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ORIGINAL RESEARCH

Derivation, characterization and expansion of fetal chondrocytes on different microcarriers

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Abstract Fetal chondrocytes (FCs) have recently been identified as an alternative cell source for cartilage tissue engineering applications because of their partially chondrogenically differentiated phenotype and developmental plasticity. In this study, chondrocytes derived from fetal bovine cartilage were characterized and then cultured on commercially available Cytodex-1 and Biosilon microcarriers and thermosensitive poly(hydroxyethylmethacrylate)-poly(N-isopropylacrylamide) (PHEMA-PNIPAAm) beads produced by us. Growth kinetics of FCs were estimated by means of specific growth rate and metabolic activity assay. Cell detachment from thermosensitive microcarriers was induced by cold treatment at 4 °C for 20 min or enzymatic treatment was applied for the detachment of cells from Cytodex-1 and Biosilon. Although attachment efficiency and proliferation of FCs on PHEMA-PNIPAAm beads were lower than that of commercial Cytodex-1 and Biosilon microcarriers, these beads also supported growth of FCs. Detached cells from thermosensitive beads by cold induction exhibited a normal proliferative activity. Our results indicated that Cytodex-1 microcarrier was the most suitable material for the production of FCs in high capacity, however, 'thermosensitive microcarrier model' could be considered as an attractive solution to the process scale up for cartilage tissue engineering by improving surface characteristics of PHEMA-PNIPAAm beads.

Keywords Fetal chondrocyte · Thermosensitive polymers · Microcarriers · PNIPAAm · Cartilage tissue engineering

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Introduction

Cartilage injuries are very common worldwide and most current cartilage-repair methods depend on cell therapy, i.e. an introduction of chondrogenic cells into the defect area. Cell therapy requires reliable cell scale up technologies to ensure the survival and integration of functional differentiated cell populations into the injured tissues. For this reason, it is mandatory to find a suitable cell source and develop a feasible large scale cell expansion system to obtain a sufficient amount of chondrogenic cell population for articular cartilage injuries (Melero-Martin et al. 2006).

In order to obtain sufficient amount of chondrocytes, usage of an alternative source like stem or progenitor cells is essential because of the limited proliferation capacity of chondrocytes (Benya and Shaffer 1982). Progenitors isolated from fetal and adult cartilage tissues seem to be the most suitable cell source for cartilage engineering. Fetal chondrocytes (FCs) are chondrogenically differentiated in part therefore, they retain a limited degree of developmental plasticity. Because of this limited differentiation capacity and the high expansion potential, these cells are demonstrating significant therapeutic potential for cell-based articular cartilage repair (Mahmoudifar and Doran 2005, 2006) just like stem cells derived from adult cartilage (Dowthwaite et al. 2004; Martin et al. 2005; Melero-Martin et al. 2006). FCs can become an alternative source for a cell-based therapy for cartilage disorders (Pioletti et al. 2006; Montjovent et al. 2009; Quintin et al. 2010).

Microcarrier cell culture system has many advantages for cartilage tissue engineering applications. It is well known that chondrocytes loose their phenotype in cell culture following prolonged expansion (von der Mark et al. 1977; Parsch et al. 2002). However, microcarriers not only promote the maintenance of the original tissue phenotype of chondrocytes, but also provide larger surface area than monolayer systems (Malda et al. 2003; Frondoza et al. 1996; Freed et al. 1993; Bouchet et al. 2000). Neverthless, microcarriers have a limited usage in cell production for tissue engineering applications because of the difficulties in cell retrieval. The removal of cells cultured on microcarriers usually requires harsh enzymatic or mechanical methods that have deleterious effects on the harvested cells.

Enzymatic methods affect the behavior and chemical make-up of the cells by distrupting cell membrane (Fujioka et al. 2003). Despite the numerous studies which have been done to investigate the effect of enzymatic applications on cells such as keratinocytes (Umegaki et al. 2004), tumour cells (Anghileri and Dermietzel 1976), epithelial cells (Reiners et al. 2000), endothelial cells (Lopes et al. 2001; Canavan et al. 2005) and kidney cells (Jung et al. 1995), there are very limited studies on the efficiency of viable cell recovery from microcarriers by enzymes. For this reason, in the presented study the efficiencies of enzymatic methods and also cold induction on cartilagenous cell recovery have been investigated by using three different types of microcarriers, i.e. commercially available Cytodex-1 and Biosilon, and thermoresponsive microcarrier, PHEMA-PNIPAAm, produced by us (Gümüşderelioğlu 2011). To synthesize the thermosensitive microcarrier, N-isopropylacrylamide (NIPAAm) was grafted to the poly(2-hydroxyethyl methacrylate) (PHEMA) beads. PNIPAAm is a smart molecule which exhibits a reversible swelling and deswelling behavior in response to thermal stimulus near physiological temperatures (Silva et al. 2007). This hydrophilic/hydrophobic transition property gives oppurtunity to control the attachment or detachment of cells by thermal stimulus. Because of its unique nature, PNIPAAm is an attractive biomaterial for tissue engineering applications. PNIPAAm-chitosan (Chen and Cheng 2006); PNIPAAm-acrylic acid (Jasionowski et al. 2004) and PNIPAAm-acrylamide (Au et al. 2003) copolymers have been used as scaffold material for chondrocyte cultivation and biocompatibility of these materials for future use has been discussed. However, only few studies have been carried out to determine the ability of PNIPAAm to serve as a microcarrier (Kim et al. 2002). In the presented study, the efficiency of PHEMA-PNIPAAm microcarriers for the production of FCs was evaluated in comparison with Cytodex-1 and Biosilon microcarriers.

Materials and methods

Isolation of fetal and adult chondrocytes

Cartilage slices were harvested from the articular cartilage of 3 months old bovine fetus joints obtained from the local slaughterhouse. Cartilage tissues were washed in sterile phosphate buffered saline (PBS, pH: 7.4) supplemented with penicillin and streptomycin and minced into small pieces by surgical blades in 100 mm culture dish. The small tissue pieces (1 mm^3) were seeded on the surface of tissue culture Petri dishes and cultured in Dulbecco's Modified Eagle Medium (DMEM/F12, Sigma) supplemented with 20% (v/v) Fetal Bovine Serum (FBS, Sigma) at 37 °C, 5% CO_2 and 95% humidity. Cells were harvested when they reached to 80–90% of confluency and then they were passaged (Freshney 2005). Adult cartilage cells were isolated from the articular

cartilage of a two-year old bovine obtained from the local slaughterhouse as described above.

Immunofluorescence characterization of cells

Fetal and adult chondrocytes were labeled with antibodies raised against Collagen type-2 (Neomarkers) and Vimentin (Sigma). Briefly, cells were rinsed with PBS and fixed in 4% (w/v) paraformaldehyde for 20 min at room temperature. Fixed cells were treated with block solution composed of 5% goat serum and Triton-X in PBS for 45 min at room temperature. After washing three times with PBS, cells were incubated with primary antibodies (Collagen type 2, 1:200; Vimentin, 1:200) for overnight at 4 °C. The next day, cells were incubated with anti-mouse IgG secondary antibody (dilution 1:128 Sigma) for 45 min at room temperature and immediately washed three times with PBS. Cells were mounted in Vectashield DAPI (Vectorlabs) containing 4′-6-diamidino-2-phenylindole (DAPI) and then observed using a fluorescent microscope (LEICA DMI6000B).

Growth factor requirements of cells

Fetal and adult chondrocytes (4×10^4 cells/mL) were inoculated into 96 well dishes in control medium which is composed of 10% FBS and DMEM/F12. After incubation for overnight, medium was drained and re-supplied with different culture media including ascorbic acid (Sigma), insulin-like growth factor (IGF-1) (Sigma), transforming growth factor (TGF-β1) (Sigma), non-essential aminoacids (Gibco) and L-glutamine (Gibco) at different concentrations. DMEM with high glucose (Invitrogen) was also used in some groups to observe the effect of medium enriched with glucose. After 4 days of cultivation, media were drained and cells were washed with PBS. Afterwards 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) assay was applied. Results were expressed as relative optical densities to control group (DMEM/F12, 10% FCS).

Calculation of population doubling time

Population doubling time (PDT) was calculated by using the standard procedure as described below. For each well of a 24-well plate, 1 mL of cells were seeded in culture medium at a density of 2×10^4 cells/mL (X_0: inoculated cell concentration). After 72 h cultivation with DMEM/F12 + 20% FCS, plates were removed from the incubator, harvested cells were counted by hemacytometer and the plate was returned to the incubator. Cell counts were repeated at the following 3 days. PDT was calculated using the following equations:

$$\ln(X/X_0) = \mu t \quad (1)$$

$$PDT = \ln 2/\mu \quad (2)$$

where, μ is specific growth rate (h^{-1}), X is harvested cell concentration (cells/mL), X_0 is inoculated cell concentration (cells/mL), t is time period (h) and PDT is population doubling time (h).

Microcarriers

Cytodex-1 (GE Healthcare) microcarrier beads were prepared according to manufacturer's instructions. In brief, dry Cytodex microcarriers were hydrated with PBS by shaking for 4 h at 37 °C. They were washed twice with PBS and then autoclaved for 15 min at 115 °C. Residual PBS was removed and the Cytodex microcarriers were washed and resuspended in warm medium prior to use. Biosilon (Nunc) microcarrier beads were washed with PBS and then, autoclaved for 15 min at 115 °C. They were suspended in 10% (v/v) FCS containing medium prior to use.

PHEMA-PNIPAAm microcarriers

Thermoresponsive PHEMA-PNIPAAm microcarriers were produced and characterized as reported by Gümüşderelioğlu (2011). At first, crosslinked PHEMA beads were prepared by suspension polymerization (Kiremitçi and Çukurova 1992). Then, PNIPAAm was grafted onto the PHEMA beads by aqueous atom transfer radical polymerization (ATRP). ATRP of NIPAAm was carried out according to the related literature (Kim et al. 2003). The basic properties of PHEMA-PNIPAAm microcarriers are summarized in Table 1 together with characteristics of Cytodex-1 and Biosilon.

PHEMA-PNIPAAm microcarriers were sterilized by autoclave (15 min at 115 °C) and equilibrated in PBS. Then the microcarriers were allowed to settle,

Table 1 Properties of PHEMA-PNIPAAm beads and commercial microcarriers, Cytodex-1 and Biosilon

Microcarrier	Matrix	Surface	Diameter (μm)	Specific surface (cm^2/g)	Density (g/mL)
Cytodex-1	Dextran-DEAE	Dextran-DEAE	170 ± 24	4,400	1.03
Biosilon	Polystrene	Polystrene	190 ± 52	225	1.05
PHEMA-PNIPAAm	PHEMA	PNIPAAm	327 ± 99	230	1.05

the buffer was decanted and the beads were rinsed with fresh growth medium.

Cell culture studies

Cell attachment and growth studies were carried out at stationary culture conditions. By taking into account the surface area of microcarriers FCs were inoculated at 1×10^5 cells/mL with a microcarrier concentration of 4 g/L in the case of Cytodex-1 microcarriers and 10 g/L in the case of Biosilon and PHEMA-PNIPAAm microcarriers.

Cell attachment

FCs were typsinized from Petri dishes and inoculated onto Cytodex-1, Biosilon and PHEMA-PNIPAAm which were preincubated in 10% (v/v) FCS containing DMEM/F12 at Parafilm coated 24 well plates. FCs were seeded at a density of 1×10^5 cells/mL and cultured at 37 °C and 5% CO_2 for 1, 2, 4 and 8 h. At the end of each time period, unattached cells were stained with trypan blue and were counted by hemacytometer.

Cell growth

FCs were seeded at a density of 1×10^5 cells/mL and cultured at 37 °C and 5% CO_2 for 11 days. MTT assay was used to quantify the amount of cells on microcarriers. At the desired time points, the culture medium was drained and re-supplied with fresh culture medium including 0.5 mg/mL MTT. After incubating at 37 °C for 4 h, formazan crystals were dissolved with 0.4 M HCl-isopropanol. Optical density (OD) value of each well was determined by ELISA microplate reader (BioRad) while the wavelength was selected at 595 nm. Cell morphology was observed under an inverted microscope (Zeiss Axiovert 35 M). The optical density values were correlated to the cell number on microcarriers, using a donor-specific calibration curve ($R^2 = 0.98$).

Bioreactors

Cytodex-1 microcarriers were transferred to a Sigmacote treated 100 mL spinner flask (working volume 50 mL) and FCs were applied at a density of 1×10^4 or 1×10^5 cells/mL onto 4 g/L Cytodex-1 microcarriers. Cell seeded microcarriers were cultured at 37 °C in an atmosphere with 5% CO_2 by stirring the culture at 30 rpm. Medium was refreshed by 50% every 48 h. Triplicate samples (1 mL) were taken from the bioreactor to determine cell density and viability by MTT assay on the 4th, 6th, 10th and 12th day of culture.

Cell detachment

Cell detachment was induced by enzymatic methods or cold induction. Microcarriers were allowed to settle for 10 min. The supernatant was removed and the culture was rinsed twice with Dulbecco's Phosphate Buffered Saline (dPBS) containing 0.02% EDTA (pH: 8). Cells were incubated with 0.1% (w/v) Pronase (Roche) or 0.25% (w/v) trypsin–EDTA (pH:8) (Sigma) or 0.5% (w/v) collagenase type 2 (Worthington) for 15 min at 37 °C by shaking. Enzymatic activity was inhibited by adding DMEM/F12 including 10% FCS. Microcarriers were removed from cells with a 70 μm Cell Strainer (Falcon). After centrifugation, cells stained with trypan blue were counted with a hemacytometer.

Cells proliferated on PHEMA-PNIPAAm beads were incubated with dPBS at 4 °C for 20 min. Detached cells were counted by using trypan blue and hemacytometer.

Scanning Electrone Microscope (SEM) analysis

Cell-microcarrier complexes were washed with sterile PBS and fixed with 2.5% gluteraldehyde at room temperature for 2 h. Then, they were washed three times in PBS. Finally they were taken through a series of alcohol concentrations (25, 50, 75, 100 and

100%) for dehydration and let to dry. In order to observe cell-microcarrier interactions, gold–palladium coated samples were examined under SEM (JEOL JSM 6335-F).

Statistical analysis

One-way analysis of variance (one-way ANOVA) was used to analyse differences among groups. All statistical analyses were performed using statistical software package SPSS 13.0 for Windows.

Results

Characterization of fetal chondrocytes

Cells from fetal cartilage tissue were characterized according to the expression of intermediate filament markers and the proliferative activity. FCs stained positive for collagen type-2 and vimentin at passage 5 and 12 whereas adult chondrocytes expressed only vimentin (Fig. 1). During the exponential growth phase, FCs demonstrated a higher population doubling time (19.1 ± 0.7 h) than the adult chondrocytes (25.4 ± 0.6 h) ($p < 0.05$). FCs were expanded up to 40 passages in vitro.

In this study, fifteen different media were compared in monolayer cultures in order to investigate the feeding requirements of FCs. The highest value of cell density corresponded to the highest supply of FCS throughout the culture. An increase in FCS concentration from 10% to 20%, 30%, and 40% or 50% nM ascorbic acid supplementation to the FCS caused significant improvements in adult and fetal chondrocyte density ($p < 0.05$). IGF-1 supplementation did not cause an increase in FC amount. However, mitogenic response of adult chondrocytes was evident when same amount of IGF-1 were supplied ($p < 0.05$). TGF-β1 supplementation and L-glutamin/non-essential amino acids did not induce the proliferation of fetal or adult chondrocytes (Fig. 2).

Cell culture on microcarriers

Attachment of FCs to commercial microcarriers (Cytodex-1 and Biosilon), PHEMA-PNIPAAm beads and tissue culture Petri dishes (TCPS) was investigated at intervals of 1, 2, 4, and 8 h after inoculation. At the first 2 h, there was no significant difference between the attachment rate of FCs to commercial microcarriers (63–77%) and thermosensitive surfaces (61%). However, at the 8th h after inoculation the attachment ratio of PHEMA-PNIPAAm (89%) was lower than that of Cytodex-1 and Biosilon (96–98%) ($p < 0.05$) (Fig. 3).

Cell-microcarrier interaction was visualized by SEM. The images of the PHEMA-PNIPAAm beads showed a non-porous rigid structure (Fig. 4a, b), while Cytodex-1 microcarriers have a highly porous sponge-like surface (Fig. 4c, d). The seeded chondrocytes were observed to be able to adhere, spread, multiply, and secrete their matrices onto the PHEMA-PNIPAAm beads (Fig. 4a, b).

Cell growth on microcarrier surfaces was recorded by MTT assay on the 3th, 5th and 11th day of culture that was inoculated with 1×10^5 cells/mL (Table 2). Cells attached, grew and reached confluency on Cytodex-1 microcarriers rapidly. Then, cell growth dropped down significantly from day 5 to day 11 since cells became over confluent and started to die off. However, cell growth on Biosilon and PHEMA-

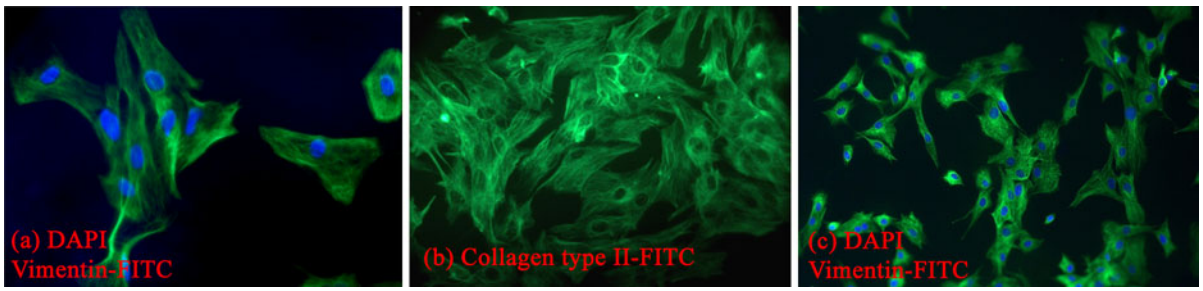

Fig. 1 Immunofluorescence characterization of fetal chondrocytes (FCs) and adult chondrocytes (ACs) at the fifth passage **a** FC Vimentin and DAPI (×400) **b** FC Collagen type-2 (×200) **c** AC Vimentin and DAPI (×400). FCs were characterized according to Collagen type-2 and Vimentin; ACs were characterized according to Vimentin

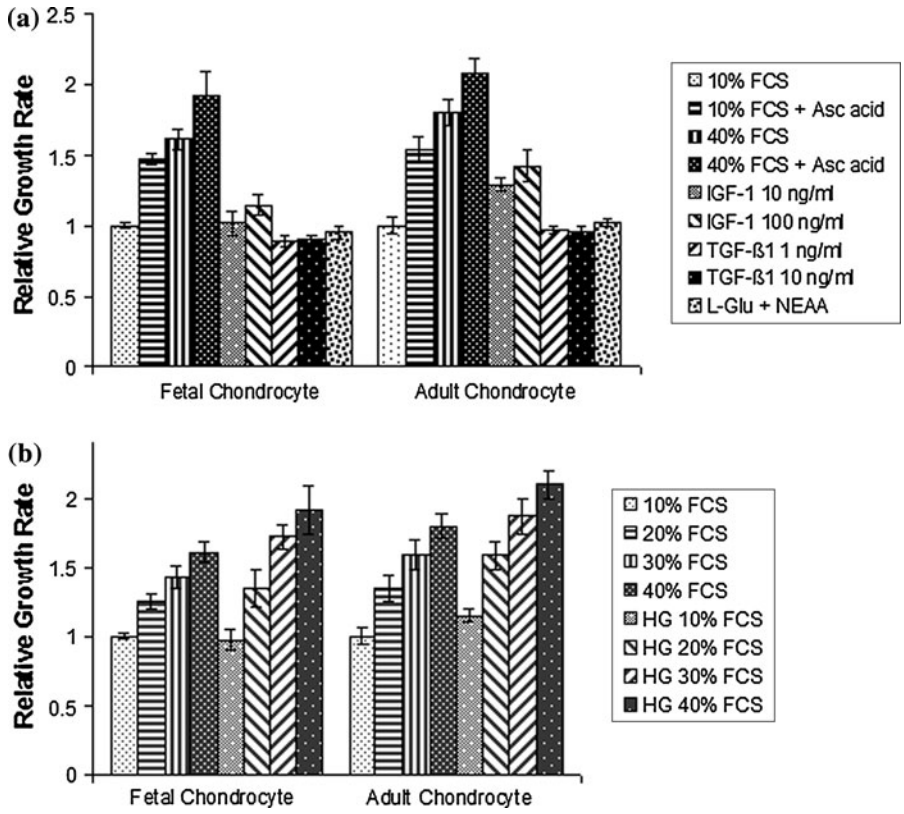

Fig. 2 The effect of different serum concentrations and DMEM with high glucose (HG) **a** and the different growth factors **b** on the proliferation of fetal and adult chondrocytes on the 5th day of culture (NEAA: Non-essential amino acids)

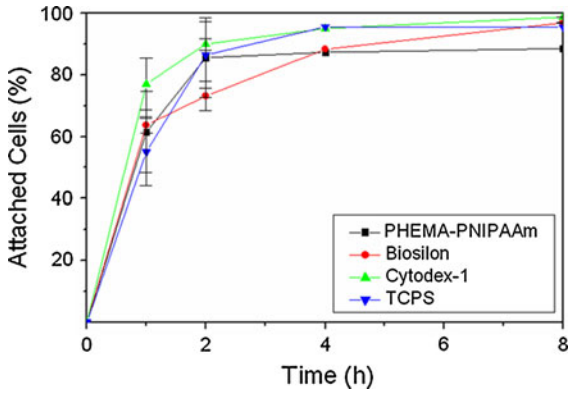

Fig. 3 Attachment of FCs to Cytodex-1, Biosilon and PHEMA-PNIPAAm microcarriers and Petri dishes (TCPS). The percentage of attached cells was determined by direct cell counts of unattached cells in the culture media. *Error bars* show standard deviation (n = 3)

PNIPAAm was slower than that on Cytodex-1, cells were still proliferating at the end of the culture period. This may be explained by the long adaptation periods of cells onto Biosilon and especially PHEMA-PNIPAAm microcarriers due to surface characteristics of the microcarriers. However, FCs displayed a significant proliferative activity on PHEMA-PNIPAAm surfaces by covering and bridging beads to each other in stationary culture after a week of cultivation (Fig. 5a, d) The inherent nature of chondrocytes, which themselves tending to aggregate, caused large cell constructs after 2 weeks of culture on both PHEMA-PNIPAAm and Biosilon microcarriers (Fig. 5).

Figure 6 shows the details of 7 days growth period on Cytodex-1 microcarriers. Here, by taking into account the high proliferative characteristics of FCs on Cytodex-1, inoculation density was decreased to 2×10^4 cells/mL. It was seen that, FCs grown on Cytodex-1 reached 3.2×10^5 cells/mL, which was at least three times higher than that on Biosilion, PHEMA-PNIPAAm and TCPS at the end of the 5th day of cultivation. Since Biosilon and PHEMA-PNIPAAm showed similar growth characteristics as TCPS within 7 days of culture period, only the data on TCPS were presented in Fig. 6.

Stationary culture results indicated that, Cytodex-1 is the most suitable microcarrier for the production of

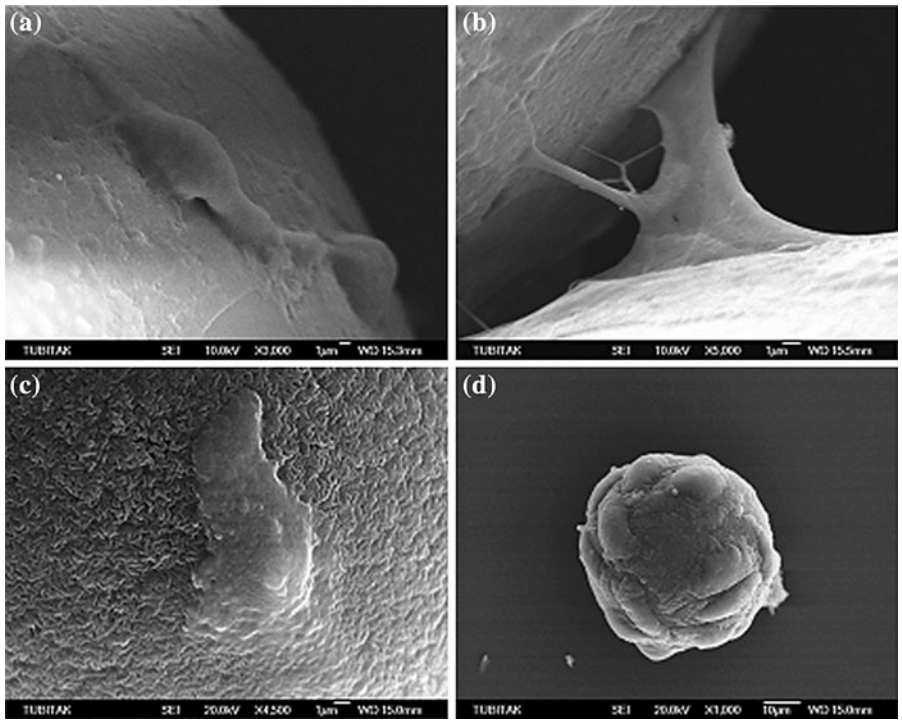

Fig. 4 SEM photographs of fetal chondrocytes cultured on (**a**, **b**) PHEMA-PNIPAAm microcarriers; (**c**, **d**) Cytodex-1 microcarriers on the 4th day of culture. *Bar* shows 1 μm in **a**, **b** and **c**; it shows 10 μm in **d**

Table 2 Comparative growth kinetics of fetal chondrocytes on Cytodex-1, Biosilon and PHEMA-PNIPAAm microcarriers on the 3rd, 5th and 11th day of static culture (Inoculation density is 1×10^5 cells/mL)

Microcarrier	Cell density (cells/mL)		
	Day 3	Day 5	Day 11
Cytodex-1	3.1×10^5	4.0×10^5	8.5×10^4
Biosilon	1.5×10^5	4.0×10^5	6.8×10^5
PHEMA-PNIPAAM	3.0×10^4	8.5×10^4	5.0×10^5

Data show the average of at least three measurements

FCs. This is why, only Cytodex-1 was tested in the spinner flask. When cells were cultured in spinner flasks at 50 mL with Cytodex-1 at 1×10^5 cells/mL, density of FCs was increased to a maximum of 1.4×10^6 cells/mL (Table 3).

Cell harvesting

Pronase, trypsin and collagenase were used in recommended dilutions to harvest FCs from Cytodex-1 microcarriers (GE Healthcare 2005). Pronase application gave the highest amount of cell retriaval in both stationary and stirred culture systems when compared with trypsin and collagenase application. Sixty-six % of the total FCs which were scaled-up in a stirred bioreactor at 50 mL, could be harvested by pronase application. However, only 29 and 26% of the total FCs population could be harvested by trypsin and collagenase (Fig. 7). Due to the highest cell retrieval amount, pronase was preferred to be used in ongoing studies. Cells were harvested from Cytodex-1, Biosilon and PHEMA-PNIPAAm by pronase application with approximately 70% yield or from PHEMA-PNIPAAm beads by cold induction with a similar cell recovery. Because of the possible enzymatic injuries or growth arrest, these cells were re-inoculated onto the Petri dishes to observe whether their proliferative activity was disrupted or not. However, there was no significant difference among the proliferative activity of harvested cells from PHEMA-PNIPAAm beads, commercial microcarriers and Petri dishes according to the MTT assay and population doubling analyses.

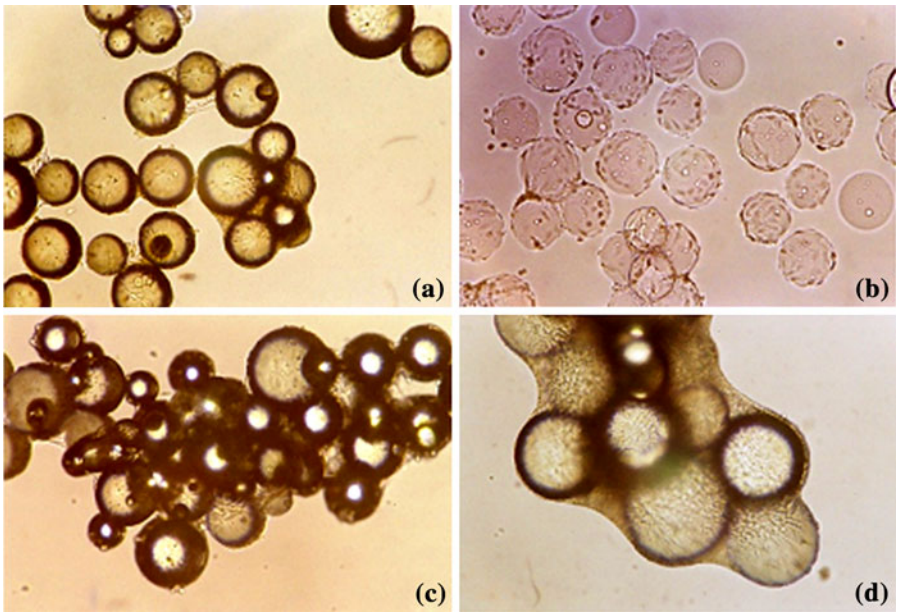

Fig. 5 Fetal chondrocytes on **a** PHEMA-PNIPAAm; **b** Cytodex-1; **c** Biosilon microcarriers after 5 days of cultivation with an inoculation of 1×10^5 cells/mL. **d** Fetal chondrocytes on PHEMA-PNIPAAm after 15 days of cultivation with an inoculation of 1×10^5 cells/mL

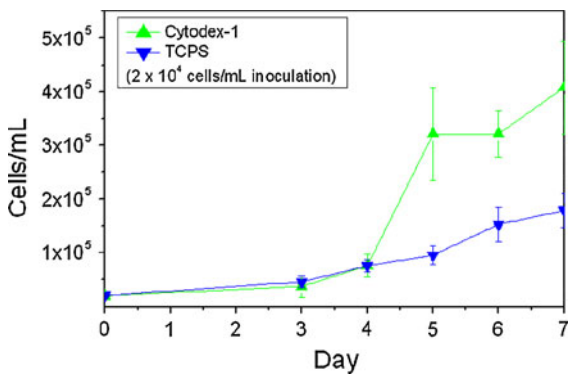

Fig. 6 Comparative growth kinetics of fetal chondrocytes on TCPS and Cytodex-1 microcarriers for 7 days of stationary culture. Cell numbers were determined according to MTT assay. Cell inoculation were density was 2×10^4 cells/mL. Data show the average of at least three measurements

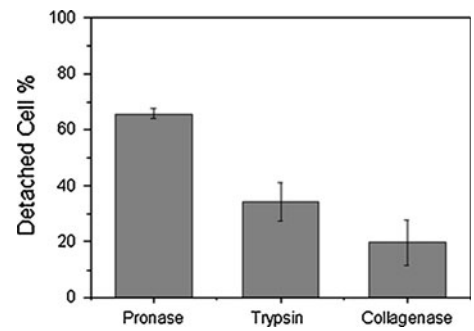

Fig. 7 The percentage of recovered cells from Cytodex-1 microcarriers by using different enzymes. Data show the average of at least three measurements

Discussion

Cells derived from fetal tissues can be an alternative source for cell therapy. Fetal stem cells represent an intermediate cell population between embryonic stem cells and adult mesenchymal stem cells regarding plasticity and proliferation rate. In this study, stem cells derived from adult cartilage tissue were observed to share the characteristics of FCs derived from bovine fetus. FCs did not show only an enhanced ability to maintain collagen type-2 expression in monolayer but also a high proliferative activity in cell culture. In contrast to stem cells in adult cartilage, FCs could be obtained in large quantities. Thus, FCs can be a unique cell source for cartilage cell therapy because of the limited differentiation capacity, high proliferative activity and quantity.

For the development of a large-scale expansion processes, it is fundamental to determine the demands of the specific cell types for optimal growth. Hence,

Table 3 Comparative growth kinetics of fetal chondrocytes on Cytodex-1 microcarriers in spinner flasks at different inoculation densities, i.e. 1×10^4 cells/mL and 1×10^5 cells/mL. Data show the average of at least three measurements

Culture period (Day)	Cell density (cells/mL)	
	Inoculation density 1×10^4 cells/mL	Inoculation density 1×10^5 cells/mL
4	1.0×10^5	6.0×10^5
6	1.9×10^5	8.0×10^5
10	2.0×10^5	1.2×10^6
12	4.0×10^5	1.4×10^6

in this study, culture conditions and feeding strategies were optimized for FCs. In contrast to previous studies carried out with chondrocytes, TGF- β1 or IGF-1 supplementation to the 10% FCS was not effective for fetal chondrocyte scale-up. Results led to the identification of important additives for the control of fetal cartilage cell proliferation. Increasing serum concentration in media supported the proliferation of both adult and FCs in direct proportion. These results were compatible with the feeding requirements of stem cells which were isolated from adult cartilage tissue (Melero-Martin et al. 2006). The proliferative effect of ascorbic acid had also important implications on the development of feeding strategies for fetal cartilage cell cultures as the use of ascorbic acid can notably reduce the need for high FCS supply. To our knowledge, this is the first report on expansion characteristics of FCs. The results of this study could lead us to proceed in the identification of cost effective growth media for cartilage tissue engineering.

Despite the low cell recovery rate (Varani et al. 1985, 1986; Weber et al. 2007), dextran based microcarriers are the most commonly used commercial ones because of the high attachment and proliferation rate of different cell types including chondrocytes (Malda and Frondoza 2006; Baker and Goodwin 1997). In accordance with these studies, our results showed that FCs derived from fetal tissues could be produced successfully by using Cytodex-1 microcarriers. The cells proliferated faster when Cytodex 1 was used instead of Biosilon or PHEMA-PNIPAAm. However, despite the high growth rate, maximal 67% of the total FCs could be harvested from Cytodex-1 microcarrier surfaces by the use of pronase.

The principal obstacle of microcarriers for tissue engineering applications and biobanking has been the difficulty of detaching cells from microcarriers. Ease of cell detachment from the substrate is not an important consideration for applications like vaccine production. However, the production of specific cell types for cell therapy and biobanking requires the release of viable cells from substrates in a functional form. Neverthless, traditional enzymatic methods for cell recovery from microcarriers are time- and labor-consuming (Giard 1986; GE Healthcare 2005).

In our opinion, the choice of microcarriers for the large scale cultivation of the chondrocytes based on their cellular characteristics i.e. adhesion, proliferation and differentation characteristics, is not enough. Along with these properties, a microcarrier model which enables cell recovery while supporting cell growth/attachment at the same time is a need for cartilage tissue engineering applications. In this study, we suggest thermosensitive biomaterials to fill this need. Although commercial thermosensitive Petri dishes are produced by Nunc for specialized cell culture studies such as membrane proteins, a commercial thermosensitive microcarrier is not available yet.

PNIPAAm supports cell attachment and proliferation only in certain molecular architectures (Silva et al. 2007). Here, we have evaluated thermosensitive beads composed of PNIPAAm and PHEMA as potential candidate for a thermoresponsive microcarrier. Results indicated that PHEMA-PNIPAAm surfaces that were synthesized by ATRP reaction supported cell attachment and proliferation. Although cell proliferation and attachment rate on PHEMA-PNIPAAm was lower than that on commercial Biosilon and Cytodex-1 microcarriers, results revealed that PHEMA-PNIPAAm could be used as bulk material for the development of a thermosensitive microcarrier by improving its cell supporting characteristics.

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