the cell density C_{cell} in the tube. If the inequality holds, then cells at the center of the tube are not glucose-deficient.

$$D_{Ti}^2 + 2D_{Ti}(D_{To} - D_{Ti}) - \frac{16D_a}{k_u C_{cell}} [C_g - C_{crit}] \leq 0 \quad (4)$$

The diffusion coefficient of glucose in the tube is D_a ; it is similar to the diffusion coefficient in growth media, but corrected for the cell and microcarrier packing [30]. The rate of glucose consumption is k_u . An obvious conclusion is that larger tubes would require higher concentrations C_g in the surrounding media. Equation (4) applies to other nutrients and oxygen, as well as metabolites. To apply Equation (4) to metabolites, C_{crit} is the maximum concentration (e.g., lactic acid) cells can tolerate. The sign of k_u is reversed to reflect a production rate instead of a consumption rate. Using glucose as an example, C_{crit} denotes the lowest glucose concentration for cell viability. From our results, we know that $C_{cell} \approx 60$ million/mL at a load of 22% (v/v) microcarriers. The tube dimensions are also known (700–800 μm OD and 550–700 μm ID). Using Equation (4), the concentration of glucose in the reactor C_g is calculated.

Next, we present a theoretical cell growth model. Let S and C be the glucose and cell concentrations, measured in mM and million cells/mL. The initial concentrations are S_0 and C_0 . It is known that cell growth is limited by the availability of glucose and the available sites for cell attachment on the MCs. A Monod model is used for glucose and a Verhulst model for the available attachment surface.

$$\frac{dC}{dt} = k_{max} \frac{SC}{k_1 + S} \left(1 - \frac{C}{C_{max}} \right) \quad (5)$$

$$\frac{dS}{dt} = -Y \frac{V_t}{V} C = -k_2 C \quad (6)$$

k_{max} is the maximum growth rate, k_1 is the half-saturation constant for glucose, and Y is the rate at which glucose is consumed. The lumen volume is $V_t = 50 \mu\text{L}$ and the growth medium volume is $V = 5 \text{ mL}$ (cf. Section 2.4). The parameter values are listed in Table 1.

Table 1. Parameter values for the Monod model.

k_{max}	k_1	Y	V_t	V
1.386/day	12 mM	0.004 mmol/million cells/day	50 μL	5 mL

The black and red plots in Figure 4a are the solutions for Equations (5) and (6) for MC fractions of 22% and 30%. Note that the equations are integrated over periods of 24 h to match the media exchanges (media exchanges occurred daily). Starting conditions for each interval are $S_0 = 20.5$ mM and C_0 , where C_0 equals the endpoint of the previous interval (continuity of $C(t)$). The experimental data points are also plotted, and they match the growth model very well.

5. Conclusions

This study has shown that it is feasible to culture adherent cells on microcarriers in alginate tubes. Culturing cells in alginate tubes addresses three problems which are plaguing their production in stirred tank reactors: (1) cells are shielded from shear stress, (2) uncontrolled cluster growth is curbed by the inner diameter of the alginate tube and (3) scaling is simpler because the micro-environment in the tubes, which determines cell fate, is conserved, regardless of the number of tubes. Cells grow to high densities in the tubes. At 22% and 30% (v/v) densities of 60 million /mL and 80 million/mL were obtained. These high cell densities enable a significant process intensification. To illustrate this point, if alginate tubes with a 4 mL lumen volume are expanded to confluency in a 50 mL reactor, then more than 300 million cells can be harvested, which is 6 million cells/mL of reactor volume. The optimum fraction of microcarriers to maximize cell density has not been determined because fractions of 50% and more caused clogging during extrusion.

Cell densities of 6 million cells/mL are almost an order of magnitude more than current reactors [3], but other technologies are also being developed to create 3D scaffolds with large surfaces [31,32]. Electro-spun PLGA fibers with poly-lysine grafting have produced yields that have exceeded conventional 2D methods. But these advances must be calibrated; there are many factors aside from cell densities which determine the efficacy of an expansion process [33], like tri-lineage differentiation potential, epigenetic changes and changes in proliferation and viability. A typical upper limit of 5–10 passages triggers the onset of senescence and the occurrence of phenotypical changes and expansion protocols must balance cell proliferation and conservation of multi-stemness. In an excellent review of the limitations of large-scale MSC manufacturing [34] the key elements include replicative senescence, bio-processed induced mechanical stress, oxygen and nutrient gradients and functional decline. The technology described in this study addresses several of these pain points, the 100-fold expansion in a single passage, the shielding from shear stress in the tubes and the control of intratubular oxygen and nutrient gradients, by selecting tube diameters and microcarrier volume fractions in advance. To summarize, this technology has the potential to advance current manufacturing practices of MSCs.

Author Contributions: Conceptualization, J.R. and H.V.; methodology, J.R. and H.V.; formal analysis, J.R. and H.V.; investigation, J.R.; writing—original draft, H.V.; writing—review and editing, J.R., L.G.V.-D., and H.V.; visualization, J.R., and H.V.; supervision, H.V.; project administration, J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Further information and requests for resources should be directed to the lead contact, Hendrik Viljoen (viljoen@cellgrotechnologies.com).

Acknowledgments: The work was supported by a Phase II R&D grant awarded by the Nebraska Department of Economic Development.

Conflicts of Interest: Authors HV and JR were employed by the company CellGro Technologies. The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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