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Journal of Chromatography B, 861 (2008) 74–80
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Separation of mesenchymal stem cells with magnetic nanosorbents carrying CD105 and CD73 antibodies in flow-through and batch systems

S. Odabaş [a], F. Sayar [a], G. Güven [a], G. Yanıkkaya-Demirel [b], E. Pişkin [a,*]

[a] *Hacettepe University, Department of Chemical Engineering and Bioengineering Division and Biyomedtek, Ankara, Turkey*
[b] *Centro Laboratories, İstanbul, Turkey*

Received 26 June 2007; accepted 16 November 2007
Available online 23 November 2007

Abstract

The aim of this study is to develop magnetically loaded nanosorbents carrying specific monoclonal antibodies (namely CD105 and CD73) for separation of mesenchymal stem cells from cell suspensions. Super-paramagnetic magnetite (Fe_3O_4) nanoparticles were produced and then coated with a polymer layer containing carboxylic acid functional groups (average diameter: 153 nm and polydispersity index: 0.229). In order to obtain the nanosorbents, the monoclonal antibodies were immobilized via these functional groups with quite high coupling efficiencies up to 80%. These nanosorbents and also a commercially available one (i.e., microbeads carrying CD105 antibodies from Miltenyi Biotec., Germany) were used for separation of $CD105^+$ and $CD73^+$ mesenchymal stem cells from model cell suspension composed of peripheral blood (97.6%), human bone marrow cells (1.2%) and fibroblastic cells (1.2%). The initial concentrations of the $CD105^+$ and $CD73^+$ cells in this suspension were measured as 5.86% and 6.56%, respectively. A flow-through separation system and a very simple homemade batch separator unit were used. We were able to increase the concentration of $CD105^+$ cells up to about 86% in the flow-through separation system with the nanosorbents produced in this study, which was even significantly better than the commercial one. The separation efficiencies were also very high, especially for the $CD73^+$ cells (reached to about 64%) with the very simple and inexpensive homemade batch unit.

Keywords: Mesenchymal stem cell; Isolation; Flow-through and batch systems; Magnetic polymeric nanosorbents; CD105 and CD73 antibodies

1. Introduction

Adult stem cells have unique properties as they have self-renewal capacity and multipotency. Their main role is to maintain and repair the tissue where they are found. Adult stem cells can be isolated from a wide variety of tissues and in general their differentiation capacity may reflect the local environment [1].

The increasing experimental and clinical interest by researchers reveals that mesenchymal stem cells (MSCs) have unique properties: they exhibit transdifferantiation. MSCs can differentiate not only into osteoblasts, chondrocytes and adipose tissues which have their own origin, but also into other cell types including muscle cells, cardiac myocytes, nerve cells and blood cells [2,3]. Especially with these unique properties, MSCs are being considered as cells which have a great potential in regenerative medicine for diverse therapeutic applications such as myocardial infarction, muscular dystrophy, lung fibrosis, segmental bone defects, tendon defect, etc. [1].

For stem cell therapies, the first step is to obtain the required amount of specific stem cells. They have to be isolated and cultured *in vitro* in order to increase their number and, if it is necessary, should be differentiated to the desired cell types. They can be isolated from various sources like bone marrow [4,5], peripheral blood [6], umbilical cord [7], Wharton jelly [8], amniotic fluids [9] and deciduous teeth [10]. Note that in the bone marrow, MSCs represent a very small fraction, 0.001–0.01% of the total nucleated cell [5]. They can be expanded under defined culture conditions.

There has been a controversy about defining mesenchymal stem cells. To address this issue "The International Society for

* Corresponding author.
E-mail addresses: piskin@hacettepe.edu.tr, erhanpiskin@biyomedtek.com (E. Pişkin).

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doi:10.1016/j.jchromb.2007.11.017

Cellular Therapy" (ISCT) declared the minimal criteria's of how a cell can be defined as MSCs [11]. According to this report, MSCs must express at least CD105, CD73 and CD90 and lack expression of CD34, CD45, CD14, CD11b, CD19 and HLA-DR.

There are several ways for isolation of stem cells from the sources. One important approach is to use magnetic separation techniques in which magnetically loaded beads carrying specific antibodies are being used [12–14]. Magnetic separation is an easy technique for purification and enrichment of the desired biological entities (biological molecules, cells, etc.) from various biological media and has several advantages comparing to the other non-magnetic conventional techniques, such as chromatography or centrifugation. It allows isolation of target cells from the biological source like bone marrow, peripheral blood or tissue homogenates directly with in a quite fast and simple way. The sheer forces for binding and elution are relatively low and the washing steps are less. Thus, the isolation can be achieved more easily, effectively and rapidly. In cell separation with magnetic systems, the cells remain unaltered and viable [12].

Magnetic particles carrying specific antibodies, referred also as "immonomagnetic sorbents" have been used for cell detection and isolation, successfully. Linuma et al. [15] have separated $CD45^+$ cells from the cell suspension with commercial MACS microbeads (Miltenyi Biotech, Germany) for the detection of tumor cells in peripheral blood in patients with colorectal cancer. Perez et al. [16] have characterized the porcine bone marrow progenitor cells by separating the cells with immunomagnetic techniques. Schwalbe et al. [17] have produced carboxymethyldextran coated magnetite nanoparticles with the size of 200–300 nm for discrimination of the tumor cells from leukocytes in peripheral blood. Chen and his colleagues have used amino silane modified nanoparticles with a core diameter of 60 nm for purification of $CD34^+$ hematopoietic stem cells from umbilical cord blood [18].

In these studies, usually micron but also nano-size, commercially available or homemade/individualized magnetic particles have been used. The small sized (50–200 nm) magnetic particles have some advantages over larger ones. They have significantly larger surface area per mass, which in turn results higher binding efficiencies. The labeling of the cells can be done quickly without requiring any mixing. Large size particles may form aggregates and block the specific binding regions on the cells, which may decrease the separation yield considerably. Usually agitation is needed with larger particles to prevent aggregation. However, the need of using high gradient magnetic separators can be consider as a minor disadvantage of using nanoparticles for the isolation because currently many high gradient magnetic separators are in lab scale and one can easily make one's own.

In our recent studies, we have also produced nanoparticles, as identification and separation platforms [19,20]. Here, we have attempted to further modify these nanoparticles with specific monoclonal antibodies and produced nanosorbents for both stem cells isolation and culture, by focusing on MSCs. This paper reports our affords towards to prepare a simple and inexpensive batch separation technique based on magnetically loaded nanosorbents, which will be easily adapted into many laboratories working with stem cells.

2. Materials and methods

2.1. Materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, 99%), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, 99%), tetramethylammonium hydroxide (TMAOH), MTT (3-[4,5-dimetiltiazol-2yl]-2,5- difeniltetrazolium were purchased from Sigma (Germany) and used as received. The surfactants, sodium dodecyl sulfate (SDS), the initiator, potassium persulfate (KPS), the activation agent 1-ethly-3-(3-dimethylaminopropyl) carbodimidehydrochloride (EDC) and sulfuric acid (H_2SO_4, 98%) were obtained from Aldrich (Germany). The monomers methyl metacrylate (MMA) and the co-monomer acrylic acid (AAc) were purchased from Fluka (Germany). MMA was treated with NaOH (10%) before use for removing the inhibitor.

The monoclonal antibodies (i.e., CD34-PE, CD45-FITC, CD73-PE, CD105-PE), the CD105 and CD73 purified ones and the Goat anti-Mouse-FITC were obtained from Becton Dickinson (USA). They were used for isolation and flow cytometry analyses.

Bone marrow derived mesenchymal stem cells, peripheral blood sample and human subcutaneous fibroblastic cells are kind gift of Genkord Stem Cell Laboratories (Istanbul). MidiMACs magnetic separations system was purchased from Miltenyi, Biotech (Germany) and used as described in User Manual.

2.2. Preparation of nanoparticles

2.2.1. Magnetite (Fe_3O_4) nanoparticles

The details of production, optimization and the characterization of the magnetite (Fe_3O_4) nanoparticles were given elsewhere [19], which was briefly as follows: production was achieved in a reactor system, as schematically described in Fig. 1A. One hundred-twenty milliliters of aqueous solution of Fe^{2+} and Fe^{3+} salts (total 1.25 M) and 120 ml of 5 M NaOH solution were added into the reactor containing 160 ml distilled water at 80 °C under N_2 atmosphere, by vigorous mixing. A black precipitate was formed at the early phase, and the medium has been continuously stirred for 2 h at a given stirring rate and temperature followed by the slow addition of 10 ml 25% (w/w) TMAOH to stabilize the magnetite particles.

The average particle size and size distribution were determined by Zeta Sizer (Malvern Instruments, Model 3000 HSA, UK). Magnetic properties of nanoparticles and their polymer-coated forms were determined using PAR-150A parallel field vibrating sample magnetometer (VSM) (USA) and Varian E-line 9 Electron spin resonance (ESR) spectrometer (Germany).

2.2.2. Magnetic polymeric particles

The magnetite nanoparticles produced in the previous step were coated with a polymer layer containing carboxylic acid functional groups by microemulsion polymerization of MMA (the main monomer) and AAc (the comonomer for carboxylic acid groups) in a shaking reactor unit as described before (Fig. 1B) [19], which was briefly as follows: The magnetite nanoparticles with an average particle size of 76.2 nm with a par-

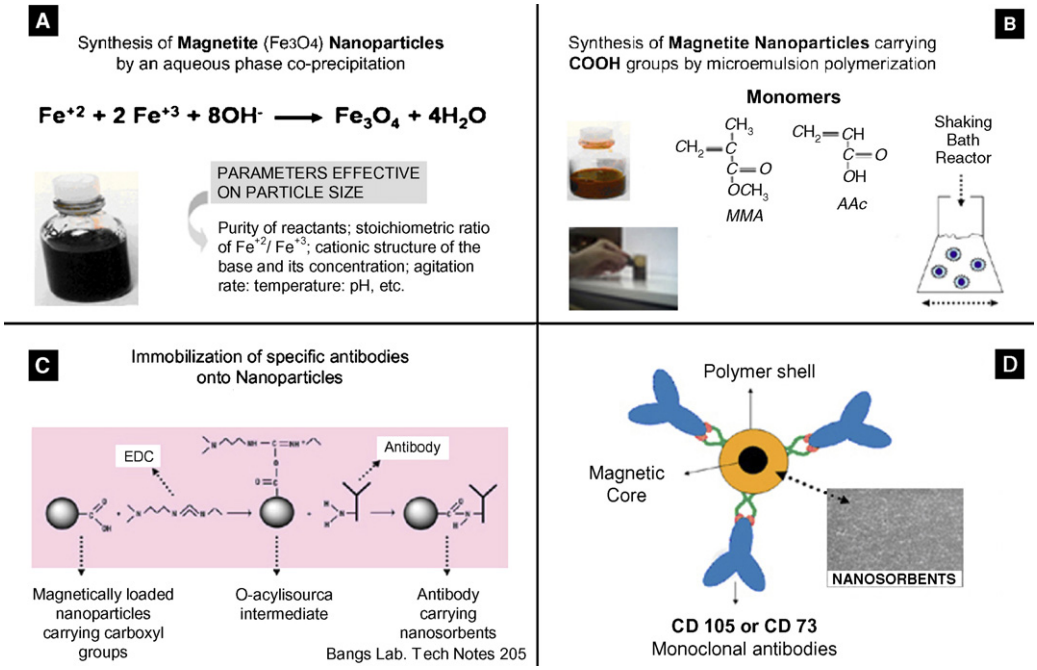

Fig. 1. Production of magnetic nanosorbents for separation of MSCs.

ticle size distribution of 0.194 (i.e., polydispersity index: PDI) were used in this second step. They were coated with a polymer by microemulsion polymerization of MMA and AAc with a comonomer ratio of 90/10 conducted in an oil-in-water system. The surfactant (SDS) and total monomer concentrations were 9.33 and 6.34 wt%, respectively. A water-soluble initiator, KPS with an initial concentration of 2.5 mM was used. Polymerizations were carried out in a constant temperature-shaking bath at 65 °C, under nitrogen atmosphere for 24 h. The magnetic nanoparticles were collected using a magnet and cleaned with methanol and distilled water. The non-coated (naked) magnetite nanoparticles were removed by immersing in 0.1 M of H_2SO_4 solution for 48 h, and then polymer-coated forms were further washed with distilled water to remove acidic residuals. Particle size and size distribution, and magnetic properties of these nanoparticles were obtained as described in the previous step.

2.2.3. Nanosorbents

In the final step, the "nanosorbents" carrying two different type of purified monoclonal antibodies (as probe or specific ligand), namely CD105 or CD73, which are both specific to the receptors on mesenchymal stem cells were prepared (Fig. 1D). The magnetic nanoparticles, with a magnetite core and polymer coating carrying carboxylic acid (–COOH) groups with an average particle size of 153 nm (with rather narrow particle size distributions, PDI of 0.229) produced in the previous step were selected by considering both size and magnetic properties, were selected and used here. For immobilization of antibodies onto the magnetic nanoparticles a quite well-known and widely used protocol was applied (see also Fig. 1C) [21–26]. In the preliminary studies, we have changed concentration of both the nanoparticles and the antibodies, and also the incubation temperature and time in order to optimize the recipe and conditions. After these studies, we decided to use the following recipe and conditions to produce the nanosorbents carrying antibodies used in this study: nanoparticle concentration in the suspension: 5 mg/ml; antibody type and concentration: CD105 and CD73 antibodies with an initial concentration of 500 μg/ml; aqueous immobilization medium with a pH of 5.2 adjusted with MES buffer; temperature: 25 °C; incubation time: 24 h; and immobilization reactor: a rotator with a gentle mixing.

After this immobilization step, the magnetically loaded nanosorbents carrying probe antibodies (see also Fig. 1D) were removed from the medium, washed several times with PBS (0.1 M K_2HPO_4–KH_2PO_4 with pH 7.4) and kept in PBS until use. Coupling efficiencies were determined measuring the initial and final (before and after the immobilization step) concentrations of the respective antibodies within the medium using a UV-spectrophotometer (Shimadzu, Japan).

2.3. Use of nanoparticles

2.3.1. Cytotoxicity

Cytotoxicity of the nanoparticles was determined by MTT assay [27]. For the assay, bone marrow derived mesenchymal stem cells (MSCs) (supplied from Genkord-Stem Cell Laboratory, Istanbul-Turkey) were used. Note that these cells were uniformly positive for CD73 and CD105 and were negative for CD34 and CD45. Here, briefly, 96-well plates containing MSCs (1×10^4 cells per well) in RPMI-1640 containing 10% FBS and 1% antibiotics were used. Different concentration of nanosorbents (1, 10, 50, and 100 ng nanoparticle per cell) were put into the wells except the control wells. Freshly prepared 13 μl MTT solution and 100 μl fresh medium added to each well. The

plates were kept in the incubator at 37 °C in 5% CO_2 for 4 h, the medium was replaced with fresh medium, and incubated under the same conditions for 24 h and 48 h. Following this incubation, the medium was discarded and 100 μl fresh isopropanol (0.04 N) added to each well and mixed gently. The absorbance of the well read at 570 nm in a microplate reader (Biotek Instruments, USA), and the viability of the cells with respect to the control was determined.

2.3.2. Isolation of mesenchymal stem cells

As the separation medium, a mixture of cell suspensions were prepared, containing peripheral blood mononuclear cells, human bone marrow cells and human subcutaneous fibroblastic cells in the relative concentrations of 97.6%, 1.2% and 1.2%, respectively, obtained from Genkord-Stem Cell Laboratory (Istanbul-Turkey). The nanosorbents developed in this study carrying CD73 or CD105 antibodies (*called here as* NanoPar/CD73 and NanoPar/CD105), and also a commercially available magnetically loaded sorbent (*referred here as* MicroBeads/CD105) from Miltenyi Biotec (Heidelberg, Germany) were used in these parallel cell isolation studies. For the isolation process, the protocol described in the data sheet of Miltenyi Biotec was applied [28]. Here, briefly 5×10^7 cells were washed with fresh medium and supernatant was removed. 400 μl fresh PBS buffer was added. Subsequently 100 μl nanosorbents were added, mixed well and incubated for 15 min at 6–12 °C. After incubation, cells were washed with fresh PBS buffer and cells were re-suspended in 500 μl PBS buffer.

A flow-through separation system with a LS separation column (Miltenyi Biotec, MidiMACS, Heidelberg, Germany) was used. The cell suspension was circulated through the column, in which the positive cells (the cells to be separated) bound to the magnetic nanosorbents and trapped, while the other cells (the probe antibody-negative cells) freely passed through. The column was then rinsed with the appropriate amount of PBS buffer. The column was then removed from the separator and the probe antibody-positive cells were collected.

The batch separation was also applied by using our very simple homemade system, which consists of a cylindrical column with a permanent magnet having a magnetic force of 5000 Gauss. Briefly, the tubes containing the cells suspension interacted with the nanosorbents was kept into the batch separator for 10 min. The supernatant was discarded and the desired cells (the probe antibody-positive cells) remained in the tube were collected after washing few times with PBS buffer.

Surface antigens on cells were analyzed by a flow cytometry (FACS Calibur, Becton Dickinson, USA) before and after the separation. Laser alignment, calibration and standardization of the system was checked daily with CaliBrite beads (Becton Dickinson, USA) and followed up with Lewey Jennings graphics on monthly basis. Before separation, to determine the cell surface antigen properties of the cell mixture suspension, 100 μl of the cell suspension (containing $1–3 \times 10^6$ cells per ml) were placed in the sample tubes and were stained with CD73 and CD105 monoclonal antibodies conjugated with phycoerythrin (PE). A non-stained sample was used as the negative control. After separation, 100 μl of the sample was stained with Goat Anti-Mouse-FITC to determine the $CD73^+$ and $CD105^+$ cells. Analyses were done with FS/SS, FL1 and FL2 scattergram and histograms. 10,000 cells were counted for each tube. Cell Quest OX.9 software (Becton Dickinson, USA) was used for data analysis. Sample preparation, data analysis and interpretation were done by different researchers.

3. Results and discussion

3.1. Nanoparticle properties

We have produced magnetite nanoparticles with different size in the range of 75–150 nm by changing several parameters including stirring rate, temperature, precipitation agent concentration, and pH which were reported in one of our previous papers [19]. Considering the final particle size of our nanosorbents which would suitable for stem cell separation, we decided to apply the recipe and conditions given in the previous section and obtained the magnetite nanoparticles with an average particle size of 76.2 nm and size distribution (PDI) of 0.215, which were determined with Zeta-Sizer (Malvern 3000, USA).

These magnetite nanoparticles were then used as seed for production of the polymeric nanoparticles (i.e., with a magnetite core and polymer coating), namely "NanoPar", which was 153 nm, with rather narrow particle size distributions, PDI of 0.229. Note that after coating with a polymer layer, the particle size distributions were still narrow, which was assumed as an indication of no aggregate formation. The results obtained with the vibrating sample magnetometer (VSM) and electron spin resonance spectrometer (ESR) showed that magnetite nanoparticles are super paramagnetic and they did not loose their magnetic property after the coating, also reported in detail elsewhere (see Fig. 1B) [19].

The immobilization of the CD105 and CD73 antibodies to the nanoparticles was achieved with well-known carbodiimide chemistry by using EDC (1-ethly-3-(3-dimethylamino propyl) carbodimidehydrochloride). There are several reports about using EDC chemistry for chemical conjugation of two molecules in which one of the molecules contains primary amine and the other carries carboxylic acid group [23–26]. For instance protein molecules can be immobilized onto carboxylic acid containing surfaces by this conjugation reaction, as we have also applied in this study. Here, we were able to immobilize CD73 and CD105 antibodies with quite high coupling efficiencies, i.e., 79.85% and 77.98%, respectively.

3.2. Cytotoxicity of the nanoparticles

Cytotoxicities of the nanoparticles ("Nanopar") were determined with MTT assay using bone marrow derived mesenchymal stem cells, as described in the previous section. The results of the MTT assay are given in Table 1.

Table 1 demonstrates that the cell viabilities depend on the amount of nanoparticles used per cell. It is quite high, about 87% when 1 ng nanoparticles are used per cell. Higher amounts especially after 50 ng/cell causes significant cell lost (cytotoxi-

Table 1
Cytotoxicities of NanoPar nanoparticles

Nanoparticle concentration (ng nanoparticles/cell)	Cell viability (%) after 48 h
1	87 ± 10
10	61 ± 3
50	32 ± 2
100	12 ± 2

city). But it should be noted that the incubation in this tests is 48 h, however, in cell isolation protocols that we have applied in this study, the cells are treated with nanosorbents only 15 min by using nanoparticles with an amount around 1 ng/cell. Therefore, we concluded that nanosorbents could be used very safely in cell separation protocols while carefully considering the amount and treatment time.

3.3. Separation of mesenchymal stem cells

As mentioned in the previous sections, we first prepared a separation medium, which was a mixture of peripheral blood (97.6%), human bone marrow cells (1.2%) and fibroblastic cells (1.2%). Then, before the separation process, CD105 and CD73 cell surface antigen expressions of the cells in this medium were determined by a flow cytometry. All mononuclear cells were gated by analyses. Typical representative graphical data are given in Fig. 2. Note that the "M1" indicates the autofluorescence. The flow cytometry data demonstrated that there were total 1.5×10^6 cells in 1 ml of the suspension and 5.86% and 6.56% of the total cells were CD105 positive ($CD105^+$) and CD73 positive ($CD73^+$), respectively.

Cell separation was conducted as explained in the previous sections, by using both batch and flow-through magnetic separators. The Mouse Anti-Human CD105 and Mouse Anti-Human CD73 antibodies were purified antibodies. In order to get the $CD105^{(+)}$ and $CD73^{(+)}$ cells after the separation steps we labeled the purified antibodies with Goat Anti-Mouse-FITC. The nanosorbents were incubated with Goat Anti-Mouse-FITC for 15 min before flow cytometry data acquisition. With this labeling, we were able to assure that the nanosorbents do carry the specific probe antibodies (Mouse Anti-Human CD105 and CD73 antibodies) and they are immobilized properly on the surfaces of the nanoparticles.

Table 2
Separation of $CD105^+$ and $CD73^+$ cells in flow-through and batch systems

Cell percentage (%)	
Flow-through separation	
NanoPar/CD105	86.90 ± 4.7
NanoPar/CD73	70.22 ± 6.8
Microbeads/CD105*	77.90 ± 1.3
Batch separation	
NanoPar/CD105	54.47 ± 0.9
NanoPar/CD73	63.52 ± 0.7

*Commercial product by Miltenyi Biotec., Germany.

In the flow-through magnetic separation process, two nanosorbents prepared in this study, namely NanoPar/CD73 and NanoPar/CD105, and also a commercial product, MicroBeads/CD105 (Miltenyi Biotec, Germany) which also carriers CD105 antibodies as specific probe for stem cell separation were used in parallel studies. Note that no commercial product carrying CD73 monoclonal antibodies is available yet. They were used first time in this study. After the separation protocols, the cells separated were analyzed by a flow cytometry as explained above. Typical representative graphical data are given in Fig. 3.

Table 2 gives the percentages of the $CD105^+$ and $CD73^+$ cells reached within the medium after both flow-through and batch separations applied. As seen here, the percentage of the $CD105^+$ cells after separation was increased to $77.90 \pm 1.3\%$ with the commercial microbeads. While it was $86.90 \pm 4.7\%$, considerably higher, when we used the same separation probe antibody (CD105 monoclonal antibodies) with our nanoparticles (i.e., NanoPar/CD105). Even with our nanosorbents carrying CD73 monoclonal antibodies (i.e., NanoPar/CD73), which was the first time used in the separation of the mesenchymal stem cells in this study, the percentage of the $CD73^+$ cells was quite high, and was about $70.22 \pm 6.8\%$.

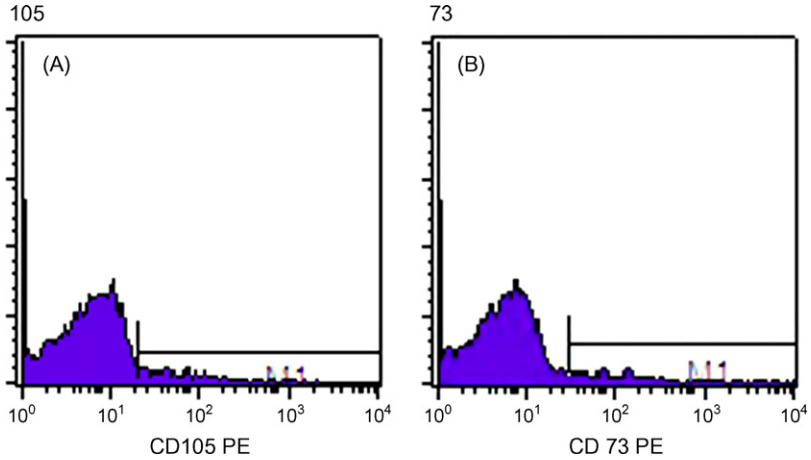

Fig. 2. CD105 and CD73 surface antigen expressions of the cells within the separation medium. A typical data obtained in the flow cytometer before the separations process: (A) CD105 antibodies and (B) CD73 antibodies.

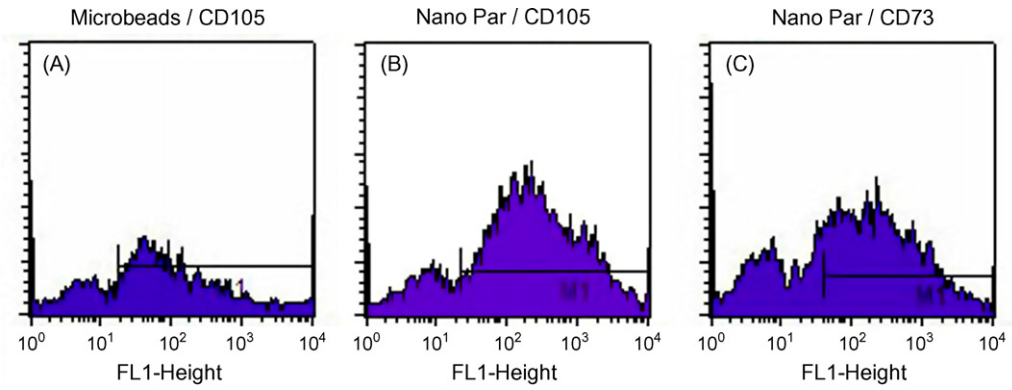

Fig. 3. Typical representative graphical data obtained with the flow cytometer after separation with the flow-through system using: (A) Microbeads/CD105; (B) NanoPar/CD105; and (C) NanoPar/CD73.

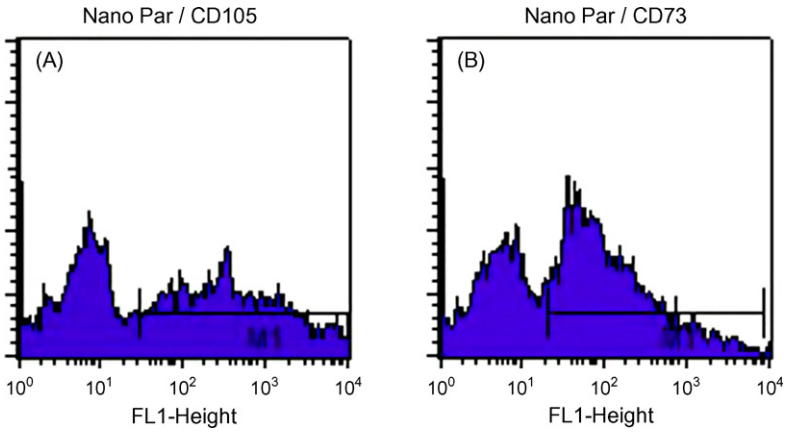

Fig. 4. Typical representative graphical data obtained in flow cytometer after separation with batch system using: (A) NanoPar/CD105; and (B) NanoPar/CD73.

In the batch magnetic separation process, only NanoPar/CD73 and NanoPar/CD105 were used in parallel studies. After the separation protocols, the cells separated were analyzed by flow cytometer as explained above. Typical representative graphical data are given in Fig. 4. The percentage of the $CD105^{+}$ cells reached after separation with NanoPar/CD105 nanoparticles was about 55% (Table 2). When we used our nanosorbents carrying CD73 monoclonal antibodies (i.e., NanoPar/CD73) with the same protocol, the percentage of the $CD73^{+}$ cells was even better and reached to about 64%. The cell separation efficiencies are somewhat higher in flow-through separation system. However, the batch system is very simple, and can be easily built in any lab, and the efficiencies reached with this system are quite high and in acceptable limits, which is one of the significant finding/achievement of the present study.

Similar separation yields have been reported with other cell types in which magnetic nanoparticles. Chen et al. [18] have successfully isolated $CD34^{(+)}$ hematopoietic stem cells from umbilical cord using a amino silane magnetite nanoparticles in a batch magnetic separation unit. They reported the isolation yield for $CD34^{(+)}$ was nearly 75%. Schwalbe et al. [17] were able to separate tumor cells from peripheral blood with carboxymethyldextran coated magnetite nanoparticles. They achieved very high separation yields to 90%.

4. Conclusion

During last decade, there has been so many properties were revealed about stem cells, it is widely agreed that stem cells, especially mesenchymal stem cells, with their great differentiation capacities, are going to take more important part of the cell based therapies, in which a quite high number of healthy cells are needed. Development of rapid, simple inexpensive and maybe the more importantly specific separation techniques are among the main technological targets in this direction. Magnetic separation has been considered as quite easy, effective and very rapid technique to isolate cells unaltered and viable [12,15–18].

Following this trend, in order use in our ongoing studies related to tissue engineering of bone and cartilage, we attempted to produce magnetic nanosorbents carrying specific probe antibodies, CD105 (is being used in commercial magnetic sorbents) and CD73 (first time used in this study) for separation and also culture of MSCs. Besides the other advantage mentioned in Section 1, the rational of using nanoparticles around 100 nm for separation of large (micron size) cells are as follows: We aimed that the magnetic nanosorbents, which are much smaller than cells to be separated, are bound the receptors molecules (specific antigens) on the cell surface, or even may be uptake by

the cells. In common approach, for cell separation, cells are adsorbed specifically on the surfaces of the sorbents, and then are detached (eluted) for further step, which is usually an *in vitro* cell culture (expansion) to reach desired cell numbers for further applications. In this study, as one of the main differences from the previous similar studies, we attempted to apply two steps (separation and expansion) subsequently. We are proposing to separate the target cells by magnetic nanoparticles, and then, without applying any detachment (elution) step (which may be harmful on the cells), culture them directly in the culture media (or in bioreactors) [29,30]. The new cells (which obviously do not carry any magnetic nanoparticles) are separated from the seeding cells (which are carrying the magnetic nanoparticles) very easily using a simple magnet.

We conducted a series of experiments using these two different nanosorbents and also a commercially available one, which was carrying only CD105 for comparison, both by using a commercial flow-through separation system and a very simple homemade batch separator. We used a model cell suspension composed of peripheral blood (97.6%), human bone marrow cells (1.2%) and fibroblastic cells (1.2%) with the initial $CD105^+$ and $CD73^+$ MSCs concentrations of 5.86% and 6.56%, respectively. We were able to increase the concentration of MSCs up to about 86% in the flow-through separation system. We also demonstrated that quite high separation efficiencies up to about 64% were also reached with the batch separation unit. It should be noted that this homemade unit is very simple, inexpensive and easily built in any laboratory, even without any experience on these systems. In addition, we were able to demonstrate that the nanosorbents carrying CD73 antibodies work well similar to commercially available CD105 carrying sorbents. Even, they perform better in batch separation units better then our nanosorbents carrying CD105 antibodies similar to the commercially available ones.

Acknowledgement

This study, which was performed in the contest of EU-FP6-NoE: Expert tissues project and Graduate Programme of Hacettepe University (a part of M.Sc. thesis of Mr. S.Odabas under the supervision of Prof. E. Pişkin), was supported by Turkish Scientific and Technological Council (Turkish Scientific and Technological Research Council, TÜBİTAK, Project no: SBAG-3024, 104S669) as a collaborative research project between Hacettepe University and Genkord Stem Cell Laboratory. Erhan Pişkin was also supported by Turkish Academy of Sciences (TUBA) as a full member. Sedat Odabaş has received a graduate student-scholarship from TÜBİTAK, during these studies.

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