Effects of Corning® CellBIND® Surface medium on growth of human umbilical cord mesenchymal stem cells

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Abstract

BACKGROUND: Stem cells expansion technology *in vitro* is affected by the microenvironment of the culture system. So, to find an effective method is particularly important to promote cell adherent and growth.

OBJECTIVE: To compare the effects of different culture media on cell expansion.

METHODS: Human umbilical cord mesenchymal stem cells at a density of 5.0×10^4 cells/cm^2 were inoculated into Corning® polystyrene culture dishes coated with or without poly-L-ornithine and Corning® CellBIND culture dishes. Cell adhesion and proliferation were observed, and expressions of cell adhesion proteins and cell markers were detected.

RESULTS AND CONCLUSION: Cell adhesion was promoted when cells were cultured in Corning® CellBIND® Surface medium coated with poly-L-ornithine for 24 hours, and the cultured cells grew at the logarithmic phase. The cell proliferation was also enhanced, and the cells expressed cell adhesion protein but not the cell markers of CD73, CD90, CD105. Corning® CellBIND® Surface medium was superior to Corning® polystyrene culture medium in the improvement of cell adhesion and proliferation. Additionally, both of these two media showed no influence on cell phenotype. These findings indicate that Corning® CellBIND® Surface medium can promote cell adhesion and proliferation, but shows no effects on cyclin and cell phenotype. This medium coated with poly-L-ornithine can further accelerate cell adhesion and proliferation, and stably express cell phenotype of human umbilical cord mesenchymal stem cells.

Subject headings: mesenchymal stem cells; cell culture techniques; cell proliferation; ornithin

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INTRODUCTION

Mesenchymal stem cells (MSCs) are used as the seed cells of regenerative medicine. MSCs have attracted great interest in clinical therapy because of their pluripotency[1-4]. MSCs are capable of tissue reconstruction and recovery of coordinating function. Human umbilical cord MSCs (hUMSCs) are considered to be the most suitable stem cell line for cell therapy[5].

For the technology of Corning® CellBIND® Surface culture dish, a microwave plasma process is used for treating the culture surface. This process improves cell attachment by incorporating significantly more oxygen into the cell culture surface, rendering hydrophilicity (wettable) and increasing surface stability. Corning® CellBIND® Surface culture dish is made of high-grade polystyrene and subsequently treated with a combination of energy and gas to create the surface roughness and hydrophilicity that is necessary for protein adsorption and cell attachment[6-8]. Poly-L-ornithine is a component of extracellular matrix and also the main part of the microenvironment in which cells survive. In this study, we examined the effect of Corning® CellBIND® Surface culture dish on cell growth, and compared the changes of cell attachment, cell proliferation and cell surface marker expression after the culture dish was coated with poly-L-ornithine to analyze the underlying relationship.

MATERIALS AND METHODS

Design

Cytology *in vitro* study.

Time and setting

The study was completed at Jilin Province Key Laboratory of Tissue Engineering Research from October 2012 to May 2013.

Materials

Cells

hUMSCs were donated with the consent of the healthy patients and approval of the Research Ethics Committee in Siping

Central Hospital (Jilin, China). hUMSCs were harvested from fresh human umbilical cords using plastic adherence method, and then cells expanded in culture flasks.

Culture dish

Corning® tissue culture polystyrene dish and Corning® CellBIND® Surface culture dish were produced by Corning, New York, USA. According to the following procedures: the 0.01% poly-L-ornithine solution diluted 10 times with sterilized injection water. The dishes were coated with 5 mL of the diluted poly-L-ornithine solution at 37 ℃ for 2 hours, and after solution removal, the dishes were aired in the biosafe cabinet[9-11]. For Corning® CellBIND® technology, a microwave plasma process is used for treating the culture surface. This process improves cell attachment by incorporating significantly more oxygen into the cell culture surface, rendering the hydrophilicity (wettable) and increasing surface stability.

Other reagents

Other reagents

Reagents	Sources
Anti-proliferating cell nuclear antigen(PCNA), anti-cyclin A, anti-cyclin E, anti-fibronectin	Santa Cruz Biotechnology
Anti-β-actin antibody	Abcam, Hong Kong, China
Poly-L-ornithine regent	Sigma-Aldrich, Shanghai, China
CD73-fluorescein isothiocyanate (FITC) conjugated; CD90-FITC conjugated; CD105-phycoerythrin (PE)	Becton-Dickinson Co., New Jersey, USA
Anti-mouse PE-conjugated secondary antibody	Guava Technologies, CA, USA

Methods

hUMSCs isolation and culture

hUMSCs were detached from culture flasks using Trypsin/EDTA seeded to the wells at a density of 5.0 × 10^4 cells/cm^2 and cultured at 37 ℃ in 5% CO_2. At culture termination, the cells were washed once with PBS before further processing as described in the respective method.

Cell grouping

There were four groups, namely Corning® polystyrene culture dishes, Corning® polystyrene culture dishes coated with poly-L-ornithine; Corning®CellBIND® Surface culture dish and Corning® CellBIND® Surface culture dish coated poly-L-ornithine. The changes in cell proliferative protein and adhesion protein expressions, cell density, cell proliferation and cell morphology were detected.

MSCs attachment and proliferation detected by microscope

MSCs (5.0×10^4) were plated onto 60 mm Corning® tissue culture polystyrene dish and Corning® CellBIND® Surface dish coated with or without poly-L-ornithine. Triplicate dishes were collected by trypsinization at the indicated periods of time and the cells were counted using Olympus CKX41SF microscope.

Expression of cell surface markers detected by flow cytometry

hUMSCs were characterized using flow cytometry (FACSCalibur, Becton, Dickinson and Company, NJ, USA), and were incubated with the following anti-human primary antibodies: CD73-FITC conjugated; CD90-FITC conjugated; CD105-PE conjugated; unconjugated markers were reacted with anti-mouse PE-conjugated secondary antibody. A total of 500 000 labeled cells were analyzed and the data obtained were analyzed using BD CellQuest software[12-14].

The expressions of cell adhesion and cell proliferative proteins detected by western blot assay

To verify that the adhesion proteins are adsorbed to the cell culture dishes coated with or without poly-L-ornithine, and the changes of hUMSCs proliferative protein expression in different culture dishes, fibronectin, cyclinA, cyclinE and PCNA expressions were detected by western blot analysis. The cells were harvested and washed with cold PBS. Then 30–50 μg of cellular or nuclear extract was subjected to SDS-PAGE in 10%–15% gradient gels. Proteins in the gel were transferred onto polyvinylidene fluoride membranes and probed with fibronectin, cyclinA, cyclinE and PCNA antibodies in 5% skim milk PBS containing 0.1% Tween-20 (PBST) for 24 hours at 4 ℃. The blots were then incubated with 1:2 000 dilutions of horseradish peroxidase-conjugated goat anti-rabbit or horseradish peroxidase-conjugated goat anti-mouse IgG for 1 hour at room temperature. The membrane was washed extensively before detection using the chemiluminescence BeyoECL plus kit (Thermo Fisher, USA). The membranes were then stripped and reprobed with β-actin antibody as the loading control. Judgment based on the band depths of color was conducted to analyze the expression of the primary antibody.

Main outcome measures

(1) The effect of different culture dishes coated with poly-L-ornithine on cell adhesion protein, cell proliferation, cell cyclin expressions; (2) the effect of different culture dishes coated with poly-L-ornithine on cell phenotype expressions.

Statistical analysis

The data were expressed as mean±SD and statistically processed by one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. Calculations were performed using the GraphPad Prism 5.0 program (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Effects of Corning® CellBIND® Surface dish coated with poly-L-ornithine on cell adhesion

Cell numbers and morphology were observed in different culture dishes within 24 hours as shown in **Figure 1**. Cells cultured in the Corning® culture dish were most adhered

and spindle-shaped, resembling fibroblasts. Corning® CellBIND® Surface culture dish also exhibited the powerful effect on cell growth[15-22], and the number of adherent cells was increased with the time prolonged within 24 hours. Depending on the treatment of microwave plasma process for treating the culture surface, Corning® CellBIND® Surface culture dish coated with poly-L-ornithine exhibited a better effect on cell attachment than Corning® tissue culture polystyrene dish coated with poly-L-ornithine (**Figure 2**).

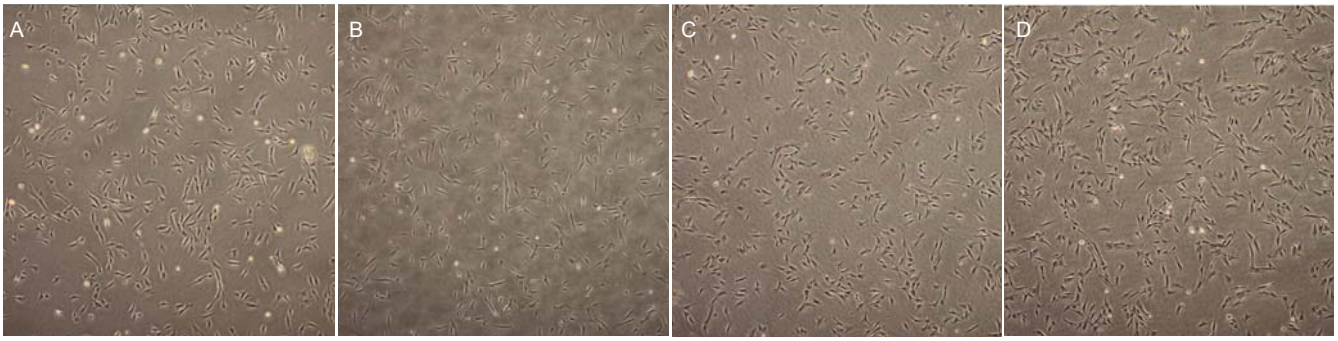

Figure 1 The morphology of human umbilical cord mesenchymal stem cells (hUMSCs) in different culture dishes within 24 hours (×40)

Note: (A) hUMSCs cultured in Corning® tissue culture polystyrene culture dish); (B) hUMSCs cultured in Corning® tissue culture polystyrene culture dish coated with poly-L-ornithine; (C) hUMSCs cultured in Corning® CellBIND® Surface culture dish; (D) hUMSCs cultured in Corning® CellBIND® Surface culture dish coated with poly-L-ornithine).

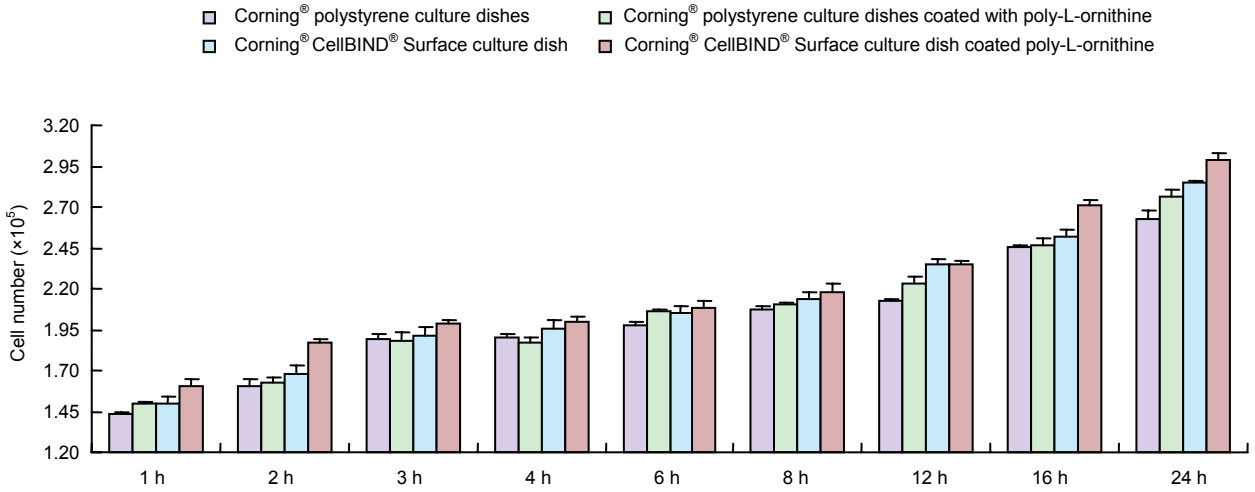

Figure 2 Cell proliferation after 24 hours culture in different dishes

Note: The data were expressed as mean±SD, *n*=3 for all conditions.

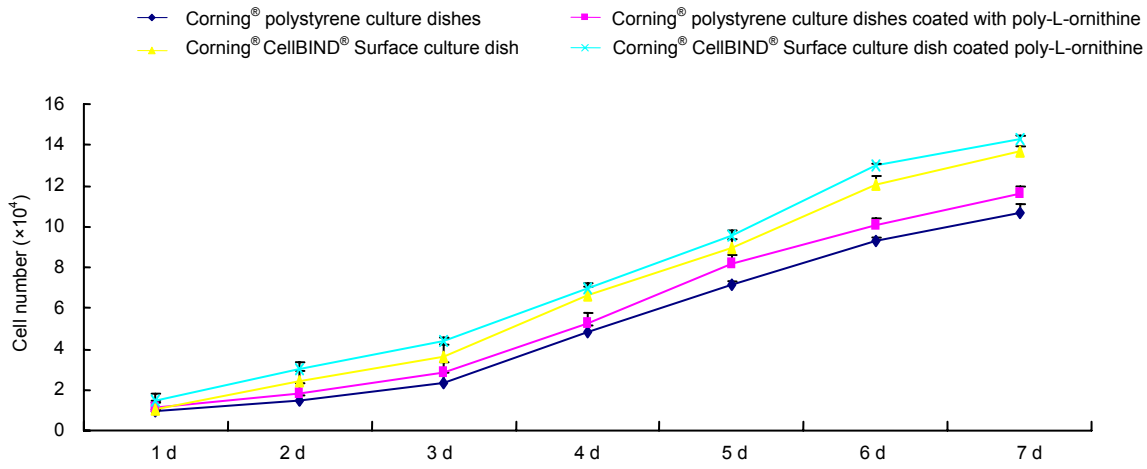

Figure 3 Cell proliferation in the surface of different culture dishes within 7 days

Note: Data were expressed as mean±SD, *n*=3 for all conditions. There was no significant difference.

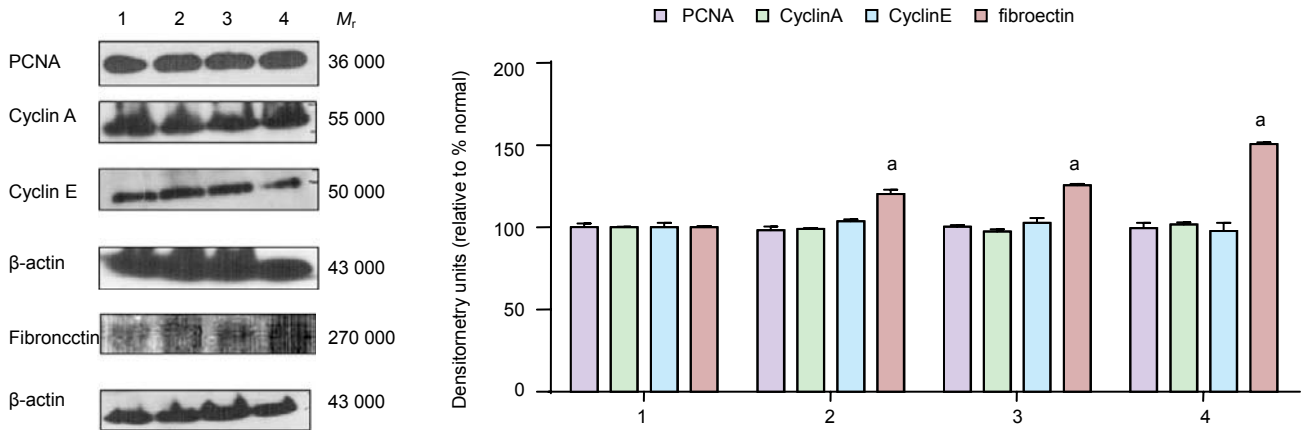

Figure 4 The expressions of proliferating cell nuclear antigen (PCNA), cyclinA, cyclinE and fibronectin in different culture dishes within 7 days

Note: Data are expressed as the mean±SD of three independent experiments. [a]$P < 0.01$, *vs*. Corning® tissue culture polystyrene culture dish; 1: Corning® polystyrene culture dishes; 2: Corning® polystyrene culture dishes coated with poly-L-ornithine; 3: Corning® CellBIND® Surface culture dish; 4: Corning® CellBIND® Surface culture dish coated poly-L-ornithine.

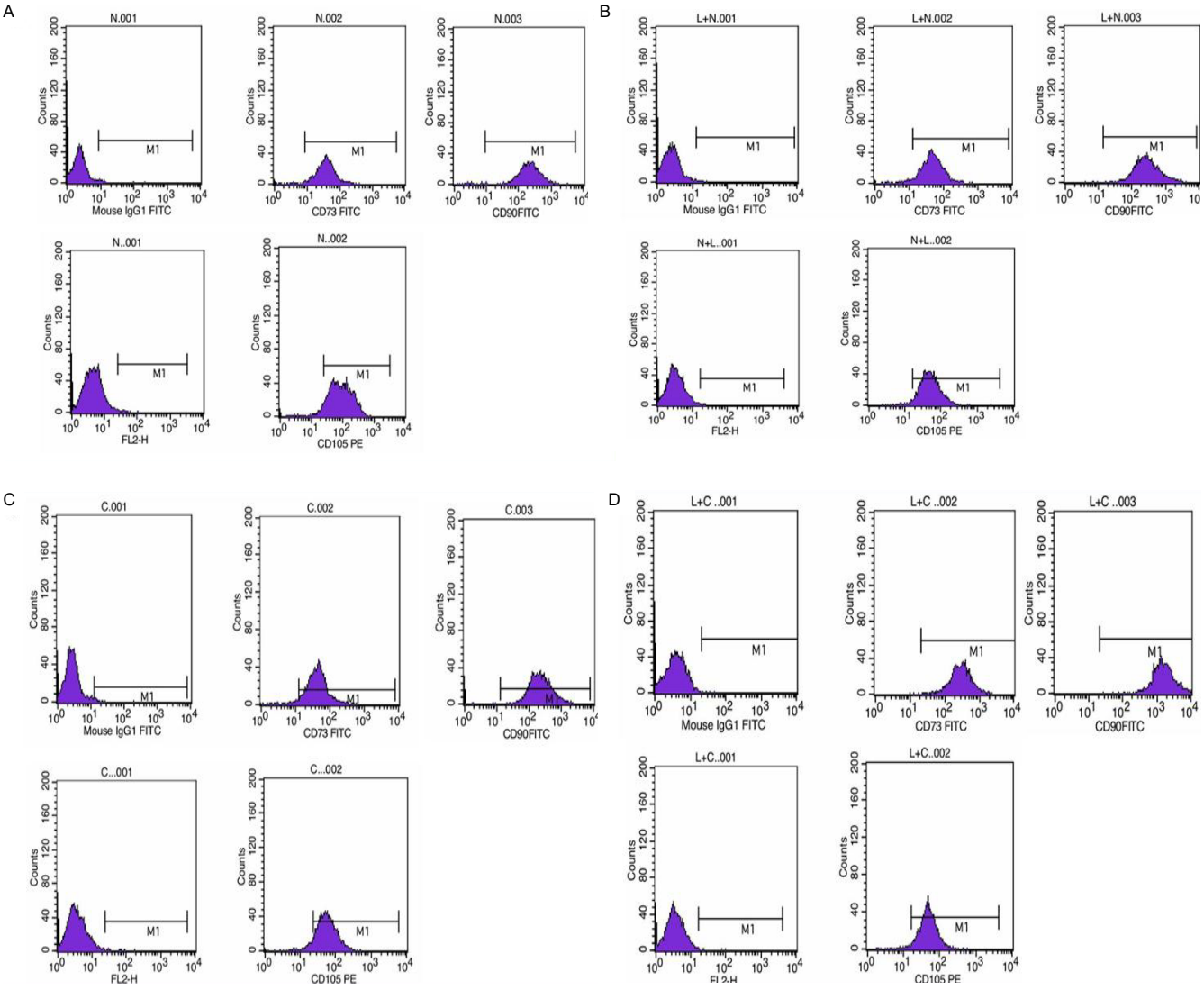

Figure 5 The cell phenotypes CD73, CD90, CD105 of human umbilical cord mesenchymal stem cells after cultured 7 days in different culture dishes

Note: A: Corning® tissue culture polystyrene dish group; B: Corning® tissue culture polystyrene dish coated with poly-L-ornithine group; C: Corning® CellBIND® Surface culture dish group; D: Corning® CellBIND® Surface culture dish group coated with poly-L-ornithine group.

Effects of Corning® CellBIND® Surface dish coated with poly-L-ornithine on cell proliferation

Cells proliferating in the different culture surfaces were observed in 7 days as shown in **Figure 3**. The results showed the growth curve of hUMSCs presented inverted "S", and cells exhibited logarithmic growth on days 3, 4, 5 and 6. Owing to the effect of poly-L-ornithine on cell adhesion, Corning® tissue culture polystyrene dish and Corning® CellBIND® Surface dish coated with poly-L-ornithine improved cell growth at 7 days compared with the culture dishes that coated without poly-L-ornithine. However, Corning® CellBIND® Surface dish exerted the better effect on cell proliferation under any treatment on the surface of dish compared with Corning® tissue culture polystyrene dish (**Figure 3**).

Effects of Corning® CellBIND® Surface dish coated with poly-L-ornithine on cell adhesion and cell proliferative protein expressions

To test the effect of Corning®CellBIND® Surface culture dish on cell attachment and cell proliferative protein expressions, first, we detected cell proliferative protein including cyclinA, cyclinE and PCNA expressions. The results showed that neither Corning® CellBIND® Surface culture dish, Corning® tissue culture polystyrene dish nor those coated with poly-L-ornithine influence the expressions of PCNA, cyclinA or cyclinE by western blot analysis ($P > 0.05$). Furthermore, the expressions of adhesion cytokines were examined and the expression of fibronectin was significant increased in Corning® tissue culture polystyrene dish coated with poly-L-ornithine and Corning® CellBIND® Surface culture dish coated with or without poly-L-ornithine ($P < 0.05$) (**Figure 4**).

Effects of Corning® CellBIND® Surface dish coated with poly-L-ornithine on expression of cell surface markers

We previously demonstrated that similarly processed hUMSCs expressed high levels of matrix receptors (CD73, CD90 and CD105) as assessed by flow cytometry. In addition, all adherent hUMSCs did not express CD45, CD34, CD14, CD19 or HLA-DR (data was not shown). In order to determine the effect of Corning® CellBIND® Surface culture dish on hUMSCs phenotype, we examined the levels of CD73, CD90 and CD105 in the four different groups. As our predicted, hUMSCs highly expressed CD73, CD90 and CD105 in the four groups as same as our isolated cells from human umbilical cords before. It implied that Corning® CellBIND® Surface culture dish coated with or without poly-L-ornithine did not affect the cell phenotype (**Figure 5**).

DISCUSSION

Extracellular matrix is an important ingredient for cell survival microenvironment, which depending on the interaction with the adaptor of cytomembrane to improve cell growth and cell attachment. Poly-L-ornithine is usually used as a component of extracellular matrix to simulate cell microenvironment during the cell culture *in vitro*[23]. Studies about the effect of the extracellular matrix on biological activity of stem cells are still few. In this study we mainly evaluated the influence of Corning® CellBIND® Surface culture dish coated with poly-L-ornithine on cell cycle, cell proliferation, cell attachment and other characteristics. At present, there are few studies on observing the proliferation and adhesion of stem cells that are cultured in cell culture dish coated with poly-L-ornithine.

A suitable copolymer surface has been developed for cell culture[24-30]. By comparing Corning® CellBIND® Surface culture dish with that coated with poly-L-ornithine, our results indicated that Corning® CellBIND® Surface culture dish promoted cell attachment and cell proliferation faster than Corning® culture dishes. Importantly, cells cultured on the special surface show neither induced differentiation under standard growth conditions nor a rate of spontaneous differentiation greater than that seen with Corning® tissue culture polystyrene dish as indicated by hUMSCs phenotypes of CD73, CD90 and CD105. And there are no advanced effects on cell proliferative protein expressions as indicated as cyclinA, cyclinE and PCNA. It means that by using microwave plasma process for treating the culture surface, Corning® CellBIND® Surface culture dish exerts the excellent effect on cell attachment and cell proliferation and maintains the characters of hUMSCs. Poly-L-ornithine coating does not change the character of Corning® CellBIND® Surface culture dish, and also develops the ability of Corning® CellBIND® Surface culture dish[31-39]. It is for the first time suggested that the promotion effect of the extracellular matrix component-poly-L-ornithine on cell proliferation and cell adsorption. The effect excellently shown on Corning® CellBIND® Surface does not affect cell proliferation and cell adsorption, and promotes characteristics of Corning® CellBIND® Surface culture dish itself. However, the changes of hUMSCs behavior and differentiation after long-term culture in poly-L-ornithine coated culture dish need to be further studied.

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Corning® CellBIND®表面培养皿促进脐带源间充质干细胞生长

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文章亮点：

1 应用流式细胞仪检测技术考察细胞表面标志物并证明人脐带源间充质干细胞经过 Corning® CellBIND®表面培养皿培养后细胞标志物没有改变。

2 Corning® CellBIND®表面培养皿能够发挥较好的促进人脐带源间充质干细胞贴壁和增殖的作用；包被多聚鸟氨酸能够促进人脐带源间充质干细胞贴壁，不影响 Corning® CellBIND®表面培养皿本身促进细胞贴壁增殖特性的发挥。

3 人脐带源间充质干细胞经 Corning® CellBIND®表面培养皿培养后，对细胞周期蛋白及细胞增殖蛋白的表达没有影响，但是能够促进细胞黏附蛋白的表达，这可能与多聚鸟氨酸本质特性有关。

关键词：

干细胞；脐带脐血干细胞；干细胞基础实验；Corning® CellBIND®表面培养皿；细胞贴壁；细胞增殖；细胞表型；脐带源间充质干细胞；多聚鸟氨酸

主题词：

间质干细胞；细胞培养技术；细胞增殖；鸟氨酸

摘要

背景：培养体系微环境影响干细胞体外扩增，寻找有效的促进细胞贴壁和增殖的培养方法尤为重要。

目的：比较不同细胞培养材质对细胞体外扩增的影响。

方法：将 5.0×10^4 培养的人脐带源间充质干细胞分别接种于包被/不包被鸟氨酸 Corning® 聚苯乙烯培养皿或 Corning® CellBIND®表面培养皿中培养，分别观察细胞的贴壁、增殖状态、与细胞黏附和细胞增殖有关的蛋白的表达及细胞标志物的表达。

结果与结论：与其他类型的培养皿相比，包被多聚鸟氨酸的 Corning® CellBIND®表面培养皿在 24 h 内能够促进人脐带源间充质干细胞贴壁，同时也能使培养的人脐带源间充质干细胞较早的进入对数生长期，细胞增殖数量相对较多，虽然对人脐带源间充质干细胞表面标志物 CD73，CD90 和 CD105 的表达没有影响，但能促进细胞中黏附蛋白的表达。且 Corning® CellBIND®表面培养皿较 Corning®聚苯乙烯培养皿更能促进人脐带源间充质干细胞的贴壁和增殖，同时也对细胞表型的表达无影响。提示 Corning® CellBIND®表面培养皿能够促进细胞吸附，增加细胞数量，且对细胞周期蛋白以及细胞表型的表达没有影响，且包被多聚鸟氨酸能够进一步促进细胞的贴壁和增殖，并稳定表达人脐带源间充质干细胞的细胞表型。

作者贡献：第一作者进行实验设计，实验实施为第三、四、五作者，实验评估为第六作者，资料收集为第二作者，第一作者成文，第六作者审校，第一作者对文章负责。

利益冲突：课题未涉及任何厂家及相关雇主或其他经济组织直接或间接的经济或利益的赞助。

学术术语：细胞外基质-是由大分子构成的错综复杂的网络，为细胞的生存及活动提供适宜的场所，并通过信号转导系统影响细胞的形状、代谢、功能、迁移、增殖和分化。

作者声明：文章为原创作品，无抄袭剽窃，无泄密及署名和专利争议，内容及数据真实，文责自负。

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