研究报告

无血清共培养条件下脐带间充质干细胞对奶牛乳腺上皮细胞的增殖作用

赵艳坤,邵伟,雒诚龙,余雄*

(新疆农业大学动物科学学院,新疆肉乳用草食动物营养实验室,乌鲁木齐 830052)

【摘要】 目的 旨在探究无血清共培养条件下脐带间充质干细胞(umbilical cord mesenchymal stem cells, UC-MSCs)对奶牛乳腺上皮细胞(bovine mammary gland epithelial cells, BMECs)的增殖作用。**方法** 选取生长状态最佳的 UC-MSCs 和 BMECs,将两种细胞以直接接触和间接接触两种共培养方式作为试验组,直接接触是按照 UC-MSCs:BMECs 分别为 2∶1、1∶1、1∶2、1∶3、1∶4、1∶5、1∶10 的浓度梯度混合共培养,间接接触则是提取 UC-MSCs 的上清液作为条件培养基重悬 BMECs,对照组为 UC-MSCs 和 BMECs 单纯培养组,并设阴性空白对照,于 0、4、8、12、24、36、48、60、72 h 时观察各组细胞的生长变化,采用 CCK-8 法检测各组细胞增殖情况。**结果** 在 48 h,条件培养基 BMECs 组 A 值显著高于对照组($P<0.05$),而 1∶2浓度组 A 值达到峰值,极显著高于对照组($P<0.01$),显著高于其他各浓度组和条件培养基 BMECs 组($P<0.05$)。**结论** 无血清条件下,UC-MSCs 和 BMECs 共培养可以促进 BMECs 增殖,直接接触促增殖效果优于间接接触,且最适比例为 1∶2,最佳时间是 48 h。

【关键词】 脐带间充质干细胞;乳腺上皮细胞;无血清;共培养;增殖

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Proliferation-promoting effect of umbilical cord mesenchymal stem cells on co-cultured bovine mammary gland epithelial cells

ZHAO Yan-kun, SHAO Wei, LUO Cheng-long, YU Xiong*

(College of Animal Sciences, Xinjiang Agricultural University, Xinjiang Key Laboratory of Meat & Milk Production Herbivore Nutrition, Urumqi 830052, China)

【Abstract】 Objective To explore the proliferation-promoting effect of bovine mammary gland epithelial cells (BMECs) co-cultured with umbilical cord mesenchymal stem cells (UC-MSCs) in serum-free culture mediuum. **Methods** Bovine UC-MSCs and BMECs were selected for co-culturing in direct or indirect contact. In the direct contact culture groups, UC-MSCs and BMECs were co-cultured at concentration ratios of 2∶1, 1∶1, 1∶2, 1∶3, 1∶4, 1∶5, and 1∶10, respectively. In the indirect contact culture group, the supernatant of UC-MSCs was used as the conditioned medium to resuspend BMECs. In the control groups, UC-MSCs and BMECs were cultured alone. The cell growth status in each group was observed at 0, 4, 8, 12, 24, 36, 48, 60, 72 h after culture, and cell proliferation was detected by cell counting kit-8 (CCK-8) assay. **Results** At 48 h, the optical density of the conditioned medium-BMECs group was significantly higher compared with the control groups ($P<0.05$). Meanwhile, the optical density in the direct contact group at a concentration ratio of 1∶2 reached the peak, which was extremely significantly higher compared with the control groups ($P<0.01$) and significantly higher compared with the other direct contact culture groups and the conditioned medium-BMECs group ($P<$

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[**作者简介**]赵艳坤(1990 –),女,博士生,专业:动物营养与饲料科学。Email:452349621@ qq. com

[**通讯作者**]余雄(1958 –),男,教授,研究方向:反刍动物营养。Email:yuxiong8763601@ 126. com

0.05). **Conclusions** Co-culture of UC-MSCs and BMECs in serum-free culture medium is capable to promote the proliferation of BMECs, and the co-culture by cell-to-cell contact has a better effect. The optimal concentration ratio of UC-MSCs to BMECs is 1∶2, and the optimal culture time is 48 h.

【Key words】 Umbilical cord mesenchymal stem cells; Mammary gland epithelial cells; Serum free medium; Co culture; Cell proliferation; Bovine

Corresponding author: Yu Xiong (1958 -), male, professor. Research direction: Ruminant Nutrition. Tel: 0991 - 8763601; Email: yuxiong8763601@126.com.

The proliferation and differentiation of bovine mammary gland epithelial cells (BMECs) run through the whole process of mammary gland development. BMECs maintain lactation by continuous proliferation, which is regulated by many hormones and growth factors [1]. UC-MSCs have a pluripotency to differentiation, self-renewal, and low immunogenicity. They can secrete multiple cytokines, and are involved in the construction of cell microenvironment[2]. UC-MSCs can be used as seed cells to multiply BMECs and promote their growth, providing a new idea for the application of breast tissue engineering and regulation of lactation.

In a previous study, our team had injected UC-MSCs into rats, sheep, and Holstein bull calves. It was found that UC-MSCs did not induce immune rejection in these animals, and they influenced different tissues by exerting the function of stem cell homing. These in vivo experiments verified that UC-MSCs have remarkable effects on promoting growth, accelerating mammary gland development, and reducing inflammation[3-6]. To further clarify how UC-MSCs promote the mammary gland development, this experiment used the direct- and indirect-contact method to co-culture UC-MSCs and BMECs under serum-free condition, and CCK-8 assay was used to detect the proliferation-promoting effect of UC-MSCs on BMECs. This article offers a novel method for enhancing the growth and proliferation of BMECs in vitro and establishes a theoretical basis and technique for further exploring the mammary gland development and the approach of raising milk yield.

1 Materials and methods

1.1 Design of experiment

The experiment was carried out from December 2015 to June 2016 in the Xinjiang Key Laboratory of Meat & Milk Production Herbivore Nutrition. The UC-MSCs and BMECs with the best growth performance were chosen for co-culturing by direct or indirect cell-to-cell contact. In the direct contact groups, UC-MSCs and BMECs were co-cultured at concentration ratios of 2∶1, 1∶1, 1∶2, 1∶3, 1∶4, 1∶5, and 1∶10, respectively. In the indirect contact groups, the supernatant of UC-MSCs was used as the conditioned medium to resuspend BMECs. In the control groups, UC-MSCs and BMECs were cultured alone. A negative control group was further arranged. The cell growth status in each group was monitored at 0 h, 4 h, 8 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h of co-culture, and cell proliferation was detected by CCK-8 assay.

1.2 Materials

1.2.1 Cell source. UC-MSCs: umbilical cord-derived mesenchymal stem cells were isolated from Holstein dairy cattle in our previous experiment and cultured in vitro (the cells have been identified). BMECs: purchased from Guangzhou Jennio Biotech Co., Ltd.

1.2.2 Main apparatus and reagents. Inverted microscope (Motic-AE31), CO_2 incubator (HF151UV), T75 flask (Corning T), 96-well plate (Bogoo, Shanghai), H-DMEM (Hyclone), RPMI-1640 (Hyclone), fetal bovine serum (Gibco), 0.25% trypsin + ED (Hyclone), CCK-8 reagent (7Sea Biotech, Shanghai), and microplate reader (Thermo).

1.3 Methods

1.3.1 Purification and amplification of UC-MSCs and BMECs. When growing to 90% confluence, UC-MSCs and BMECs were subcultured at the ratio of 1∶2 or 1∶3, using trypsin digestion.

1.3.2 Co-culture of UC-MSCs and BMECs. UC-MSCs and BMECs were purified and multiplied at first.

Some of them were co-cultured in 96-well plates at different concentration ratios. For the indirect contact group, supernatant of UC-MSCs was used as the conditioned medium to re-suspend BMECs, which were then inoculated in culture plates. In the control groups, both BMECs and UC-MSCs were cultured alone. For each group, the same inoculum density was adopted and three parallel experiments were conducted. Each culture plate was marked and incubated at 37℃ in 5% CO_2. Serum-free medium was used to replace the culture medium.

1.4 Determination of viable cells

10 μL of CCK-8 solution (prepared with serum-free medium) was added in each well, and incubated at 37℃ in 5% CO_2 for 4 h. Subsequently, an automatic microplate reader was used to determine the optical density at 450 nm.

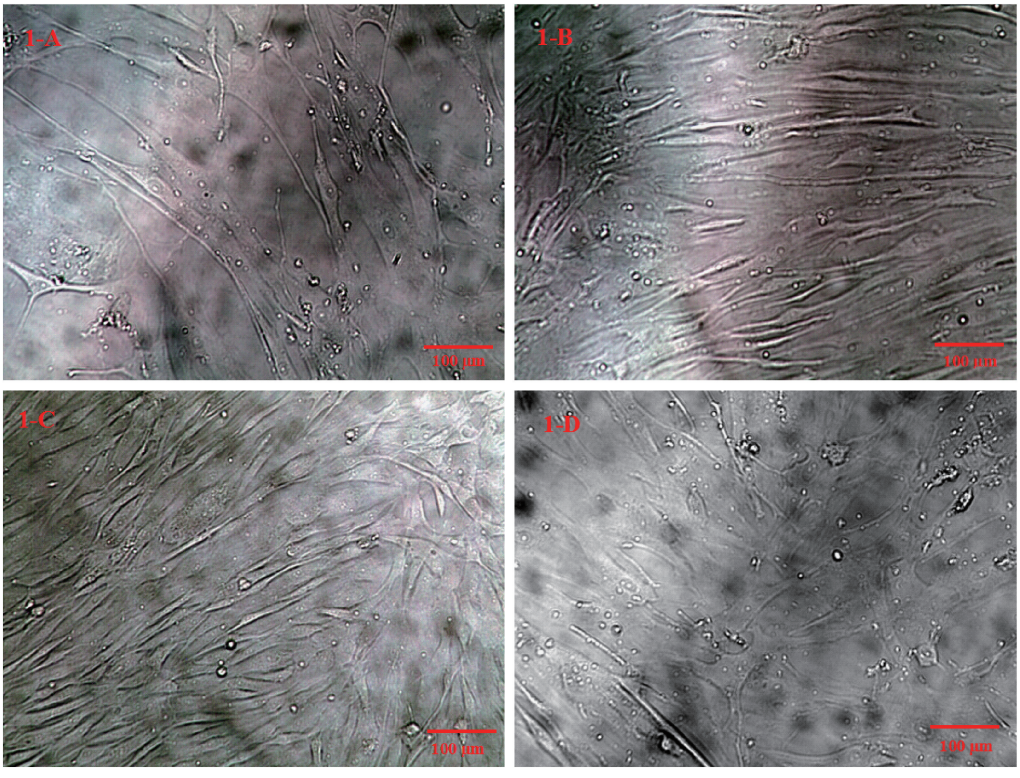
1.5 Data statistics and analysis

Variance analysis and Duncan's multiple range test were performed using SPSS 18.0. $P < 0.05$ was considered as statistically significant, and $P < 0.01$ extremely significant. Results were expressed as average ± standard deviation ($X \pm S$).

2 Results

2.1 Morphological observation of UC-MSCs during the purification and amplification

Through the inverted microscope, it was seen that passage 3 (P3) UC-MSCs grew into fusiform or triangular adherent cells at the bottom of the flask; at 7 d, the UC-MSCs presented a fibroblast-like morphology (Fig. 1-A). To passage 4 (P4), most of the UC-MSCs were fusiform or fibroblast-like, while some were triangular or polygonal (Fig. 1-B). To passage 5 (P5), the number of UC-MSCs increased gradually; the colony forming and cell growth speed were faster compared with dispersed cells; the cells present a circinate arrangement, and the morphology was more consistent. (Fig. 1-C). After passage 6 (P6), the UC-MSCs changed very little in morphology, but the cell number was reduced slightly and the proliferation slowed down (Fig. 1-D).

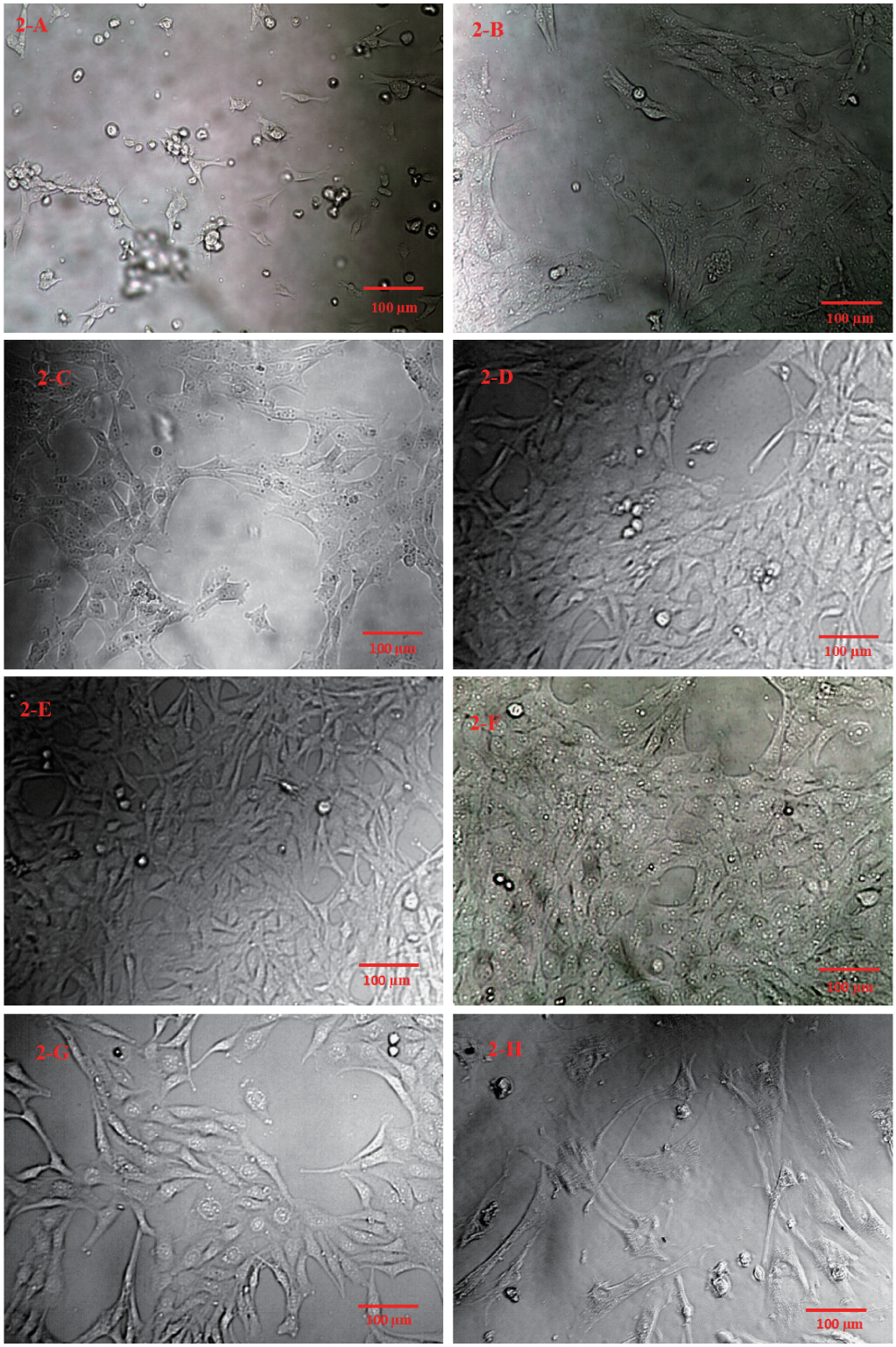


Note. 1-A: P3 UC-MSCs cultured for 7 d; 1-B: P4 UC-MSCs; 1-C: P5 UC-MSCs; 1-D: P6 UC-MSCs

Fig. 1 Recovery and subculture of the UC-MSCs (×100)

2.2 Morphological observation of the BMECs during purification and amplification

It was observed through the inverted microscope the recovery of BMECs was mixed with other cells, at first (Fig. 2-A). In the subsequent culturing, the BMECs showed an obvious "netting" feature (Fig. 2-



Note. 2-A: After the recovery of BMECs cultured for 2 days; 2-B: BMECs cultured for 6 days; 2-C: BMECs cultured for 8 days; 2-D: Purification of BMECs cultured for 12 days; 2-E: BMECs of passage 5 generations; 2-F: BMECs of passage 8 generations; 2-G: BMECs of passage 12 generations; 2-H: BMECs of passage 15 generations.

Fig. 2 Recovery and subculture of the BMECs(×100)

B). Moreover, the cell confluence enhanced and the cells exhibited a cobblestone-like growth pattern (Fig. 2-C). After passage and purification, the originally round BMECs stretched gradually into flat cells, exhibited a fusiform, triangular, or irregular polygon shape (Fig. 2-D). At P5, cells became bigger and were uniformly distributed over the flask bottom in a cobblestone-like pattern. At passage 8 (P8), the sharply increased BMECs were arranged tightly and developed into colonies. After passage 12 (P12), the growth of BMECs was slowed down and the cell number was decreased, and finally was stopped.

2.3 Results of CCK-8 assay for cells co-cultured at different concentration ratios and for different time durations

The optical densities of theUC-MSCs and BMECs inoculated at different concentration ratios were presented in Figure 3. At 8 h and 12 h, the optical densities of the groups with concentration ratio of 1∶2 was significantly higher than that of the control groups ($P < 0.05$). At 24 h, the "1∶3" group was significantly different from the control groups ($P < 0.05$). At 36 h, the "1∶2" group was extremely significantly higher than the group with the conditioned medium-BMECs group ($P < 0.01$). At 48 h, the "1∶2" group was extremely significantly higher in comparison with the control groups ($P < 0.01$) and the other concentration ratio groups ($P < 0.05$); and the conditioned medium-BMECs group was significantly higher than the control groups ($P < 0.05$). At 60 h, the "1∶2" group was extremely significantly higher than the control groups ($P < 0.01$), and significantly higher than the "2∶1", "1∶1", and conditioned medium-BMECs groups ($P < 0.05$). At 72 h, the "1∶2" group was extremely significantly higher than the control groups and conditioned medium-BMECs groups ($P < 0.01$) and significantly higher than those of the "2∶1", "1∶1", and "1∶10" groups ($P < 0.05$).

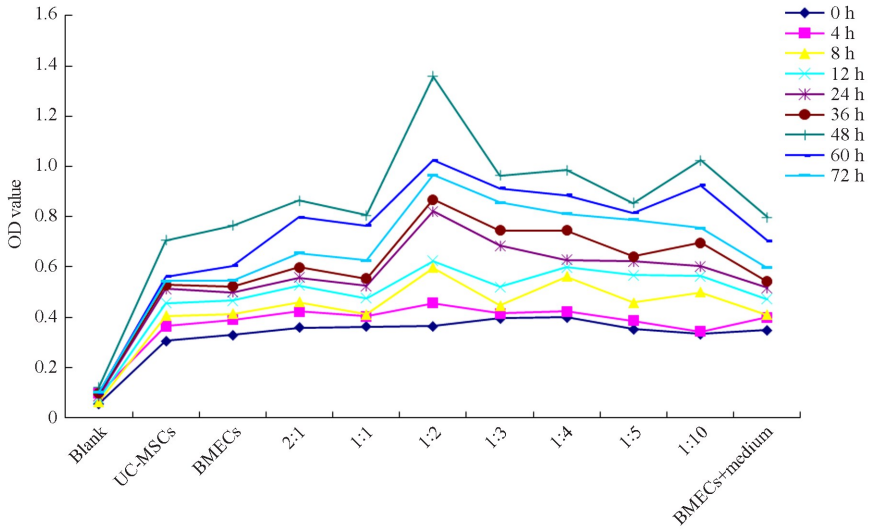


Fig. 3 Comparison of the OD values of UC-MSCs and BMECs co-cultured in different concentrations and at different time points

3 Discussion

3.1 Morphological changes of the UC-MSCs and BMECs during purification and amplification as seen in Fig. 1-B and 2-A, P3 and P4 UC-MSCs were not pure UC-MSCs, and the revivified primary BMECs were also mixed with other cells. It was reported that the UC-MSCs before P3 are highly inconsistent in morphology, such as no clear boundaries between cells and no apparent fibroblast-like colonies formed[7]. Li et al.[8] pointed out that primary BMECs are prone to fibroblast pollution and thus should be purified before culture. Hence, UC-MSCs and BMECs were gradually purified by continuous passage in the present work. The changes of cell morphology can be used to judge whether cells are proliferating or at the stage of apoptosis[9]. It is believed that the UC-MSCs at passage 5 and the BMECs at passage 8 are relatively unpolluted, with an optimal growth and high activity. In the in vitro culture process, UC-MSCs can be propagated to a desired

numbers in a shorter time with higher metabolic rate than BMECs. The in vitro metabolic rate of mesenchymal stem cells (MSCs) is related to cell proliferation and differentiation, thus influencing the biological functions of the cells[10]. These facts suggest that UC-MSCs have the power to participate in the amplification of BMECs as seed cells. That is, UC-MSCs are appropriate for subsequent trials. Chen et al.[11] reported that UC-MSCs are able to form apparent cell colonies, and the used UC-MSCs generally do not exceed the sixth passage. This is consistent with our results. Moreover, it has been reported that both UC-MSCs and BMECs could propagate for over 20 generations. However, the cell morphology will be gradually changed after passage 10 and the proliferation rate will be slowed down till death[12]. The cells in the 3rd to 18th passages have the optimal biological activity[13]. The results of these reports were not consistent with our experimental results, which could be due to differences in the cell culture environment, inoculum density, culture medium, and serum concentration.

3.2 Effect of UC-MSCs on the proliferation of BMECs cultured in different co-culture modes

In the present work, UC-MSCs and BMECs were co-cultured in two modes: direct and indirect cell-to-cell contact modes. At direct contact mode, two types of cells are mixed directly to allow sufficient interaction between them[14]. At indirect contact mode, the close physical contact or regulation between the two types of cells are cut off, but the diffusible factors secreted by a certain type of cells can penetrate through the permeable membrane and accelerate cell proliferation and differentiation[15]. Additional cells in a co-culture system can regulate the growth status of target cells[16]. Phagocytosis does not occur in the system where UC-MSCs and other cells coexist. Meanwhile, UC-MSCs can promote the proliferation of the coexisting cells and even induce their differentiation[17,18]. The results of our study show that the optical densities of the cells co-cultured in either direct contact or indirect contact modes were higher than those of control groups, and the optical densities of cells co-cultured in direct contact mode are higher than that of the conditioned medium-BMECs group. No matter direct or indirect contact mode, the co-culture of UC-MSCs and BMECs is able to enhance the proliferation capacity of BMECs, while the direct contact mode has a better effect than the indirect mode.

3.3 Possible approaches for UC-MSCs to promote BMECs proliferation

MSCs can produce manifold factors, which exert a critical supportive role through paracrine and autocrine actions[19]. It is noteworthy that the use of the supernatant containing growth promoting factors secreted by UC-MSCs as the medium of BMECs is comparable to the addition of exogenous growth stimulator. The supernatant provides better nutrition for BMECs than serum-free medium (SFM). Hence, the BMECs cultured in such supernatant grow faster, along with stronger activity and proliferation capacity. Moreover, some studies indicated that the stem cells in a direct contact co-culture system can induce the proliferation and differentiation of other cells by secreting several growth factors, which are closer to physiological dose, duration and sequenc[e][20,21]. Therefore, the proliferative effect in the co-culture system could be linked to the concentrations of the factors secreted by UC-MSCs. In the direct contact co-culture of two types of cells, neither the paracrine nor the autocrine of UC-MSCs will be interrupted. However, only paracrine is present in the indirect contact co-culture. Accordingly, the concentrations of the secreted factors in the direct contact mode are higher compared with that in the supernatant, thus generating a more remarkable proliferative effect on BMECs. On the other hand, the direct contact between cells and/or the extracellular matrix might contribute more to the proliferative effect[22]. However, this experiment is still not able to determine which type of cell causes the cell proliferation in the direct contact co-culture system and which one plays a dominant role in the regulation of proliferation. Further experiments are needed to answer these questions.

3.4 Optimal co-culturing condition of UC-MSCs and BMECs

Experimental results showed that there is no cell proliferation in each group in the first 36 h of co-cul-

ture. Previous study indicated that UC-MSCs grew slowly within 24 h, and then their growth began to accelerate at 24 – 36 h. After a stable phase, cell growth slowed down again and then entered gradually into a plateau phase[23]. Their report confirms our experimental results that the growth cycle of co-cultured cells follows the growth regularity of cells as well.

The optical density of each group reached the peak at 48 h, while the optical density of each treatment group was higher than those of the control groups. It is because that after the rapid growth phase UC-MSCs began to secrete various factors in large quantities and act on target cells, thus promoting the growth of BMECs. Our finding that UC-MSCs have an optimal proliferation promoting effect at 48 h, is in contrast with Wang's (2014) report[24]. It might be due to that SFM was adopted in this experiment, which eliminated the interference of serum on cells. This approach better verified that the outcome was attributed to UC-MSCs. Moreover, cell proliferation was slowed down at 60 h and 72 h when the proliferation-promoting effect of the co-culture system was weaker after 48 h. It was because that the cells entered the decline phase, and the proliferative ability of co-cultured cells was gradually weakened. UC-MSCs might not passage the whole co-culturing period. The growth of UC-MSCs is in periodicity within the limited time of one generation, so their secretion keeps pace with their growth cycle[25]. The secretion intensity is higher in the rapid growth phase, while the secretion level declines when cells grow slowly. At this moment, UC-MSCs failed to supply pertinent factors and to enhance the proliferation of BMECs.

Wang et al.[26] pointed out that there would be cell fusion when MSCs were co-cultured with other cells. In this experiment, cell proliferation capacity was improved by the co-culturing of UC-MSCs and BMECs at different concentration ratios, and the co-culturing at the concentration ratio of 1∶2 displayed the best effect. It was because of that the two types of cells interacting with each other when co-existing in a limited space[27]. Only when the two types of cells reach the optimal confluence at an appropriate concentration ratio, UC-MSCs can play a critical role in linking two types of the cells, thus exerting the proliferation-promoting effect. Dong[28] found that the co-culturing of osteoblasts and vascular endothelial cells at the concentration ratio of 1∶1 for 36 h was the optimal co-culture condition. Xue et al.[29] indicated that the cell proliferation effect was optimal when bone marrow-derived mesenchymal stem cells and umbilical vein endothelial cells were co-cultured at the concentration ratio of 1∶5 for 5 d. In the present study, the optimal concentration ratio of co-culturing was 1∶2 and the optimal duration was 48 h. These differences suggest that the optimal concentration ratio and culture time vary from the source of experimental cells and culture condition.

4 Conclusions

1) The P5 UC-MSCs and P8 BMECs propagated in vitro have the best morphology and favourable activity.

2) Under the serum-free condition, direct contact mode of co-culturing overmatches that of indirect contact mode in promoting the proliferation of BMECs.

3) The optimal concentration ratio for co-culturing UC-MSCs and BMECs is 1∶2 and the optimal time is 48 h for the direct contact mode co-culture.

参 考 文 献

[1] He BP, Xing YP, Lei LC, et al. Effects of IGF-I on cow mammary gland epithelial cells milk fat, milk protein [J]. Chin J Vet Med, 2012, 32(8): 1231 – 1234.

[2] Kale S, Karihaloo A, Clark PR. et al. Bone marrow stem cells contributes to repair of the ischemically injured renal tubule [J]. J Clin Invest, 2003, 112(5): 422 – 449.

[3] Shao W. Effects of intravenous umbilical cord mesenchymal stem cells injection on growth development and serous biochemical traits in Kazakhstan lamb [D]. Urumqi: Xinjiang Agricultural University, 2012.

[4] Qin XH. Research on that transplanting bone marrow mesenchymal stem cells has an effect on mastitsis of mammary mastitis model rats [D]. Urumqi: Xinjiang Agricultural University, 2013.

[5] Guzailinuer AM. The effects of two ways transplantation bone marrow stem cells to the female rat's mammary gland development in different physiology growth period [D]. Urumqi: Xinjiang Agricultural University, 2013.

[6] Zhao YK. Effects on growth performance and biochemical indexes in serum of Holstein bull calf after injecting umbilical cord mesenchymal stem cells [D]. Urumqi: Xinjiang Agricultural University, 2012.

[7] Deng CQ. Research of biological characteristics and function of umbilical cord mesenchymal stem cells [D]. A master's degree thesis. Suzhou: Soochow University, 2011.

[8] Li Y, Xu Ki, Zhao GQ. The cow mammary gland epithelial cell culture and identification [J]. Shanghai Animal Husb Vet Commun, 2014(1): 8 – 11.

[9] Chang YN, Wei W. The research progress of cell culture and its application [J]. Chin Clin Pharmacol Therap, 2009, 14(7): 827 – 830.

[10] Liechty KW, Mac Kenzie TC, Shaaban AF, *et al.* Human messenchymal stem cells engraft and demonstrate site-specific differentiation after inter transplantation in sheep [J]. Nature Medicine, 2000, 6(11): 1282 – 1286.

[11] Chen Y, Dong X J, Zhang G, et al. In vitro differentiation of mouse bone marrow stromal stem cells into hepatocytes induced by conditioned culture medium of hepatocytes [J]. J Cell Biochem, 2007, 1(102): 52 – 63.

[12] Chen JH, Tong HL, Li QZ, et al. The establishment of the cow mammary gland epithelial cell line [J]. J Animal Husb Vet, 2009, 40 (5): 743 – 727.

[13] Xu C. Biological activity and gene expression changes of long-term cultured human umbilical cord MSCs [D]. A master's degree thesis. Guangdong: Jinan University, 2011, 15 – 16.

[14] Grellier M, Bordenave L, Amédée J. Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering [J]. Trends Biotechnol, 2009, 27(10): 562 – 571.

[15] Meury T, Verrier S, Alini M. Human endothelial cells inhibit BMSC differentiation into mature osteoblasts in vitro by interfering with osterix expression [J]. J Cell Biochem. 2006, 98(4): 992 – 1006.

[16] Zhang Q, Jin RM. The research progress of cell co-culture techniques [J]. Chin J Pharmacol Toxicol, 2011, 25(3): 330 – 334.

[17] Tian Y. The study of biological characteristics of human umbilical cord mesenchymal stem cells and their co-cultivation with brain tumor stem cells [D]. Zhengzhou: Zhengzhou University, 2010, 33 – 35.

[18] Zhu H, Ye L, He J, et al. Mouse umbilical cord derived mesenchymal stem cells were induced to differentiate in vitro [J]. Chin J Lab Animals, 2015, 23(06): 622 – 627.

[19] Salvolini E, Lucarini G, Zizzi A, et al. Human skin derived mesenchymal stem cells as a source of VFGF and nitric oxide [J]. Arch Dermatol Res, 2009, 302(5): 367 – 374.

[20] Aboody KS, Brown A, Rainov N G, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas [J]. Proc Natl Acad Sci U S A, 2000, 97 (11): 12846 – 12851.

[21] Qiu PB, Liu C, Li W, et al. Inducing human umbilical cord mesenchymal stem cells to differentiate into cardiomyocytes and its therapeutic effect on myocardial infarction in mice [J]. Chin J Lab Animals, 2012, 20(05): 9114 – 9193.

[22] Li L, Chen WQ, Zhu JX. et al. Research of direct contact co-culture techniques explores the cell-to-cell interaction in vitro [J]. Chin J Sports Med. 2013, 32(4): 337 – 342.

[23] Zhou JY. The cultivation and identification of rat umbilical cord mesenchymal stem cells [D]. Guangzhou: Guangzhou Medical College, 2011.

[24] Wang LW. Experimental study on the co-culture of bovine mammary epithelial cells with bovine umbilical cord mesenchymal stem cells [D]. Urumqi: Xinjiang Agricultural University, 2014.

[25] Liu Y, Dulchavsky D S, Gao X, et al. Wound repair by bone marrow stromal cells through growth factor production [J]. J Surg Res, 2006, 136(2): 336 – 341.

[26] Wang SY, Lai PP, Han CM. The research progress of bone marrow stem cells to the epithelial cell differentiation [J]. Int J Surg, 2006, 33(4): 306 – 308.

[27] Ball SG, Shuttleworth AC, Kielty CM. Direct cell contact influences bone marrow mesenchymal stem cell fate [J]. Int J Biochem Cell Biol. 2004, 36(4): 714 – 727.

[28] Dong XH. Osteoblast-vascular endothelial cell co-culture system in the expression of IGF-I and the influence of the vascular endothelial cell biology research [D]. Luzhou: Luzhou Medical College, 2014.

[29] Xue Y, Xing Z, Hellem S, et al. Endothelial cells influence the osteogenic potential of bone marrow stromal cells [J]. Biomed Eng Online, 2009, 25(8): 34.

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