Biological Effects of the Extracellular Matrix on Rat Bone Marrow Mesenchymal Stem Cells

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Abstract **Objective** To investigate the biological effects of polylysine on bone marrow mesenchymal stem cells (MSCs). **Methods** MSCs were isolated from Wistar rats bone marrow by adherence methods and respectively plated in culture flasks that were coated with polylysine or not. Then we studied the morphology of the cells with phase contrast microscope, measured the number of adhesive cell, drawed the growth curve and examined the comprising cell cycle by a fluorescenceactivated cell sorter. **Results** Compared with the contra1 group, the MSCs growing on the polylysine showed more rapid adhesion and proliferation, as well as more typicalmorphous. **Conclusion** The polylysine of extracellular matrix can significantly increase the adhesion and proliferation capacity of MSCs in vitro, which indicated the amplification and cultivation of MSCs became more simply and efficiently.

Key words Bone marrow mesenchymal stem cell; Cell culture; Polylysine

Mesenchymal stem cells (MSCs) which have many potential to differentiation and proliferation of features, are used as the first choice of tissue engineering seed cells and gene therapy to the target cells [1,2]. The MSCs access and in vitro cultivation are an important process in above research. Regarding MSCs raise whether does need to spread the cloth extracellular matrix, at present still didn´t have the unified understanding. To this end, we choosed the poly-L-lysine as extracellular matrix packets trained in containers, and not wrapped in the extracellular matrix as control. Biological activity of rat MSCs in vitro process was observed and compared, with a view to establish an ideal training methods, and provide a good foundation for further research .

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MATERIALS AND METHODS

Materials

This experiment was carried out in Institute of Anatomy & Histology and Embryology, School of Medicine, Shandong University. Wistar Rats, weights of about 150g, were provided by Experimental Animal Center of Shandong University. L-DMEM and trypsin were purchased from Gibico Company of America; FBS was purchased from Hyclone Company of South America; poly -L -lysine was purchased from Sigma Company of America.

Cell culture

Bone marrow from 4~6 -week -old SD rat was flushed out of tibias and femurs. After 2 washings by centrifugation at 1500 rpm for 5 minutes in PBS, 5×10^6/mL density cells were plated in 25cm^2 glass flasks coated with or without 0.5% poly-L-lysine overnight. Cells were kept in a humidified 5% CO_2 incubator at 37℃, and the 10% fetal bovine serum of L-DMEM medium. The floating cells were removed after 3 days. The medium was refreshed every 3 to 4 days [3]. When the cells reached 90% confluence, they were digested l

min by 0.125% trypsin containing 0.02% EDTA. The cells were seeded at 1×10^4/mL in the glass flask coated or non-coated with the poly-L-lysine.

Methods

Observation under microscope To observe the cellular morphology and the growth situation every day under the inverted microscope.

Counting the number of adherent cells at different time The 3rd generation cells were seeded in 24-well plates coated with or without the poly-L-lysine at 5×10^3/mL, and cultured for 2h, 4h, 8h, 12h, 24h respectively, then, removed the un-adherent cells, and the number of adheren cells were counted in 4 fields under microscope stochastically.

Cell proliferation assay The 4th generation of cell were digested and seeded in the 24-well plates at 1.0×10^4/mL which were coated with or without the poly-L-lysine. The cells were digested and counted. Each group contained 3-wells, each well of cells were counted for three times.

Flow cytometric analysis of cell cycle and apoptosis The 4th passage cells were used to investigate the cell cycle and apoptosis with propidium iodide.

Statistical analysis Experimental data was treated with SPSS13.0 statistical software. The experimental result was indicated by x±s, and was assessed by the *t* test, which were considered as significant difference when $P<0.05$. Using Microsoft Excel software mapping.

RESULTS

Cytomorphologic observation

In polylysine group, the cultured cells adhered to flask more easily than the un-polylysine group. More primary cells adherent to flask at cultured 24h; a few passaged cells adhered to flask at cultured 1h; all adhered and stretched until 12h; 90% cells grew to confluence at 4 or 5d, with typical spindle, clear outline and uniform in shape; the 6th passage cells still maintain the good cellular morphology and vitality. In un-polylysine group, only a few primary cells adhered to flask at 24h and grew to fusion at 10d~14d, passaged cells adhered to flask occasionally at cultured 3h; all adhered and stretched until 24h; 90% cells grew to confluence at 6~8d; with spindle shape, but comparatively broad, faintness contour than polylysine group; the vitality of 5th passage cells became weak, with broad appearance, some vacuoles in plasma of cells. 90% cells reached confluence at 12d. Obviously, compared with the the un-polyly-

Table 1 the number of cells pasted the wall in two groups at different time(n=3, x±s)

Group	2h	4h	8h	12h	24h
Polylysine	27.41±17.31	42.16±15.09★	41.58±14.83★	50.50±18.54★	62.75±12.03
Contral	21.58±6.70	24.41±7.58	7.50±14.58	31.91±7.80	58.41±10.62

★$P<0.05$ vs Contral

Table 2 cell cycle analysis of two groups(n=3, x±s%)

Group	G0/G1	S	G2/M
Polylysine	71.70±0.43★	28.00±0.10 ★	0.30±0.34
Contral	78.09±0.17	21.31±0.30	0.59±0.36

★$P<0.01$ vs Contral

sine group, the polylysine group stem cells had typical morphology and better vitality.

The number of adherent passage cells at different times

The number of adherent cells of polylysine group were significantly more than un-polylysine group respectively after cultured 4h, 8h, 12h, but no diference between 2 groups at 24h (Table 1).

Analysis of the growth curve

In the first 3 days, the cells of un-polylysine group were in the latent periods of growth in which the cells adhered to flask; entered logarithm vegetal period in 4~6 days, in this period the cells multiplication were active, and cells number was increasing exponentially; entered platform period 7 days latter until the cells reached confluence. The latent periods of polylysine group cells were only 12~20h, 36h~4d for its logarithm vegetal periods, 5~7 days for its growth platform time(Figure 1).

Analysis of flow cytometry

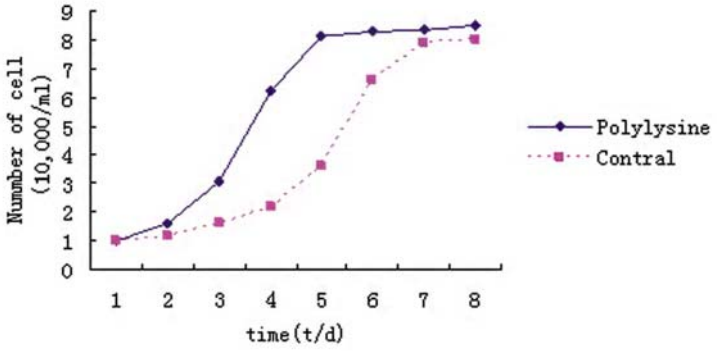

Fig. 1 Comparison with the two growth curve of two group cells

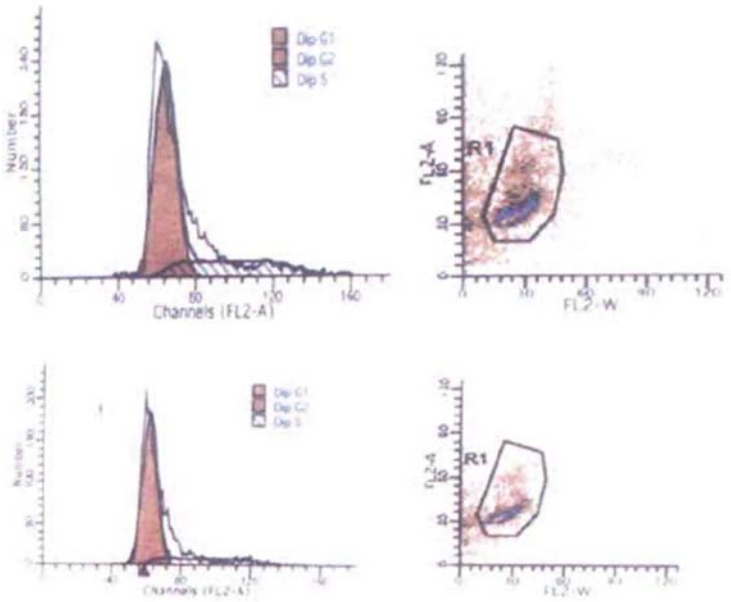

Fig. 2 Cell cycle analysis maps of two groups
Up: polylysine group Under: contral

The cellular percentage in S phase of polylysine group cells changed significantly ($P<0.01$)while the percentage of the cells in G0/G1 phase decreased ($P<0.01$), which indicated that the polylysine coud promote the conversion from G0 to S phase (Table 2). The graph represents two groups were no apoptosis, which explained that the suitable concentration of polylysine didn´t harm the cells (Figure 2).

DISCUSSION

The bone marrow stem cells include mesenchymal stem cells (MSCs) and hematopoietic stem cells(HSCs). The former is adherent growth cell, and the latter is suspended growth cell. Therefore it can be obtained by adherence methods. But MSCs were very few in number, the average 100,000 cells of adult bone marrow contains only one MSCs[4]. Therefore, to meet the needs of the study, the establishment of MSCs in vitro culture conditions to access the adequate number of MSCs in limited time appears to be particularly important.

Extracellular matrix is the main components of the physical and chemical environment to cell survival. To the adherent cells, extracellular matrix provides the necessary supports to them, then further affects their proliferation, differentiation and gene expression[5]. Moreover the different matrix have different effects to the biological activity of mesenchymal cells[6,7]. At present the common extracellular matrix with collagen, poly－L－lysine, laminin, fibronectin, their mechanism of biological activity to right adherent cell is not the same. The majority of extracellular matrix such as collagen causes the cell to mount attaches and spreads out all over on the matrix through the interactions between certain ingredients and the cellular membrane acceptor, and further affects cell proliferation, migration and differentiation [5]. Polylysine monomers from the multi－cationic polymer prices, mainly through solid surface charge to increase adhesion and cell surface affinity.

In this study, we coated flasks with polylysine to observe the growth situation of MSCs. Compared with un－polylysine group, the results showed that whether the primary or the passaged cells adherented earlier and better vitality than the un－polylysine group. The primary cells only needed 7～8d to grow to confluence, and after that, the cells were passaged about every 4d, which indicated that the suitable concentration of polylysine could contribute MSCs to adherent and extend, and caused the cells to enter the growth cycle comparatively early. Therefore, it is effective method to use the extracellular matrix to promote cell expansion. We also found that the vitality of 6th passage cells were still better in poly－L－lysine group, but the vitality of 5th passage cells in un－polylysine group had became weak. At the same time, we discovered polylysine group no significant apoptosis, which indicated appropriate concentration of the poly－L－lysine on the cell didn´t damage the function to the cell. However, polylysine belongs to the non－nutrition extracellular matrix, its longer effect on MSCs, especially on its differentiation, is also need to be studied.

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