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Expansion in microcarrier-spinner cultures improves the chondrogenic potential of human early mesenchymal stromal cells

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Abstract

Background aims. Cartilage tissue engineering with human mesenchymal stromal cells (hMSC) is promising for allogeneic cell therapy. To achieve large-scale hMSC propagation, scalable microcarrier-based cultures are preferred over conventional static cultures on tissue culture plastic. Yet it remains unclear how microcarrier cultures affect hMSC chondrogenic potential, and how this potential is distinguished from that of tissue culture plastic. Hence, our study aims to compare the chondrogenic potential of human early MSC (heMSC) between microcarrier-spinner and tissue culture plastic cultures. *Methods.* heMSC expanded on either collagen-coated Cytodex 3 microcarriers in spinner cultures or tissue culture plastic were harvested for chondrogenic pellet differentiation with empirically determined chondrogenic inducer bone morphogenetic protein 2 (BMP2). Pellet diameter, DNA content, glycosaminoglycan (GAG) and collagen II production, histological staining and gene expression of chondrogenic markers including *SOX9, S100β, MMP13* and *ALPL*, were investigated and compared in both conditions. *Results.* BMP2 was the most effective chondrogenic inducer for heMSC. Chondrogenic pellets generated from microcarrier cultures developed larger pellet diameters, and produced more DNA, GAG and collagen II per pellet with greater GAG/DNA and collagen II/DNA ratios compared with that of tissue culture plastic. Moreover, they induced higher expression of chondrogenic genes (e.g., *S100β*) but not of hypertrophic genes (e.g., *MMP13* and *ALPL*). A similar trend showing enhanced chondrogenic potential was achieved with another microcarrier type, suggesting that the mechanism is due to the agitated nature of microcarrier cultures. *Conclusions.* This is the first study demonstrating that scalable microcarrier-spinner cultures enhance the chondrogenic potential of heMSC, supporting their use for large-scale cell expansion in cartilage cell therapy.

Key Words: *cartilage, cell therapy, chondrogenic differentiation, mesenchymal stromal cells, microcarrier*

Introduction

Articular cartilage is a weight-bearing connective tissue surrounding synovial joints and is physiologically important for reducing friction for movement, bearing mechanical stress without distortion and absorbing shock [1–4]. It is avascular and consists of chondrocytes surrounded by a specialized extracellular matrix primarily made up of glycosaminoglycans (GAG) and collagen II fibrils [1–4]. Because of its poor regenerative ability, degeneration or injury to articular cartilage is responsible for diseases such as osteoarthritis, which is one of the leading causes of pain and immobility worldwide [4–6]. Current treatments for cartilage defects with allografts are inadequate because of limited cadaver tissue supply [4–6], and thus researchers have turned to tissue engineering to generate articular cartilage-like tissue *in vitro* for potential allogeneic cell therapy [7–10].

Cartilage tissue engineering efforts for cartilage replacement are centered on generating articular cartilage-like tissue *in vitro* by directing chondrogenic differentiation of stem cells into chondrocyte-like cells [7–10]. Many studies are focused on using

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human mesenchymal stromal cells (hMSC) mainly because they are easily isolated and safe to use [11,12]. Particularly, human early hMSC (heMSC) are attractive for cell therapeutic applications as they display higher growth rate, enhanced plasticity and slower senescence rate than adult hMSC [13,14]. One of the many challenges facing allogeneic cartilage cell therapy is that conventional static culture platforms on tissue culture plastic cannot supply the large quantities of cells necessary for cartilage regeneration that is projected to be at least 1.5 to 4.5×10^7 cells per patient [15–17]. Although reports have estimated that conventional static culture on tissue culture plastic can yield up to 5×10^9 cells with a 40-cell stack, this process is too expensive and labor intensive for it to be viable on a large-scale basis [15–17]. Therefore, researchers have explored the use of alternative platforms, especially scalable microcarrier-based technologies, to meet this prospective market demand for hMSC [15,18–22].

Microcarrier-based cell culture platforms were first developed by Van Wezel and his group, who cultivated adherent cells on the surface of solid particles known as microcarriers, which were then suspended in growth medium by constant impeller-based agitation [23]. This system generates a homogenous suspension culture system that is similar to what is commonly used for propagating mammalian cells in industrial-scale bioreactors [23]. It is advantageous because it (*i*) provides a high surface-to-volume ratio that can be adjusted on the basis of microcarrier concentration; (*ii*) enables efficient oxygen and nutrient transfer; (*iii*) allows easy scale-up for use in bioreactors; (*iv*) enables on line monitoring and control of culture parameters, which improves the reproducibility of cell culture for quality control; and (*v*) is cost-effective in terms of yield per unit medium [15,24]. The demonstration that microcarrier-based culture platform allows for efficient propagation of cells from a small starting population by many groups also makes it attractive for cartilage tissue engineering of hMSC.

Previous work has explored expanding cell types such as isolated bona fide chondrocytes [25–29], chondroprogenitors [30] and other types of stem cells [31] on microcarrier-based culture platforms and tested their ability to generate cartilaginous tissue *in vitro*. However, no study to date has demonstrated (*i*) how microcarrier-based culture platform affects hMSC chondrogenic potential, (*ii*) how this potential differs from that achieved with conventional static cultures on tissue culture plastic, and (*iii*) most importantly, whether microcarrier cultures pose an additional advantage of improving hMSC chondrogenic potential, in addition to their scalability, that will enable large-scale hMSC cultivation for allogeneic cartilage cell therapy.

To address this, we present the first study comparing the chondrogenic potential of heMSC after initial propagation on agitated microcarrier-spinner culture to that of conventional static culture on tissue culture plastic. We found that chondrogenic pellets derived from microcarrier-spinner cultures displayed enhanced chondrogenic potential in terms of pellet diameter, DNA content, glycosaminoglycan and collagen II production; histological staining; and gene expression of chondrogenic markers, including *SOX9*, *S100β*, *MMP13* and *ALPL*. Similar trend in improving chondrogenic potential was also achieved with another microcarrier type, suggesting that the mechanism behind this enhancement is likely due to the agitated nature of the microcarrier cultures and not the surface coating of the microcarriers.

Methods

heMSC static culture on tissue culture plastic

heMSC were isolated, characterized and approved for use by the Domain Specific Review Board of National Healthcare Group in Singapore (DSRB-2006-00154), as previously described [20]. heMSC (passage 8–9) were plated at a density of 2400–2800 cells/cm^2 in either T175 cm^2 cell culture flasks or Nunc EasyFill Cell Factory Systems in MSC growth medium consisting of Minimum Essential Medium α, 10% fetal bovine serum and 1% penicillin-streptomycin (all from Gibco). The medium was changed every 2–3 days. heMSC were passaged at ~70% confluency when they were harvested using 0.25% trypsin-EDTA (Gibco) for 5 min at 37°C. Viability and cell count assays were performed with the automated NucleoCounter NC-3000 (Chemometec). All cultures were maintained at 37°C in a 5% CO_2 humidified incubator (Thermo Scientific).

heMSC microcarrier-spinner culture

Cytodex 1 (size: 147–248 μm) and Cytodex 3 (size: 141–211 μm) microcarriers were prepared in accordance to the manufacturer's instructions (GE Healthcare) and sterilized by autoclaving at 121°C for 20 min. The microcarriers were washed three times in MSC growth medium before use. 4.8×10^4 or 9.6×10^4 heMSC were seeded onto 2.7 mg/mL of Cytodex 1 or 8 mg/mL of Cytodex 3 microcarriers, respectively, which is equivalent to a four cells/bead ratio. Cells were cultivated in 500-mL disposable spinner flasks (Corning) at an agitation rate of 30–40 rpm (unless otherwise stated) with MSC growth medium. Fifty percent medium change was done every other day. After 7 days of expansion in microcarrier-spinner culture, heMSC were dissociated from the microcarriers with 0.25% trypsin-EDTA for 5–15 min