Research Article

Dissolvable gelatin-based microcarriers generated through droplet microfluidics for expansion and culture of mesenchymal stromal cells

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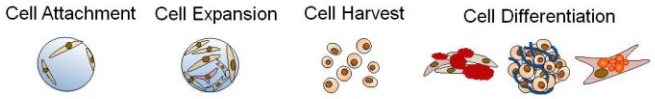
Abstract

Microcarriers (MC) are synthetic particles used in bioreactor-based cell manufacturing of anchorage-dependent cells to promote proliferation at efficient physical volumes, mainly by increasing the surface area-to-volume ratio. Mesenchymal stromal cells (MSCs) are adherent cells that are used for numerous clinical trials of autologous and allogeneic cell therapy, thus requiring avenues for large-scale cell production at efficiently low volumes and cost. Here, a dissolvable gelatin-based microcarrier was developed for MSC expansion. This novel microcarrier showed comparable cell attachment efficiency and proliferation rate when compared to several commercial microcarriers, but with higher harvesting yield due to the direct dissolution of microcarrier particles and thus reduced cell loss at the cell harvesting step. Furthermore, gene expression and in vitro differentiation suggested that MSCs cultured on gelatin microcarriers maintained tri-lineage differentiation with similar adipogenic differentiation efficiency and higher chondrogenic and osteogenic differentiation efficiency

when compared to MSCs cultured on 2D planar polystyrene tissue culture flask; on the contrary, MSCs cultured on conventional microcarriers appeared to be bipotent along osteochondral lineages whereby adipogenic differentiation potential was impeded. These results suggested that these gelatin microcarriers are suitable for MSC culture and expansion, and could also potentially be extended for other types of anchorage-dependent cells.

Keywords: Cell Manufacturing, mesenchymal stromal cells, microcarrier, multipotency, regenerative medicine.



Microcarrier-based cell expansion has low cell recovery efficiency during harvesting step. In this work, a gelatin-based dissolvable microcarrier is developed for cell expansion. High cell yield is achieved through direct dissolution of microcarrier for cell recovery. In addition, mesenchymal stromal cells expanded on gelatin microcarrier maintain tri-lineage differentiation potency. Thus, gelatin microcarrieer could be a promising tool for cell manufacturing.

Abbreviations

CoV, coefficient of variation; CCE, counter-flow centrifugation elutriations; ISCT, International Society for Cellular Therapy; MC, microcarrier; MSC, mesenchymal stromal cell; OPN, osteopontin; PPARγ, Peroxisome Proliferator Activated Receptor Gamma; RUNX2, Runt-related transcription factor 2; sGAG, sulfated glycosaminoglycan; TCF, tissue culture flask; TFF, tangential flow filtrations.

1 Introduction

Mesenchymal stromal cells (MSCs) comprise multipotent cells that can undergo tri-lineage differentiation into adipo-, chondro- and osteogenic mesenchymal tissue lineage. A subset of these cells can be classified as mesenchymal stem cells, but it is now generally accepted that the MSC population that is expanded *in vitro* exhibits heterogeneity of physical and phenotypic attributes[1-3]. In regenerative medicine applications including cell therapy, MSCs are of great current interest as they can be isolated readily from various adult tissues and further expanded to treat diseases such as osteogenesis imperfecta[4], graft-versus-host disease[5, 6]. bone and cartilage defects[7], and myocardial infarction[8]. MSCs or a subset thereof exhibit the capability to self-renew to replace damaged tissue[9,10], but can also act as a "drug factory" when administered *in vivo* to secrete beneficial factors that speed repair by paracrine signaling to endogenous cells and tissues[11]. For such MSC-based treatment, there is a need to expand MSCs in great quantity, either to produce sources of cells defined by critical quality attributes from a donor source to treat that donor as also the patient (autologous MSC therapy) or a source for multiple patients (allogeneic MSC therapy), or to meet the required cell dose per patient depending on the type of disease[12-14].

Conventional 2D planar culture is the current standard practice for expansion of anchorage-dependent cell type such as MSCs. However, the associated large physical space occupied by culture flasks and culture media fluid volumes required of a planar culture surface is incompatible with cost-efficient cell population scale-up, and can also result in gradients of nutrients and gases that affect cell phenotype[15]. Therefore, microcarrier (MC) technology was developed to provide attachment surfaces for anchorage-dependent cells to adhere to and proliferate inside bioreactor vessels. Advantages of MC culture include high surface area-to-

volume ratio which allows more cells to attach per culture volume, scalability for parallel processing, achievable homogeneous environmental conditions in a stirred MC suspension culture, and minimal shear stress on cells as MCs are freely moving in a stirred culture[16]. To date, different types of MCs have been developed and are commercially available. MCs typically take the form of a sphere which has a maximum high surface area-to-volume ratio, and can be macroporous (e.g., CultiSphere-S), microporous (e.g., Cytodex-1 and Cytodex-3) or nonporous (e.g., SoloHill Collagen Coated (Sartorius [C102-1521]) and Synthemax).

MC technology has been widely used for MSC expansion. Tavassoli et al. has reviewed several types of MCs used for MSC expansion[17]. Also, several works have been reported to develop a scalable and robust cell manufacturing process by growing MSCs on microcarriers in vessels of various volume and size, ranging from a small spinner flask[18] to an automated tabletop microbioreactor[19], to a 5-50 L bioreactor setup[20,21]. Conventional MCs are designed with a focus on promoting initial cell attachment efficiency and proliferation rate of cells on the microcarrier particles. However, when applied to cell manufacturing that aims to deliver just the cells to the patient (separated from those microcarriers), the yield of resuspended cells can be compromised in those steps if that separation of the cells from the MCs is inefficient[22]. One challenge in cell harvesting is the use of enzymes to detach cells from the microcarriers. While the detachment efficiency of cells from microcarrier is inferior compared to that of the 2D planar surface with longer incubation time and lower cell retrieval rate, repeated and long exposure to enzymes have shown to modify the cells down to the molecular level[23,24]. A rapid cell detachment and harvesting strategy are therefore preferred to maintain cell quality. Also, there is a lack of available suitable technologies particularly at the liter-scale to efficiently separate detached cells from the microcarrier suspension. Conventionally, membrane filters of

appropriate pore size could be used to separate cells from the particles. The filtering step with membrane filters (e.g., cell strainer for small volume or Steriflips for up to 50 mL filtration) could lead to cell loss during the cell-particle separation, as cells could be trapped in between the particles and the filter membrane[25]. Collectively, both harvesting and separation steps present a persistent challenge in efficient cell manufacturing.

The cell harvesting step is technically challenging, and cell loss can be due to the user's technique or increased level of aggregation that may deter cell release during enzymatic treatment. As such, more sophisticated harvesting protocols have been developed by introducing mechanical agitation during the harvesting step. Nienow et al. has reported >95% cell recovery rates upon separation with multiple steps of spinner flask agitation[26,27]. Ultimately, this requires optimization of the process on different culturing platforms (e.g., agitation speed and duration) and with different types of enzymatic reagent; these improvements can require technical practice and expertise for such modifications to result in improved separation of cells from microcarriers in spinner flasks. Another approach for cell harvesting was explored by developing dissolvable microcarriers, which can be readily dissolved through enzymatic degradation of the protein substrate or chemical destabilization of the polymer substrate, depending on the material used to fabricate the microcarrier. Corning recently introduced a dissolvable MC made of polygalacturonic acid (PGA) polymer chains crosslinked with calcium ions. These MCs are coated with Synthemax® II substrate, which is a synthetic peptide-based matrix developed by Corning to facilitate cell adhesion. Dissolution of MCs is achieved through calcium ion chelation, which destabilizes the PGA crosslinking with the addition of EDTA, and pectinase to degrade the overall polymer. Increased cell recovery was reported for human induced pluripotent stem cells[28]. That recent report showed that there is

still room for improvement in designing MCs, with the potential to simplify or speed downstream cell manufacturing processes.

Here, we developed a gelatin-based MC through droplet microfluidics. Our evaluation of the performance of these gelatin, dissolvable MCs compared to commercialized MCs allowed us to assess possible simplifications in cell manufacturing downstream bioprocesses. We obtained monodisperse MC with narrow particle diameter distribution. These gelatin MCs were readily dissolvable by a commercialized protease mixture, without agitation. The short incubation time limits the negative effects of enzymatic treatment and obviates mechanical stress on cells[15]. We also characterized MSCs post-culture on gelatin MC through several phenotypic *in vitro* assays. With gelatin as a natural extracellular matrix protein (denatured collagen) for cell adhesion and growth, we showed that this material and the processing of diameter-uniform MC particles provide an efficient platform for MSC to grow while retaining multipotency and achieving excellent yield at the final cell harvesting step through facile MC dissolution.

2 Materials and methods

Materials and methods are included in the Supporting Information A: Materials and methods.

3 Results

3.1 MC Particle Diameter Distribution

Gelatin MC was fabricated through droplet microfluidics **(Figure 1a)**. By measuring the diameters of the MC particles of each MC type from bright-field images **(Figure 1b)**, we computed the coefficient of variation (CoV) for the microcarrier particle diameter of each MC type as an indicator of particle diameter uniformity. The CoV for particle diameter distributions were 15.87%, 15.07%, 12.55% and 4.52% for Cytodex-1, Cytodex-3, SoloHill Collagen and gelatin MCs, respectively **(Figure 1c)**. According to the specifications given on each of the commercialized MC, Cytodex-1 MCs have a particle diameter range of 131-220 μm; Cytodex-3 from 133 to 215 μm; and SoloHill Collagen from 125 to 212 μm. In principle, the diameter of the gelatin microcarrier is tunable; this feature depends on the channel size of the microfluidic chip designed for particle fabrication. With the use of a droplet microfluidics platform, the fabricated particle diameter showed high uniformity with CoV below 5%.

3.2 MSC Expansion on Microcarriers

The material comprising MCs plays a role in affecting cell adhesion, cell spreading, and cell proliferation. According to the manufacturer's description, Cytodex-3 and SoloHill Collagen are coated with a microlayer of denatured collagen, and should therefore share similar properties to gelatin, which is also a denatured form of collagen. We first investigated the attachment efficiency of MSCs onto different types of MCs after 24 hours of intermittent agitation inoculation. Cell attachment efficiencies of MSCs onto MCs were generally >95% (Cytodex-1: 97.52 ± 0.29%, Cytodex-3: 97.41 ± 0.16%, SoloHill Collagen: 96.84 ± 0.16%, gelatin: 95.62 ± 0.18%) **(Figure 2a)**.

Population doubling times for MSCs on Cytodex-1, Cytodex-3, SoloHill Collagen and gelatin MCs were 3.41 ± 0.13 days, 4.89 ± 0.50 days, 5.27 ± 0.55 days and 3.67 ± 0.19 days, respectively **(Figure 2b)**. Total cell count calculated on day 10, one day prior to harvesting, is listed in Table 1. Expansion-folds calculated at day 10 (relative to day 0) were 6.11 ± 0.42 for Cytodex-1, 3.57 ± 0.47 for Cytodex-3, 3.26 ± 0.43 for SoloHill Collagen, and 5.28 ± 0.48 fold increase for gelatin MCs **(Figure 2c)**.

To obtain a growth kinetic curve, we conducted a dynamic culture of MSCs with MCs in spinner flasks to investigate the cell proliferation performance on these different MCs. The proliferation of MSCs on four different types of MCs **(Figure 2d)** indicated that Cytodex-1 and gelatin MCs exhibited similar performance in terms of promoting cell growth (proliferation); cell growth on Cytodex-3 and SoloHill Collagen MCs was relatively slower (Table 1). The observation of WST-1 activity of MSCs was consistent with our cell counting, wherein MSCs cultured on Cytodex-1 and gelatin MCs showed higher overall activity, which translated to greater total cell numbers **(Figure 2e)**. Bright-field images **(Figure 2f)** and confocal images (Supporting Information B1, Table B1) of the MC cultures also provided a means to estimate cell number per particle, and imaging results were in concordance with the previous measurement with cell counting and WST-1.

Table 1. Cell count (million cells) on day 4, 7 and 10 of culture.

MC type	Day 4	Day 7	Day 10
Cytodex-1	3.52 ± 0.23	7.38 ± 0.89	15.27 ± 1.04
Cytodex-3	2.98 ± 0.13	4.48 ± 0.54	8.93 ±1.16

SoloHill Collagen	2.80 ± 0.54	4.65 ± 0.67	8.09 ± 1.09
Gelatin	3.82 ± 0.34	6.95 ± 0.79	13.21 ± 1.20

3.3 MSC Harvest and Cell Recovery

Downstream processing of cell manufacturing involves cell harvesting after cell expansion. Various groups have reported the use of different enzymatic solutions such as trypsin[19, 21], TrypLE[29,30], and collagenase[31]. We tested several enzymatic solutions and observed that Pronase (Sigma) showed the best performance in detaching MSCs from MCs (Supporting Information B2). In this process of cell recovery with enzymatic incubation, we observed that cells did not fully detach from the Cytodex MCs even after 30 min of enzymatic treatment (**Figure 3a** bright-field images). In contrast, gelatin MCs were fully dissolvable with Pronase treatment. Particles could be dissolved within 5 minutes with 0.1% Pronase solution without agitation, thus eliminating the need to perform separation of cells from the particles **(Figure 3b)**.

To quantify the cell harvest efficiency process on each type of MCs, it is critical to determine the number of cells present in the culture accurately, in which this number will be used as the baseline for harvest efficiency computation. As cell counting is inherently affected by the inefficient cell harvesting process, it is difficult to determine the true number of cells in the culture especially at high cell density. Therefore, we designed a scaled-down experiment to determine the harvesting efficiency. We seeded 0.4 million of MSCs and MCs with a total surface area of 50 cm^2 into a 24-well ultra-low attachment well plate (Corning) for overnight incubation

inside a 37°C with 5% CO_2 incubator. Cell proliferation in the first 24 hours was minimal, and this allowed us to approximate the total cell number that could be harvested from the MCs when we performed the enzymatic detachment for the cell harvest step and MC-cell separation in the filtering step. For all conditions, incubation time with Pronase was fixed at 30 minutes and all detached cells were passed through a 70 μm cell strainer to filter out MC particles or cell aggregates. Overall cell harvesting efficiencies were 60.55 ± 8.25%, 58.75 ± 5.74%, 68.41 ± 7.48%, 92.95 ± 3.56% for MSCs harvested from Cytodex-1, Cytodex-3, SoloHill Collagen and gelatin MC cultures, respectively.

While the scaled-down experiment might not be fully representative of the actual situation of harvesting cells at high cell density with the presence of MC-cell agglomerates, it provided a means to compare cell harvesting efficiencies among all four types of microcarriers under controlled conditions that minimized additional confounding effects among the MC-cell combinations such as differing proliferation rates. As an alternative consideration of whether cell harvesting was quantifiably different for the different MCs, we also reflected on the data that we collected throughout the 11 days of MSC expansion on MCs: we compared the total cell number harvested on day 11 against total cell number calculated on day 10 (1 mL aliquot sampling). The cell harvesting efficiency calculated, using this total cell count at day 10 as the baseline cell number just before harvesting, were as follows: 69.68% for Cytodex-1, 75.48% for Cytodex-3, 85.66% for SoloHill Collagen and 92.42% for gelatin MC. We note that the actual cell harvesting efficiency is likely to be a bit lower than these calculated efficiencies, as MSCs would continue to proliferate from day 10 to day 11 (see Figure 2D). While this analysis is also of limited accuracy, in that cell count on day 10 was conducted based on 1 mL of sampling that includes some potential for error as compared with analysis of the total working volume; this is

an inherent limit of knowing the full cell count of MC-attached MSCs just before harvesting that same population. Others have analyzed a half-total volume that they considered representative of the harvested population, conferring similar approximations of accurate pre-harvested cell count[26]. Nevertheless, our results of harvesting efficiency estimated from two different methods confirmed that gelatin MCs provided the highest cell harvesting efficiency when compared to the commercial MCs harvested under the same conditions.

Separately, we calculated the fold increase by day 11, dividing the number of cells harvested on day 11 to the initial cell seeding number on day 0. This comparison includes the steps of cell expansion, cell harvesting, and cell separation (from MCs, needed for non-dissolvable MCs). Total cell number harvested on day 11 were as follows: 1.08×10^7 cells for Cytodex-1, 6.74×10^6 cells for Cytodex-3, 6.93×10^6 cells for SoloHill Collagen, and 1.22×10^7 cells for gelatin MC. We found that fold increase by day 11 was 4.32 ± 0.21 for MSCs on Cytodex-1, 2.70 ± 0.43 for Cytodex-3, 2.77 ± 0.26 for SoloHill Collagen, and 4.87 ± 0.23 for gelatin MC after the cell recovery step **(Figure 3c)**. We also analyzed cytotoxicity to confirm that the hydrolyzed gelatin that improved overall efficiency of MSC yield from the gelatin MCs did not cause any observable cytotoxic effect to the cells (Supporting Information B3).

3.4 Characterization of MSC Cultured on Various Type of MCs

3.4.1 MSC Plastic Adherence and Immunophenotypic Cell Surface Markers

According to the criteria established by the International Society for Cellular Therapy[32], we evaluated plastic adherence of MSCs, cell surface markers via flow cytometry, and *in vitro* tri-

lineage differentiation. MSCs harvested from all MC culture demonstrated plastic-adherent property after re-seeding onto a 2D plastic plate in standard culture conditions (result not shown). For immunophenotypic surface marker analysis, CD73, CD90 and CD105 were MSC positive markers and CD34 and CD45 were used as negative markers. MSCs harvested from 2D planar culture and from3D gelatin MC exhibited positive staining (>95%) for CD73, CD90 and CD105, and negative staining (<5%) for CD34 and CD44 (Supporting Information B4, Figure B4). *In vitro* tri-lineage commitment of MSCs was also assessed successfully. These results suggested that MSCs grown on gelatin MC met current ISCT criteria of identity and multipotency.

3.4.2 Multipotency of MSCs

MSCs can exhibit *in vitro* differentiation upon chemical induction along the tissue cell lineages of bone, cartilage, and fat; this attribute is termed tri-lineage multipotency. As this analysis of multipotency is considered a standard or expected attribute of culture-expanded MSCs under basal culture conditions, we sought to confirm whether the expansion of MSCs on gelatin MCs either restricted or maintained this attribute post-expansion. Cells cultured on MCs generally showed higher expression levels for chondro- and osteo-differentiation genes (SOX9, OPN and RUNX2) compared to 2D planar culture. For adipogenic differentiation, MSCs on commercial MC showed lower expression of PPARγ, while gelatin MC-cultured MSCs showed an upregulation in PPARγ expression when compared to 2D TCF culture **(Figure 4a and Supporting Information B5)**. Statistical analysis of the gene expression was conducted on delta-CT value rather than on the expression-fold change by using one-way ANOVA and post hoc Tukey test (when ANOVA determines the significant difference between groups). The results were summarized in Supporting Information B6.

We further conducted chemical induction experiments to assess later markers of tri-lineage differentiation. MC-cultured cells exhibited higher calcium deposits and normalized sGAG content when compared to 2D tissue culture flask-cultured cells, as measured by Alizarin Red staining and sGAG assay, respectively. However, cells expanded on Cytodex-1, Cytodex-3 and SoloHill Collagen showed lower oil deposits after 10 days of adipogenic differentiation when compared to 2D tissue culture flask-cultured cells **(Figure 4b,c)**. Gelatin MC-cultured MSCs produced fewer oil droplets (specifically, produced less Oil Red O staining per image surface area) than cells on 2D tissue culture flasks, but the difference was not statistically significant ($p > 0.05$).

4 Discussion

To date, the development of microcarriers (MCs) has focused on tissue engineering purposes, where cells of interest are seeded on or encapsulated inside MC particle and are subsequently implanted directly into the human body at the injury site. Therefore, most results-focused on cells in or on the MC particle as the repairing unit, and most studies are restricted to considering a single type of application[33-38]. Relatively little focus has been given to developing MC to expand cells for cell-based therapy in regenerative medicine, though this is of current interest in cell therapy manufacturing process development. In this work, we focus on discussing from the cell manufacturing perspective, where cells could be harvested from the particles and injected into the body for clinical cases that leverage the secretory-based function of MSCs *in vivo*. As such, the performance of the MCs in terms of expanding cells is critical to reaching the required cell number for treatment dosage.

We compared our gelatin MC to commercial MCs to evaluate performance in MSC expansion under basal culture conditions. Our results indicated that gelatin MCs exhibited similar or superior performance when compared to several commercial MCs **(Figure 5)**. All microcarrier types tested in this work showed >95% MSC attachment efficiency. Cell expansion rate on gelatin microcarriers was comparable to an existing microcarrier such as Cytodex-1 and exceeded proliferation rates of other MC types tested by approximately twofold. However, as gelatin microcarriers were highly uniform in particle diameter, similar confluency could potentially be reached on each gelatin microcarrier at any given time point. This could plausibly lead to a more homogeneous MSC population, to the extent that confluency influences population heterogeneity related to MSC subpopulation proliferation rates[39]. Such hypotheses for the rationale of this effect of MC physical cues on expansion rates warrant further studies. In the cell harvesting step, direct dissolution of the gelatin microcarriers allowed more efficient recovery of cells, and thus led to a higher overall yield of the harvested cells. Finally, MSCs cultured on gelatin microcarriers showed multipotency, as cells readily differentiated into all three lineages; on the contrary, MSCs displayed lower adipogenic differentiation capacity after expansion on commercial microcarriers. The analysis herein was limited to consideration of multipotency at the gene expression and metabolic activity levels *in vitro*. This could plausibly lead to a more homogeneous MSC population in terms of phenotype, to the extent that MC particle curvature affects cell-cell and diffusive signaling interactions.

Notably, cell harvesting is deemed as a key bottleneck of adherent cell expansion on MCs. While Nienow et al. has published several protocols to facilitate cell release from microcarriers[25,26], the step of cell-microcarrier separation remains a practical implementation challenge[40].

Current and nascent separation techniques include tangential flow filtrations (TFF), counter-flow centrifugation elutriations (CCE), and dead-end sieving; these require sophisticated equipment for such separation process[42]. Simpler membrane-based filtration faces the disadvantage of clogging (cake formation) during the cell-particle separation step[25]. Membrane-less separation approaches such as microfluidic-based sorting have also been explored to separate cells from particles[40]; nevertheless, this additional cell-particle separation step is time-consuming and can present contamination or dead volume (cell loss) risks with the incorporation of another microfluidic module into the cell manufacturing workflow. Our approach of direct dissolution of gelatin MC eliminates the need for separation of cells from MCs. Moreover, the dissolved gelatin did not induce detectable cytotoxic effects towards these cells (Supporting Information B3). For these reasons, our gelatin MC offers a promising and attractive approach to aid efficient cell manufacturing.

As gelatin has been used widely as scaffold or coating material for cell culture, it is not surprising that gelatin offers good cell attachment efficiency as well as proliferation rate. Also, MSCs cultured on gelatin exhibited enhanced chondro- and osteo-differentiation while maintaining a similar level of adipo-differentiation when compared to 2D monolayer culture. Gelatin has also been demonstrated as a substrate for 2D monolayer culture, for which others have found that adipogenesis and osteogenesis differentiation were enhanced[41,42]. This suggested that gelatin is a suitable substrate for the growth of MSC while maintaining cell differentiation capacity and multipotency. Also, as gelatin has been demonstrated as a safe material for human exposure and consumption (at least as an edible), there is relatively low but to no risk in the delivery of any highly degraded gelatin with the therapeutics of cell suspension. Therefore, we do not anticipate elevated safety issues posed by highly dissolved, degraded

gelatin that may be retained in the delivered cell suspension. However, we note that we did not directly assess animal or human response in this study. Different modes of product finish and fill or administration to the patient may affect this safety profile, and direct assessment of that risk would be required in future studies.

The MC production protocol described herein is a basic cross-junction microfluidic chip, and therefore the throughput is on the low end of microfluidics technologies (droplet generation of ~0.15 mL/hour, which is roughly equivalent to a total surface of 50 cm^2 of gelatin MC surface area). This means that 10 hours of MC production were required to produce the MCs sufficient for the spinner flask experiment (100 mL total working volume) described above. We can extrapolate that without modification of our production method, 1000 hours would be required to produce a sufficient volume of MCs for a 10 L bioreactor production run. However, there are many ultra-high-throughput droplet generation techniques using microfluidics that could be adapted for scalable production of these gelatin MCs. For instance, Jeong et al. demonstrated droplet/bubble production of 3 L/hour[43], and Yadavali et al. developed arrays of droplet generators to increase throughput by 10,000x compared to microfluidics with a single generator[44]. This would mean that only 1 hour would be required for sufficient production of gelatin MC used in a 10 L bioreactor for allogeneic MSC manufacturing. Thus, by adopting microfluidic technologies for ultra-high throughput droplet generation, we believe the gelatin MC production process is scalable to manufacture MSCs at volumes or throughputs of interest for allogeneic or cell bank applications.

To our knowledge, these microcarriers thus fabricated confer a unique combination of properties including microcarrier size (diameter range 150 to 250 μm that is achievable through droplet microfluidics fabrication), low variation in particle diameter (coefficient of variation of <5%), and rapid dissolution with Pronase (~5 min, in the absence of mechanical agitation or centrifugation) to enable retrieval of viable MSCs. Naturally, more developmental work remains by us and the wider community.

5. Conclusion

In this work, we demonstrated the potential use of a dissolvable gelatin-based MC platform for manufacturing of anchorage-dependent cells, using mesenchymal stromal cells as a case study relevant to cell therapy production requiring efficient scale-up. This platform allows rapid release of cells from the MCs and could potentially increase the yield of the cell product from the culturing process. This inducible dissolution of MCs also eliminated an extra separation step to retrieve the cells from the MCs, thus reducing the complexity of cell manufacturing. MSCs expanded on gelatin MCs expressed immunophenotypic surface markers consistent with the current ISCT criteria, and maintained tri-lineage differentiation capacity *in vitro*. Collectively, we showed that the gelatin MC could facilitate the efficient production of MSCs while retaining the critical quality attributes of those retrieved cells. Thus, this gelatin MC offers a promising tool for the cultivation of MSCs and other anchorage-dependent cell types in process development and cell therapy manufacturing settings.

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Supporting Information

Acknowledgement

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure legends

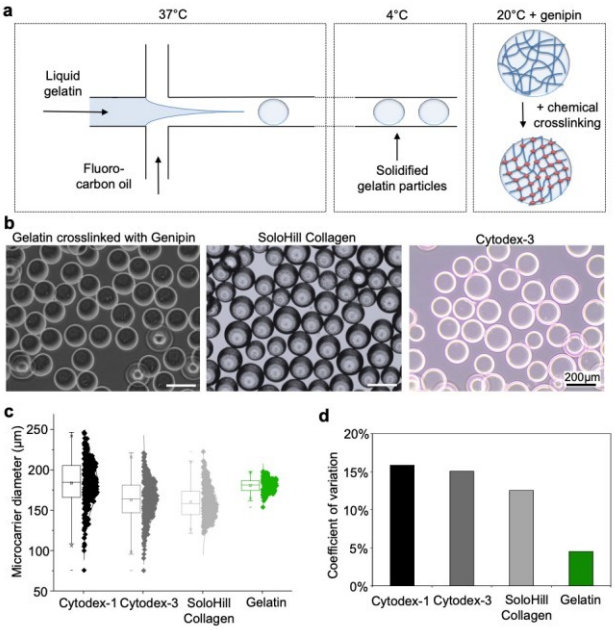

Figure 1. Gelatin microcarriers fabrication and comparison of diameter uniformity with existing microcarriers. (a) Gelatin microcarrier particles fabrication through droplet microfluidics. (b) Particle diameter uniformity of various MC particles: gelatin MC, SoloHill Collagen and Cytodex-3. Scale bars = 200 µm. (c) Particle diameter distribution of Cytodex-1, Cytodex-3, SoloHill Collagen and gelatin MC where n = 300 microcarriers analyzed for each MC type. Every point represents one measurement of one microcarrier particle. d) Coefficient of variation (CoV) indicates a degree of MC diameter uniformity. N = 1 experiment from which CoV was obtained from the 300 microcarriers analyzed in each MC type.

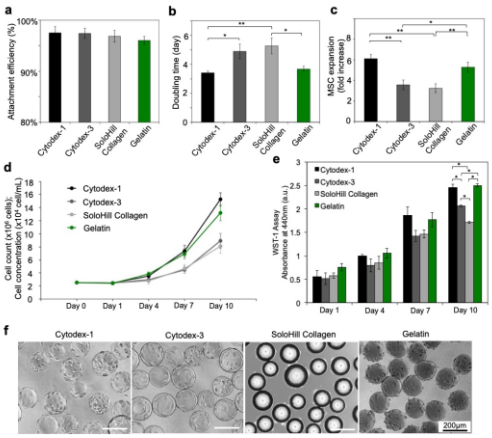

Figure 2. MSC culture on microcarriers. (a) Attachment efficiency of MSCs onto different types of microcarrier particles after 24 hours. Cell counts were obtained in triplicate (n = 3 technical replicates) for each MC type to obtain attachment efficiency. No significant difference was identified among different types of microcarrier (one-way ANOVA). (b) Doubling time of MSCs when cultured on different types of microcarrier particles. Doubling time was calculated based on n = 3 technical replicates (wells). (c) Total MSC expansion-fold on different microcarrier particle types after 10 days of culture. Expansion-fold was calculated based on n = 3 technical replicates (wells) on day 10. (d) Growth curve of MSCs cultured on different of microcarrier particle types from day 1 to day 10. Note that the vertical axis expressed as cell count or equivalent cell concentration (n = 3 at day 1, 4, 7 and 10). (e) MSC proliferation as detected by WST-1 assay measured on days 1, 4, 7 and 10 (n = 3 technical replicates (wells) for WST-1 quantification). Significant differences detected by day 10. (f) Bright-field images of MSC culture on different microcarrier particle types on day 7. Scale bars = 200 μm. All data were expressed as arithmetic mean ± standard deviation for technical triplicates. One-way ANOVA and post hoc Tukey test were used to analyze results with technical triplicates (*$p < 0.05$, **$p < 0.01$).

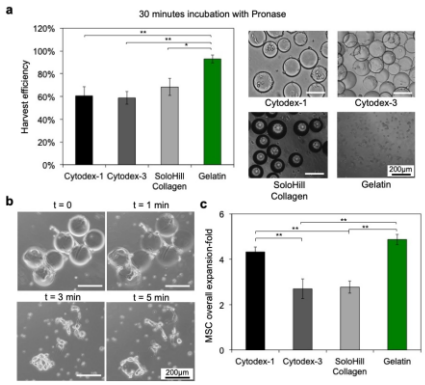

Figure 3. Cell harvest from different microcarriers. (a) Harvest efficiency of MSCs with Pronase enzymatic solution (30 minutes incubation). Cell counts obtained in triplicate (n = 3 technical replicates or wells for each MC type). Scale bars = 200 μm. (b) Bright field time-lapse images of the gelatin MC dissolution process when incubating gelatin microcarriers in Pronase enzymatic solution. Scale bars = 200 μm. (c) The overall yield of MSC expansion-fold characterized the ratio between the total number of cells collected from microcarrier culture on day 11 against initial cell seeding density as quantified by day 1 cell count. Overall expansion-fold of cells calculated from technical triplicates at day 11 (n = 3 technical replicate wells for each MC type). All data were expressed as arithmetic mean ± standard deviation for technical triplicates. One-way ANOVA and post hoc Tukey test were used to analyze results with technical triplicates (*$p < 0.05$, **$p < 0.01$).

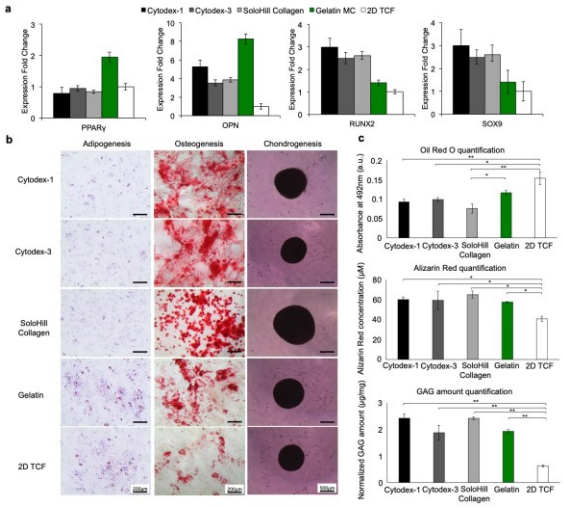

Figure 4. *In vitro* multipotency of MSCs harvested from various microcarriers and 2D planar culture. (a) The expression level of differentiation-related genes of MSCs cultured under different conditions. RT-PCR obtained in technical triplicate (n = 3 wells for each MC type). (b) Images and (c) quantification of adipo-, osteo- and chondrogenic differentiation levels were obtained through Oil Red O staining, Alizarin Red staining and sGAG assays, respectively. For Oil Red O staining, oil droplets were stained in red and cell nuclei were stained by hematoxylin in deep blue-purple; for Alizarin Red staining, calcium deposits were stained in red. Differentiation assays conducted in technical triplicate (n = 3 wells for each microcarrier type). Scale bars = 200 μm for adipogenesis and osteogenesis images, and 500 μm for chondrogenesis images in (b). All data were expressed as arithmetic mean ± standard deviation for technical triplicates. One-way ANOVA and post hoc Tukey test were used to analyze results with technical triplicates (*$p < 0.05$, **$p < 0.01$).

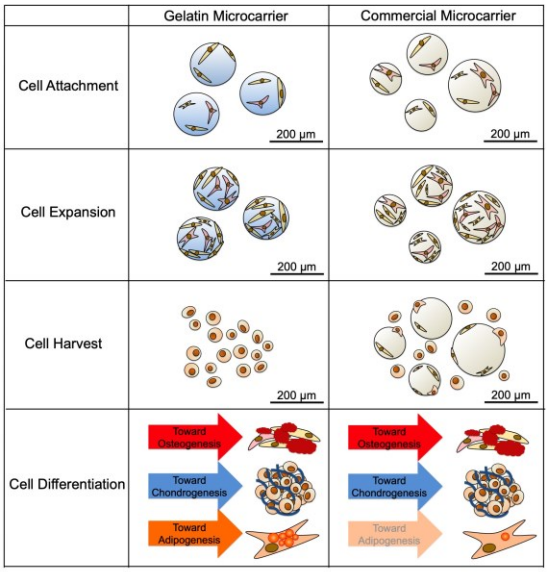

Figure 5. Microcarrier comparison. Schematic summary, illustrating that mesenchymal stromal cell (MSC) expansion on microcarriers as a platform for subsequent retrieval of expanded cells for study or cell therapy applications. In this study, gelatin microcarriers of relatively more uniform particle diameter supported MSC adhesion and comparable or faster expansion as compared with several existing commercial microcarriers. Direct dissolution of the gelatin microcarriers allowed efficient recovery of cells as well as *in vitro* trilineage multipotency.