



REVIEW

Recent advances in the use of microcarriers for cell cultures and their ex vivo and in vivo applications

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Abstract Microcarriers are 100- to 300-micron support matrices that permit the growth of adherent cells in bioreactor systems. They have a larger surface area to volume ratio in comparison to single cell monolayers, enabling cost-effective cell production and expansion. Microcarriers are composed of a solid matrix that must be separated from expanded cells during downstream processing stages. The detachment method is chosen on the basis of several factors like cell type, microcarrier surface chemistry, cell

confluency and degree of aggregation. The development of microcarriers with a range of physiochemical properties permit controlled cell and protein associations that hold utility for novel therapeutics. In this review, we provide an overview of the recent advances in microcarrier cell culture technology. We also discuss its significance as an ex vivo research tool and the therapeutic potential of newly designed microcarrier systems in vivo.

Keywords Bioreactor · Cell culture · Cell expansion · Drug delivery · Microcarriers


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Introduction

Microcarriers are support matrices that enhance the growth of cells in bioreactor systems (Park et al. 2018; Scheffler et al. 2018). For cell expansion, cells are grown on the surface of small solid particles that have been suspended in growth medium to enable cell adherence (De Silva Thompson et al. 2019; Yekrang-Safakar et al. 2018). The discovery of microcarriers is not a recent event as their initial characterization dates back as far as 1967 in studies by van Wezel and colleagues (Van Wezel 1967). The recent advances in microcarriers relate to their range of designs and surface modifications that have extended their repertoire to a plethora of clinical and ex vivo applications. In their simplest form, microcarriers are 100- to 300-micron-beads that have sufficient density to maintain suspension during stirring. A number of microcarriers have been synthesized and commercially utilized, including glass, diethylaminoethyl (DEAE)-dextran, acrylamide, polystyrene, collagen, and alginate (O'Connor et al. 2017). Alginate acid, for example, is a widely used biomaterial for cell encapsulation and drug delivery. Cartilage cells cultured in an alginate carrier have the ability to synthesize an extracellular matrix (ECM) that mimics natural cartilage. These materials can be subject to further surface modifications to modulate their behaviours upon cell/protein attachment (Dias et al. 2017).

All classes of cultured animal cells, namely mammalian, avian, fish and insect cells, were successfully cultured on microcarriers (Kubis et al. 2016). Cells of broad histotypic origin, including primary cells, diploid cell strains, established or transformed cell lines, hybrid cell lines and tumor cells, can be cultured on microcarriers. Selection of the suitable microcarrier depends on the cell types and applications. In general, microcarriers reduce the time, expenditure, and complexity of the apparatus necessary for the routine propagation of anchorage-dependent cells (Tavassoli et al. 2018). This is exemplified by Synthemax® II microcarriers which create synthetic surfaces that promote stem cell adherence and expansion for long-term cultures (Pennington et al. 2015). Other commercially available microcarriers include alginate-based carriers (GEM, Global Cell Solutions), dextran-based carriers (Cytodex, GE Healthcare), collagen-based carriers (Cultispher, Percell), and polystyrene-based carriers (Plastic, SoloHill Engineering

(Tavassoli et al. 2018). These microcarriers differ in their porosity, density, dimensions and surface chemistry (summarized in Table 1). The range of applications of any given microcarrier is related to its chemical composition. With technological advances, the microcarrier field is rapidly expanding and exciting new applications continue to emerge (Fig. 1). These applications will be reviewed herein.

Microcarrier culture techniques and their advantages

Microcarriers can be made of synthetic or natural polymers. Most of the early microcarriers were synthetic polymer-based, including those based on poly(lactide-*co*-glycolide) (PLGA), polyhydroxyethylmethacrylate, acrylamide, polystyrene and polyurethane (Li et al. 2015a). Whilst microcarriers produced from synthetic polymers show high reproducibility, they tend to lack cell recognition sites, which limits their use in cell expansion applications (Yang et al. 2002). Recently, increasing focus has been placed on natural polymers and their derivatives, as they are easy to obtain and display biocompatibility (Wu et al. 2018). Examples of natural polymers used to fabricate microcarriers include Gelatin (GELI-BEAD, Ventregel, and Cultispher), collagen (Verax), cellulose (Cytopore), chitosan, alginate acid, etc. Examples of the utility of both classes of microcarriers are discussed in this review. On the basis of their physical properties, microcarriers are further subdivided into solid and liquid microcarriers. Among these, solid microcarriers possess the advantages of adherence and cell expansion (Table 1). In cell cultivation methods using liquid/liquid systems i.e., liquid microcarriers, cells adhere and grow at the interface between the culture media and hydrophobic liquids (Hanga et al. 2017). Perfluorocarbons are used as the hydrophobic liquids since they do not show adverse effects on cell growth and have high oxygen solubility (Pilarek et al. 2013). It has been demonstrated that the liquid/liquid systems are flexible (independent of vessel shape), fully scalable and do not require proteolytic enzymes, scaffolds or inserts typically employed for the 3D-culture of animal cells (Pilarek et al. 2013). In addition, the anchorage-dependent mammalian cell growth is not restricted by the confluence effect.

Table 1 Examples of commercially available microcarriers

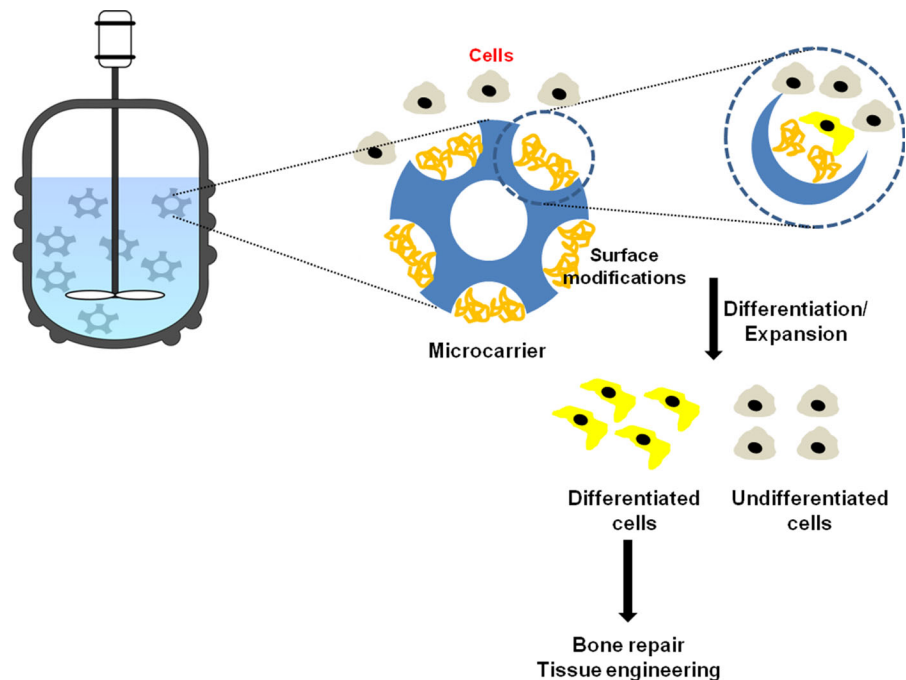
Name	Type	Diameter (μm)	density (g/mL)	Matrix and surface modifications	Properties/applications	Refs.
Cytodex 1	Solid	190 \pm 58	1.03	Dextran matrix with positively-charged diethylaminoethyl groups	Substrates for the propagation of articular bovine chondrocytes	Van Wezel (1967)
Cytodex 2	Solid	135–200	1.04	Dextran matrix with N,N,N-trimethyl-2-hydroxyaminopropyl groups	High biocompatibility and injectable for cartilage regeneration	Jakob et al. (2016)
Cytodex 3	Solid	175 \pm 36	1.04	Dextran beads coated with denatured porcine-skin collagen	Natural microcarrier that permits prolonged and rapid cell growth	Sart et al. (2013)
Hillex II-170	Solid	170 \pm 10	1.12	Polystyrene with treated surface	Use in cell culture systems for bioreactor scale up	Li et al. (2015a) and Tavassoli et al. (2018)
ProNectin® F	Solid	169 \pm 44	1.02	Polystyrene with Recombinant proteins	Shaped 3D cell carrier for 3D culture analysis	Derakhti et al. (2019)
FACT III	Solid	169 \pm 44	1.02	Charged polystyrene and collagen coated	Cell expansion	Wu et al. (2018)
CGEN 102-L	Solid	169 \pm 44	1.02	Polystyrene coated with Type I porcine collagen	Good biocompatibility, for tissue-healing processes	Li et al. (2015a)
Cytopore 1, 2	Macroporous	240 \pm 40	1.03	Cross-linked cellulose with positively-charged diethylaminoethyl groups	Suitable for stirred-tank cultures	Van Wezel (1967)
Cultispher G, S and GL	Macroporous	255 \pm 125	1.04	Cross-linked Type I porcine collagen	Suitable for all types of cultures	Li et al. (2015b)

Microcarriers are usually cultured in stirred-tank bioreactors, but other support systems, such as rotating wall microgravity bioreactors, Wave bioreactors and fluidized-bed bioreactors, have also been employed (Shekaran et al. 2016). They possess the advantage of enhanced production capacity as the high surface area-to-volume ratios achieved lead to high cell yields (Alkhatib et al. 2017; Reibetanz et al. 2016). Microcarrier systems are flexible and surface areas can be adjusted to promote cell-growth. The homogeneity of the microcarrier systems permits the monitoring and control of parameters, such as pH, $p\text{O}_2$, and nutrient and metabolite concentrations. Improved control of the system leads to more reproducible cell cultures, a key characteristic for commercial cell-based protocols (Jakob et al. 2016; Wang et al. 2015). Microcarriers also facilitate scalability through large-scale cultures using conventional technologies, including the stirred-tank bioreactors used for suspended mammalian cell

cultures. They also allow scale-up via bead-to-bead cell transfer by adding fresh microcarriers, without the necessity for enzymatic dissociation. Compared to static culture, higher cell densities (number of cells per mL) in relatively low volumes are achieved due to their high surface-to-volume ratio. Hence, microcarrier technology minimizes laboratory and storage space as well as labor required for routine production, ultimately reducing costs (Jin et al. 2016; Kubis et al. 2016). The number of handling stages of cells (opening and closing of culture vessels) is also reduced, decreasing the risk of damage or media contamination. All these advantages highlight the application of microcarriers for a wide range of laboratory and commercial applications.

In recent years, recombinant therapeutic proteins have garnered intense research attention (O'Connor et al. 2017; Park et al. 2018; Scheffler et al. 2018). This has driven the design of microcarriers that maximize

Fig. 1 Representation of microcarrier cell culture technologies. Porous microcarriers are presented here as an example



protein yields. Mammalian, microbial, and insect expression systems have been optimized for therapeutic proteins/vaccines (Gupta et al. 2019). High throughput devices, modern bioreactors and scale-out technology have enabled significant improvements in the final yields and product qualities obtained from mammalian-based culture systems since they allow consistent scalability and control of culture conditions. Computational tools and simulation models have also increased product yields. These advances are exemplified by improvements in vaccine production, evidenced by the large-scale (200 L scale) production of inactivated EV71 virus using a microcarrier-based suspension bioreactor system (Wu et al. 2015). The efficiency of this system is superior to the previously described roller-bottle vaccine production system, yielding approximately 2–3 times more EV71 vaccine within 70 days (Wu et al. 2015).

Microcarriers for disease treatment

Microcarriers continue to attract intense clinical interest due to their ability to repair tissue and bone defects (Kornmuller et al. 2017; Moradian et al. 2017; Saltz and Kandalam 2016; Wang et al. 2018b; Yan et al. 2018; Yu et al. 2017; Zhang et al. 2017, 2016;

Zhou et al. 2019). In particular, injectable microcarriers have value as cell carriers to injured sites using only small incisions (Hoareau et al. 2018; Perteghella et al. 2017; Wang et al. 2018b; Yan et al. 2018; Yu et al. 2017; Zhang et al. 2017; Zhou et al. 2019). Due to its non-toxicity, ease of functionalization, biocompatibility and biodegradability, pullulan (PULL) is considered an ideal material for tissue engineering (Mocanu et al. 2014). Aydogdu and colleagues designed PULL microspheres to deliver cells for bone tissue engineering (Aydogdu et al. 2016). PULL was cross-linked using trisodium trimetaphosphate to enhance its stability. In addition, improved cell compatibility was achieved through the use of silk fibroin coating and surface biomimetic mineralization following treatment with simulated bodily fluids. Silk fibroin coating induced higher osteogenic property relative to uncoated and simulated body fluid-coated PULL microspheres. In vitro, the PULL microspheres displayed slow degradation rates and supported the formation of new bone tissue (Aydogdu et al. 2016).

In a novel approach, Kankala et al. fabricated poly(lactic-co-glycolic acid)-based highly open porous microspheres (HOPMs) using microfluidics to harbour proliferating skeletal myoblasts. Their feasibility was then evaluated for cell delivery in situ (Kankala et al. 2019). The HOPMs were 280–370

microns in size and had open pores of 10–80 microns that provided an environment favourable for cell arrangements into elongated morphologies with deposited ECM. This facilitated cell adhesion, proliferation, and myogenic differentiation *in vitro*. *In vivo* results in mice demonstrated enhanced vascularization and partial myoblast differentiation. Since the use of porogens and multistep processing may alter the polymer structure leading to cytocompatibility issues, the authors validated the biosafety of the HOPMs using cell cultures *in vitro*, hemocompatibility assays *ex vivo* and acute toxicity studies *in vivo* in nude mice.

Cultivation of mesenchymal stem cells (MSCs) on materials which induce osteogenic differentiation is a promising strategy in tissue engineering and regenerative medicine. In related studies, Goncharenko and co-workers investigated the proliferation and differentiation of MSCs cultured on fibroin microcarriers (Goncharenko et al. 2017). Effective cell proliferation was noted on the surface of the microcarriers *in vitro*, which enhanced mineralization (Goncharenko et al. 2017). A range of alginate microcarriers have also been reported as candidates to accommodate MSCs in 3D environments, particularly during craniofacial bone tissue engineering (Saltz and Kandalam 2016).

Microcarriers can deliver proteins, nucleic acids, and cells, and can promote both neuroprotection and neurorepair (Andre et al. 2016). Treatments for neurodegenerative disorders have been revolutionized by stem cells and neuronal progenitor transplants (Kandalam et al. 2017). For instance, Kandalam et al. integrated human marrow-isolated adult multilineage-inducible (MIAMI) stem cells and fibronectin-coated microcarriers into an injectable silanized-hydroxypropyl methylcellulose hydrogel (Kandalam et al. 2017). This delivery system displayed a prolonged release of brain-derived neurotrophic factor (BDNF) encapsulated in the microcarriers. In neurological disorders, the released BDNF and the augmented secretion of growth factors by the stem cells could have a synergistic effect on damaged neural tissues.

In a novel multidisciplinary approach, Moradian and colleagues used bone marrow-derived MSCs (BMSCs) that were engineered to overexpress neurotrophin-3, a known neuroprotective agent and potent stimulator of neuronal differentiation (Moradian et al. 2017). PLGA microcarriers were designed as injectable scaffolds and their surface was functionalized by collagen as a bioadhesive agent to enhance

BMSC attachment. The overexpression of neurotrophin-3 using these microcarriers enhanced dopaminergic neuron differentiation and resulted in an elevated secretion of dopamine (Moradian et al. 2017). Thus, the combination of engineered BMSCs capable of growth factor overexpression, adhesion molecules and 3D structure in the same polymeric scaffold represents a potential therapeutic strategy for Parkinson's disease.

Daviaud et al. reported the functional recovery of hemiparkinsonian rats following treatment with human MIAMI cells attached to neurotrophin-3-releasing pharmacologically-active PLGA microcarriers (Daviaud et al. 2015). The released neurotrophin-3 from the microcarriers markedly increased cell survival and neuronal differentiation, conferring neuroprotection in an *ex vivo* organotypic model of Parkinson's disease. Furthermore, vascular endothelial growth factor A (VEGFA) was identified as the potential mediator of the neuroprotective effect of MIAMI cells.

Microcarriers with oxygen-delivering capacity are of interest to wound healing applications and molybdenum disulfide quantum dots have been integrated into responsive porous microcarriers to permit controllable oxygen-delivery to wounds (Liu et al. 2019). These specific gelatin methacryloyl porous microcarriers were produced from inverse opal microparticles decorated with hemoglobin. As the microcarriers were porous, they displayed good biocompatibility and oxygen delivery (Liu et al. 2019). An additional advantage was their photoresponsive oxygen release. These remarkable properties show clinical promise for future applications in wound healing and tissue engineering systems.

Microcarriers for cell expansion

In spite of potential cell-damaging effects by affecting critical cell membrane proteins (Baumann and Doyle 1979), enzymatic cell dissociation using proteases in combination with chelating agents is still the most common strategy due to optimum cell recovery and reliability for an extensive range of cells (Merten 2015). A number of advances for maximizing the efficiency of dissociation using recombinant proteolytic enzymes have been reported. With the aim of developing an animal-component-free enzymatic

dissociation, Rourou et al. investigated TrypLE Select (recombinant trypsin) and Accutase as trypsin substitutes. Subsequently, they demonstrated that TrypLE Select and soybean trypsin inhibitor were efficient for in situ Vero cell detachment from Cytodex-1 microcarriers and enzyme deactivation in bioreactors, without any effect on cell growth and proliferation (Rourou et al. 2013). Furthermore, Caruso et al. employed TrypLE Express (recombinant trypsin) for the dissociation of MSCs from Cytodex-3 with high cell viability and no damaging effects on the cells (Caruso et al. 2014). Nienow et al. developed a scalable method based on a short period of intense agitation in the presence of trypsin–EDTA for harvesting human MSCs from microcarriers (Nienow et al. 2014). The overall harvesting efficiency was greater than 95% and the cells maintained all their typical attributes. This flexible protocol has the potential to be applied to various cell lines by adjusting the agitation rate and time as well as the enzyme and its concentration.

Allogeneic keratinocytes can be used as cell therapies for the treatment of burn injuries and epithelial barrier restoration (Levato et al. 2015). However, significant cell loss due to poor adhesion in the wound means more controllable transplantation methods are required. Adhesion can be enhanced using cell microcarriers embedded in a biodegradable scaffold. Martin and colleagues produced keratinocyte-laden microcarriers that retained their basal proliferative phenotype after rapid expansion (Martin et al. 2017). The cell-binding microcarriers were incorporated into collagen scaffolds prepared by plastic compression, which when cultured in vitro, enhanced cell proliferation and migration. Wound healing models showed that the cells retained their basal proliferative phenotype and improved epithelial barrier restoration.

Due to the importance of stem cell therapies, microcarriers that aid stem cell expansion and differentiation have been extensively developed. Microcarriers in bioreactor culture systems are one of the most promising advancements and several commercially available systems are now in active use. Stimulus-responsive microcarriers that can be controlled by field, temperature, and pH changes are also under development (Shi et al. 2017; Tavassoli et al. 2018; Wang et al. 2018a). The features of the microcarriers include morphology, geometry, size, composition,

nanotopography, surface coating, surface functionalization and charge. As an example, to replace lost retinal cells for the treatment of blindness, Baakdhah and coworkers used microcarriers in hypoxic stirring bioreactors to achieve cell numbers suitable for differentiation and transplantation (Baakdhah and van der Kooy 2019). The system used a combination of FACTIII microcarriers in a hypoxic (5% O₂) suspension stirring bioreactor. Moreover, up to ten-times greater tertiary retinal stem cell yields were noted compared to normoxic static T-flask cultures ($p = 0.02$). The authors hypothesized that hypoxia activated Notch signaling and its downstream Hes cascade through stabilization of HIF1- α .

Classical adherent cell expansion methods using 2D monolayer flasks are limited by their lack of scalability, labor-intensive handling and enzymatic cell harvesting. To address these drawbacks, Lee et al. designed a series of alginate/PEG network microcarriers containing disulfide bonds which support the attachment and proliferation of human umbilical cord blood MSCs (Li et al. 2016). Following expansion, the cells could be detached from the microcarriers after the addition of L-cysteine as a reducing agent to cleave the disulfide crosslinkage, decreasing cell damage in response to proteolytic enzyme treatment. In similar studies, Shekaran and co-workers cultured human early MSCs (heMSCs) on biodegradable polycaprolactone microcarriers that were coated with ECM (a combination of fibronectin and poly-L-lysine) to overcome the aforementioned challenges (Shekaran et al. 2016). Following in vitro osteogenic induction, the heMSCs displayed $\sim 68\%$ higher levels of Ca²⁺ deposition compared to monolayer culture. In mouse ectopic mineralization models, similar improvements in bone mass were observed for monolayer-expanded and microcarrier-bound heMSC implants at 16 weeks post-implantation. The polycaprolactone microcarriers were not degraded at 16 weeks post-implantation since polycaprolactone degrades over 3–4 years. Moreover, the pores formed between the spherical microcarriers supported neovascularization in vivo, which indicated that they function similar to porous scaffolds. Therefore, these biodegradable microcarrier cultures could be employed for the large-scale expansion of heMSCs and can be delivered in vivo, eliminating the need for damaging cell harvesting procedures and for scaffolds for cell delivery (Shekaran et al. 2016).

It should be noted that the use of microcarriers to expand cells has not always been successful. The epithelium of the stomach encompasses multipotent SCs which differentiate into multiple cell lineages that produce hormones and peptides required for gastric function. The 3D culture of SCs enhances their differentiation. Alkhatib and colleagues designed disk-like immobile HD silicone-rubber microcarriers to minimize toxicity and enhance mouse gastric stem (mGS) cell expansion (Alkhatib et al. 2017). Several parameters that influence initial cell attachment were identified, including the cell inoculum, serum concentrations, and the speed of agitation. While the microcarrier permitted the attachment and growth of mGSs, they did not support the functional differentiation of these cells; whether this phenomenon is specific to this cell type warrants further investigation in a range of related cell models.

Cell expansion using microcarriers in suspension stirring bioreactors can also be performed through bead-to-bead cell transfer, which allows cells to migrate from confluent microcarriers onto fresh microcarriers without the need for enzymes and degradable carriers. This process involves either adding fresh microcarriers to already confluent microcarriers or transferring confluent microcarriers to a new bioreactor containing fresh microcarriers (Merten 2015). In high-cell density cultures, bead-to-bead transfer aids the augmentation of the microcarrier concentration and hence available culture surfaces for surface-adherent cells. Direct bead-to-bead transfer was demonstrated for several continuous cell lines, such as BHK-21, CHO, hMSCs, Vero and MDCK cells (Baakdhah and van der Kooy 2019; Rafiq et al. 2018). However, this method is limited by the mobility of cells and can lead to microcarrier aggregation. For example, human fibroblasts and hepatocytes are usually unable to undergo bead-to-bead transfer. The propensity of cell transfer between microcarriers can be increased by allowing the culture to remain static for prolonged periods, with occasional stirring to eliminate microcarrier aggregation (Merten 2015).

Conclusions and future perspectives

Microcarrier technology offers the advantages of culturing greater numbers of functional cells and maintaining their specific phenotypes, as well as being

a source of cell therapy and tissue engineering. Engineered microcarrier culture systems that promote adherent cell growth represent a new paradigm in cell culture and tissue repair. The large surface area of the microcarriers enables the enhanced expansion of anchorage-dependent cells and proteins, with a range of therapeutic benefits. However, despite clear progress, several challenges remain. Due to the higher cell density, cell enumeration and harvesting in microcarrier cultures are difficult. Cells attached to the microcarriers often remain exposed to harmful adjoining surfaces, and cells growing across rounded surfaces show altered morphologies over time. Several advancements have been made to overcome these issues and expand the microcarrier repertoire, including physical immobilization and chemical modifications. Physical immobilization uses coating materials without altering the surface structure of the microcarrier. This endows microcarriers with the ability to simultaneously up-regulate cell functions and support cell proliferation. Surface functionalization is an effective strategy to overcome microcarriers' inability to specifically interact with cells like natural ECM does. Recently, technologies to engineer the shape, nanopopography and functionality of microcarriers through surface modifications have expanded this field. Smart external stimuli-responsive materials have shown promise in cell cultures and could be exploited for the design of novel microcarriers. Furthermore, the development of cost-effective biodegradable microcarriers is essential for future applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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