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*Original Article*

# Effects of PM2.5 on proliferation, differentiation, apoptosis and cytokine secretion in rat bone marrow stromal cells

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**Abstract:** Objective: Discuss the impact of atmospheric fine particles (PM2.5) on proliferation, differentiation, apoptosis and cytokine secretion of SD rat bone marrow stromal cells (BMSCs). Method: Respectively detect the function of PM2.5 on proliferation, osteogenic differentiation, cell apoptosis, cycle change and cell factor secretion on BMSCs. Result: 10, 20 μg/ml PM2.5 stimulates the proliferation of BMSCs, but 50, 100, 200 μg/ml PM2.5 inhibits this proliferation ($P<0.05$). At the same concentration, PM2.5 with low concentration stimulates ALP activity, while PM2.5 with high concentration inhibits ALP activity. Under the same time, with an increased concentration of PM2.5, the inhibition function of ALP shall be increased. With the increased concentration of PM2.5, the apoptosis rate of the cells shall be gradually increased ; 200 μg/ml PM2.5 group, G0/G1 period, compared S period with the control group, the cell cycle was significantly prolonged ($P<0.05$). PM2.5 with low concentration stimulates cytokine secretion of BMSCs, while PM2.5 with high concentration inhibits cytokine secretion of BMSCs. Conclusion: PM2.5 with a low concentration can stimulate the proliferation of BMSCs, the activity generation of ALP and the effects of cytokine secretion; PM2.5 with a high concentration can inhibit the proliferation of BMSCs and apoptosis, and reduce the toxic function of cytokine secretion.

**Keywords:** Atmospheric fine particulate matter, bone marrow stromal cells, proliferation, differentiation, apoptosis, cytokine, toxicity

## Introduction

The impact of air pollution on human health has become increasingly important. As an important part of air pollutants, PM2.5 seriously endangers human health and causes environmental and social problems [1], which are especially serious for children [2, 3]. Research indicates that PM2.5 has a very close relationship with illnesses in multiple systems [4]. PM2.5 polycyclic aromatic hydrocarbons in organic components have potential mutagenicity and long term exposure may be associated with tumorigenesis [5, 6]. Bone marrow stromal cells are the main components of the hematopoietic microenvironment, whose integrity of structure and function is very important for the maintenance of the stability of the body [7-9]. The experiment researched the impact of PM2.5 on differentiation and apoptosis of bone marrow stromal cells, in order to provide a theoretical basis for the development of hematological malignancies disease caused by air pollution. Now, the report is as follow:

## Materials and method

### *Experiment materials*

Low sugar DMEM Culture medium (HyClone company), Fetal calf serum (HyClone company), 0.25% Trypsin (USA, GIBCO company), Cell Counting Kit-8 (Biyuntian Biotechnology Research Institute), Annexin V/PI Apoptosis Kit (Association Biology company), ALP Alkaline phosphatase Kit (Nanjing Jiancheng Biology company), Osteogenic induction medium (Guangzhou Saiye Science and Technology Co., Ltd), Alkaline phosphatase staining kit (Nanjing Jiancheng Biology company; $CO_2$ Incubator (Ge-

rmany Hereus), SNP1-PM10/PM2.5 Air sampler (USA, AT company), Ultrasonic shock cleaning apparatus (Beijing Chinese and Western Technology company), Vacuum freeze dryer (UK, EDWARDS company), Inverted phase contrast microscope (Japan, Olympus company), Danny enzyme device (MK-3) (Denmark), EPLICSXL Flow cytometry (USA, Coulter company). Specimen source: SD rat bone marrow stromal cells (BMSCs) Guangzhou Saiye Science and Technology Co., Ltd.

*Method*

*PM2.5 collection and preparation:* Select the gate of Zhanjiang Guangdong Affiliated Hospital of Medical College (heavy car traffic and air pollution) as the sample point. Apply a flow sample of intelligent TSP to collect air PM2.5 from March to August of 2014 on special quality analysis filter paper. Cut the filter paper collecting the fine particulate matter into blocks of 1 cm×1 cm, and immerse in the third distilled water. Elute the particulate matter by 4°C ultrasonic oscillation at 30 min×4 times. Filter the oscillating fluid by using an 8 layer gauze, and lay into the vacuum freeze dryer to freeze and dry into powder. Finally, collect the powder to reserve under -20°C for preparation. Before use, weigh the powder of PM2.5 with the required amount. After high pressure sterilization, add PBS fluid into an ultra-clean bench. Then, dissolve the dry powder by means of ultrasonic vibration for 20 min and adopt a method of dilution to prepare the PM2.5 fluid at concentrations of 0, 10, 20, 50, 100, 200 μg/ml for preparation.

*Impact of proliferation of PM2.5 on BMSCs:* Collect BMSCs adjusted for cell concentration into logarithmic growth phases of $5\times10^5$/ml and inoculate into 96-hole culture plates. Add 100 ul 10% cell culture fluid into negative a control group (containing cell culture) and add 90 ul 10% cell culture fluid into the experimental groups (containing different PM2.5 concentrations of cells) with 10 ul concentration, as PM2.5 fluid of 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml. After cultivating for 12, 24 and 48 hours, add CCK-8 fluid of 10 ul into each hole for a period of 4 hours. Use a Danny Microplate Reader to detect the OD value of each group of cells, with wavelengths of 450 nm. Repeat 3 times during the experiment, and draw the average value to calculate the survival rate. Survival rate=OD value of experiment group/OD value of negative control group ×100%.

*Impact of PM2.5 on osteogenic differentiation ability of BMSCs:* Divide into control groups and osteogenic differentiation inducing groups (including the negative control group and the experimental groups of osteogenic differentiation). Control group has 10% fetal calf serum with a low sugar DMEM medium; the osteogenic differentiation induced group has an osteogenic differentiation inducing medium with different concentrations of PM2.5 solution. After preparation, the final concentration is 0 μg/ml (That is the negative control group of osteogenic differentiation), and osteogenic differentiation induced groups of 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml of PM2.5 solution.

Alkaline phosphatase staining and the staining of sin red pigment were identified as bone cells: Inoculate cell density of $5\times10^4$/ml of bone marrow stromal cells into a 6-hole plate, and then abandon the medium after 24 h. According to the experiment group, add a corresponding medium of 2 ml into each hole. Stain the alkaline phosphatase on the 7$^{th}$ day, and stain the alizarin red on 14$^{th}$ day.

Activity detection of alkaline phosphatase: Inoculate the third generation of BMSCs into 6-hole plate of $5\times10^4$/holes. Provide the different mediums required in the above experiment on the second day, and draw a cell supernatant for detection after inducing date of 1, 3, 7, 11, 14.

*Flow cytometry detection:* BMSCs apoptosis and cycle: Draw the third generation of BMSCs and inoculate in the 6-hole plate. After cultivating for 24 hours, change the DMEM medium into the one without serum, and abandon the medium after continuing to cultivate for 12 hours. Add 2 ml of cell culture fluid containing 10% cells into the negative control group and add 2 ml PM2.5 solution with cells of different concentration into the experimental groups. After cultivating in the culture box for 48 hours, collect BMSCs of each group and wash PBS, to make centrifugation. Lightly suspend the cell with 500 ul 1× Combined buffer. Add 5 ul Annexin V-FITC and 10 ul Propidium iodide (PI). Incubate from the light for 5 min; Meanwhile, collect BMSCs of each group, and add into a

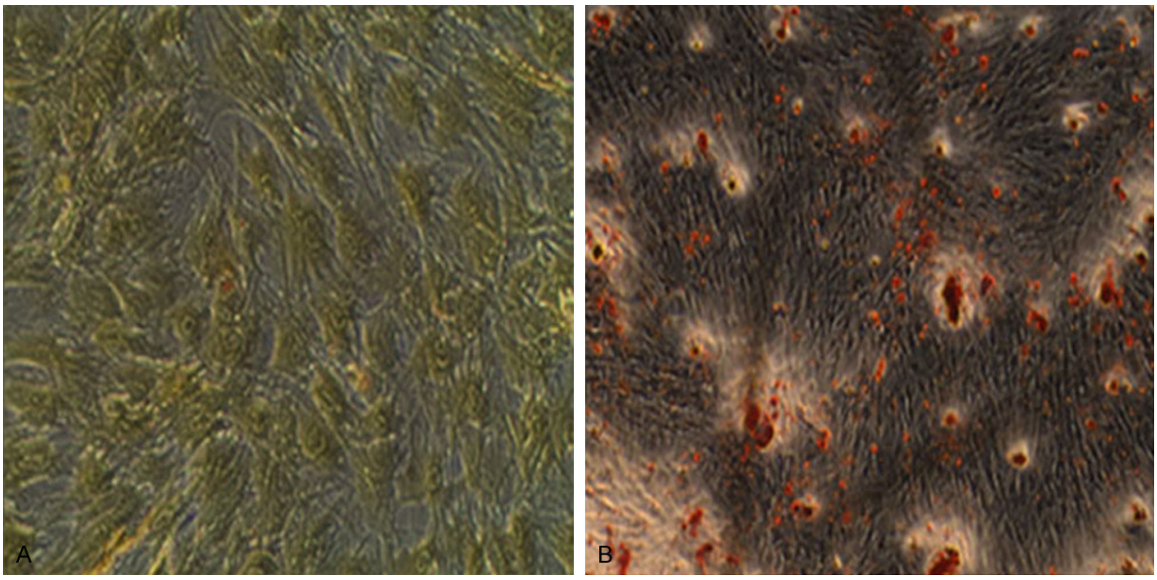


**Figure 1.** A. The alkaline phosphatase staining showed strong positive). B. Alizarin red staining assays showed positive.

**Table 1.** The impact of PM2.5 on BMSCs proliferation ($\bar{x}\pm s$, n=5)

| PM2.5 (μg/ml) | 12 h | 24 h | 48 h |
|---|---|---|---|
| 10 | 0.910±0.014▪ | 0.845±0.009▪,★ | 0.776±0.009▪,★ |
| 20 | 0.948±0.015▪ | 0.867±0.034▪,★ | 0.797±0.022▪,★ |
| 50 | 0.856±0.034▪ | 0.798±0.008★ | 0.721±0.020★ |
| 100 | 0.823±0.024▪ | 0.741±0.021▪,★ | 0.658±0.009▪,★ |
| 200 | 0.724±0.015▪ | 0.656±0.034▪,★ | 0.607±0.013▪,★ |

Note: ▪$P$<0.05, express compared with the control group at the same time; ★$P$<0.05, express compared with the same concentration of 12 h group.

pre-cooled 70% ethanol. Fix for 2 hours at 4°C and stain. Bath while avoiding sunlight at 37°C for 30 min. Flow cytometry detection.

*Cytokine secretion level detection:* Use the ELISA method to detect the negative control group and secretion of cytokines in each group after the function of PM2.5 on BMSCs for 24 hours. The concentration of standard products is a longitudinal coordinate, and the OD value of standard and blank holes is shown by the horizontal coordinates, thereby drawing the standard curve and calculating the regression equation of the standard curve. Take the OD value of sample into the equation, and calculate the sample concentration (if the sample is diluted, all the resulting value shall be multiplied by the dilution factor), regarded as the actual concentration of the sample.

Use RealTime-PCR method to detect the negative control group and expression level of mRNA in cell factor SCF, G-CSF, GM-CSF of each group after the function of PM2.5 on BMSCs for 24 hours.

Obtain the whole gene sequence from Genebank. Dalianbao Biological company design and compose rat SCF, G-CSF, GM-CSF genes and internal primers. ① Rat SCF (117 bp): Upstream primer 5'-CC TGGCTC TGGCTTCTGAAC-3', Downstream primer 5'-CCAATCCACCCGACCTA AGAC-3'. ② Rat G-CSF: Upstream primer 5'-CCGAGT-ATTGATTC C TGCCGCT-3', Downstream primer 5'-CAG AAGGGAGACCAGATGCTG-3'. ③ Rat GM-CSF: Upstream primer 5'-GACATGCGTGC TCTG-GAGAAC-3', Downstream primer 5'-GTATAGC-TTCAGGCGGGTCTG-3'. ④ Rat 18SrRNA: Upstream primer 5'-GAATTCCCAGTAAGTGCGGGT-CATA-3', Downstream primer 5'-CGAGGGCCTC-ACTAAACCATC-3'.

*Data processing*

After RT-PCR reaction is finished the gene amplification analyzer will automatically analyze and calculate the results. Ct value is the original data, indicating the number of cycles required to achieve the threshold of fluores-

**Table 2.** The impact of PM2.5 with different concentration on ALP activity

| PM2.5 (ug/ml) | ALP activity (U/L) | | | | |
|---|---|---|---|---|---|
| | 1 d | 3 d | 6 d | 9 d | 12 d |
| Control group | 0.145±0.021▪ | 0.256±0.17★,▪,△ | 0.398±0.024★,▪,△ | 0.489±0.019★,▪,△ | 0.626±0.026★,▪,△ |
| 0 | 0.212±0.015▪ | 0.552±0.018▪ | 0.932±0.023▪,△ | 1.224±0.026▪,△ | 1.525±0.031▪,△ |
| 10 | 0.217±0.013▪ | 0.423±0.014★,▪,△ | 0.753±0.021★,▪,△ | 0.995±0.028★,▪,△ | 1.265±0.019★,▪,△ |
| 20 | 0.213±0.015▪ | 0.381±0.018★,▪,△ | 0.602±0.023★,▪,△ | 0.875±0.031★,▪,△ | 1.114±0.029★,▪,△ |
| 50 | 0.219±0.023▪ | 0.301±0.025★,▪,△ | 0.239±0.013★,▪,△ | 0.189±0.022★,▪,△ | 0.129±0.011★,▪,△ |
| 100 | 0.210±0.018▪ | 0.255±0.021★,▪,△ | 0.194±0.019★,▪,△ | 0.131±0.011★,▪,△ | 0.098±0.011★,▪,△ |
| 200 | 0.211±0.013 | 0.219±0.015★,▪,△ | 0.163±0.011★,▪,△ | 0.091±0.017★,▪,△ | 0.045±0.021★,▪,△ |

Note: $^{\Delta}P<0.05$, Express with different concentration between groups comparison at the same time; $^{▪}P<0.05$, Express with the same concentration of different time between groups comparison; $^{★}P<0.05$, express with 0 μg/ml group at the same time.

cence intensity in the process of PCR. However, due to the non-linear relationship, it cannot be used as statistically. According to the Ct value, the experiment adopts a relative quantitative analysis method of Delta-delta Ct to analyze the experiment results, $\Delta Ct_{experiment\ group}=Ct_{experiment\ group\ target\ gene}-Ct_{experiment\ group\ inner\ reference}$; $\Delta Ct_{control\ group}=Ct_{control\ group\ target\ gene}-Ct_{control\ group\ inner\ reference}$; $\Delta\Delta Ct=\Delta Ct_{experiment\ group}-\Delta Ct_{control\ group}$, $2^{-\Delta\Delta Ct}$ has linear relationship, to be used in the statistics of the final data, so as to show the relative multiple relationship between the experiment group and control group.

*Statistical treatment*

The measurement data used in this experiment are shown in average value ± standard deviation ($\bar{x} \pm s$), by SPSS17.0 statistics software to conduct a statistical description. Multiple samples mean that comparison adopts a single factor analysis of variance (One-Way ANOVA), while every two samples a comparison among multiple sample average should be based on the homogeneity test of variance (Homogeneity -of-Variance). When the total homogeneity tests of variance are the same, select LSD method; Otherwise, select Tamhane's T2 method. $P<0.05$ shows statistical significance.

## Result

*CCK-8 method detects the proliferation of BMSCs*

Result indicates that a low concentration of (10 μg/ml, 20 μg/ml) PM2.5 stimulates the growth of BMSCs, while a high concentration of (50 μg/ml, 100 μg/ml, 200 μg/ml) PM2.5 inhibits cell growth. Under the same time, compare the groups of 10 μg/ml, 20 μg/ml with control group, the function of stimulating cell growth shall be increased gradually, with an increasingly strong survival rate, so the differentiation has statistical significance ($P<0.05$); from 50 μg/ml, with increasing concentration, the inhibition function of cell proliferation shall be increased, with an increasingly low survival rate, compared with control group, the differentiation has statistical significance ($P<0.05$); under the same concentration, compared with a group of 12 h, the cell survival rate of 24, 48 h shall be decreased, and the differentiation has a statistical significance ($P<0.05$).

*Impact of PM2.5 of BMSCs differentiation ability*

Osteoblast cells identification: Alkaline phosphatase activity is the early marker of BMSCs in the process of osteoblast cell differentiation, while mineralized nodules are calcium deposits formed outside the body by the mature osteoblast cells of differentiation, which is a latter marker of the differentiation process. The research adopts early and late market of differentiation to identify that BMSCs have successfully transferred into osteoblast cells differentiation. Result (as shown in **Figure 1A**) shows that visible cell cytoplasm has yellow-black particle sediment, which is an ALP positive cell. After alizarin red stains, orange red nodules can be seen to form shown in **Figure 1B**. The result demonstrates that BMSCs have successfully transferred into osteoblast cell differentiation.

Under different concentration, the impact of PM2.5 on BMSCs ALP activity during osteoblast differentiation: Result is shown as **Table 1**, negative control group and control group of osteo-

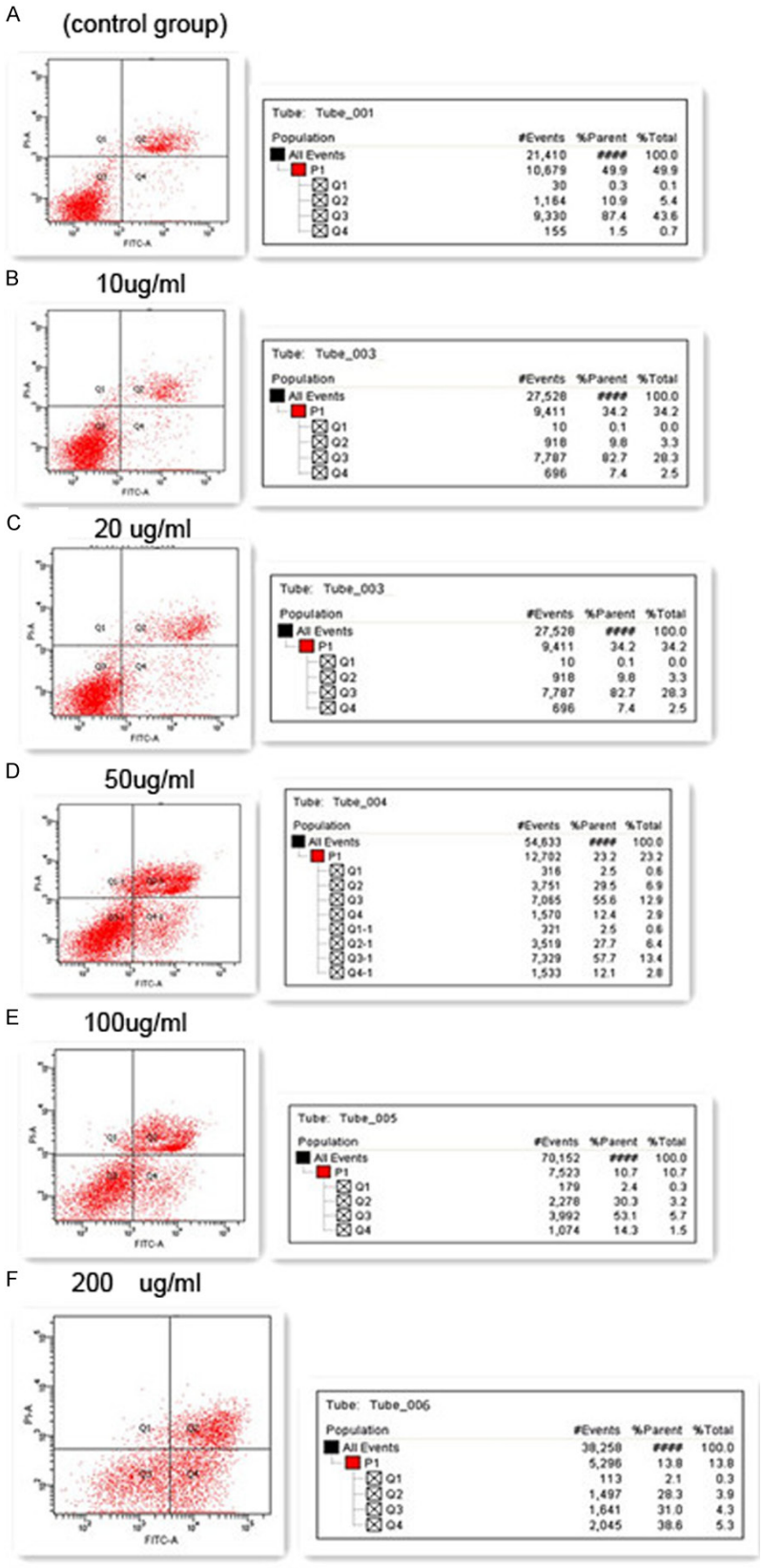


**Figure 2.** The effects of PM2.5 on BMSCs, apoptosis.

blast differentiation (0 μg/ml). ALP activity of 0 μg/ml groups is obviously higher than the value of the control group, indicating that BMSCs have successfully transferred into osteoblast cell differentiation under the function of osteoblast inducing fluid. Under the same concentration, with a prong long function time, and a low concentration (10, 20 μg/ml) of PM2.5 osteoblast differentiation inducing group, ALP activity shall be increased, indicating that the osteoblast differentiation degree for BMSCs shall be stronger over time, while in a high concentration (50, 100, 200 μg/ml) PM2.5 osteoblast differentiation inducing group, ALP activity shall be decreased. Comparing the different time groups, the differentiation has a statistical significance ($P<0.05$). After 3 days, compared with osteoblast differentiation of the negative control group, with the increased concentration of PM2.5 solution, the ALP activity shall be inhibited gradually, appearing the concentration dependency for PM2.5, which means that under the same time, compare the groups of different concentration, the differentiation has a statistical significance ($P<0.05$).

*Impact of PM2.5 on apoptosis and cycle of rat bone marrow stromal cells*

*Annexin V/PI detect the cell apoptosis:* The result is shown in **Table 2** and **Figure 2**: the function of

**Table 3.** The impact of PM2.5 with different concentration on apoptosisof BMSCs after 48 h ($\bar{x}$ ± s, n=3)

| PM2.5 (μg/ml) | Cell apoptosis rate (%) |
|---|---|
| control group | 1.31±0.21★ |
| 10 | 4.12±0.24▲,★ |
| 20 | 7.15±0.25▲,★ |
| 50 | 12.10±0.16▲,★ |
| 100 | 13.12±0.36▲,★ |
| 200 | 37.2±0.15▲,★ |
| F | 414.593 |
| P | 0.000 |

Note: ▲$P$<0.05, express compared with the control group; ★$P$<0.05, express compared with different concentration groups.

different concentration (0 μg/ml of control group, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml) of PM2.5 on BMSCs for 48 h, with the increasing concentration of PM2.5, the cell apoptosis rate shall be increased gradually. Compared with control group, the differentiation shows a statistical significance ($P$<0.05); compared with different concentration group, the differentiation has the statistics significance ($P$<0.05).

Q1 is left upper quadrant; Q2 is right upper quadrant (FITC+/PI+), indicates dead cells and apoptosis cells of later period; Q3 is left down quadrant (FITC-/PI-), indicates the normal cells; Q4 is right down quadrant (FITC+/PI-), indicates apoptosis cell of early period.

*Flow cytometry detects the cell cycle:* The result **Table 3** and **Figure 3** show that: the function of PM2.5 with different concentrations on BMSCs for 48 h, after 200 ug/ml PM2.5 is contaminated, G0/G1 period, compare S period with control group, the cell cycle has been prolonged obviously, the differentiation has a statistical significance ($P$<0.05).

*ELISA method detects secretion condition of BMSCs cell factors SCF, G-CSF, GM-CSF*

For PM2.5 with different concentrations of (0 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml) on BMSCs for 24 h, adopt ELISA method to detect the secretion condition of BMSCs cell factors SCF, G-CSF, GM-CSF, shown as **Table 4**. PM2.5 of low concentration (10, 20 μg/ml) stimulates the secretion of BMSCs cell factors SCF, G-CSF, GMCSF, while PM2.5 with high concentration (50, 100, 200 μg/ml) inhibits its secretion of BMSCs cell factors SCF, G-CSF, GM-CSF. Compare the 10, 20 μg/ml group with control group. With the increasing concentration of PM2.5, the secretion amount of BMSCs cell factors SCF, G-CSF, GM-CSF shall be increased, showing that the differentiation has a statistical significance ($P$<0.05); compare the 50, 100, 200 μg/ml group with the control group, with the increasing concentration of PM2.5, the secretion amount of BMSCs cell factors SCF, G-CSF, GM-CSF shall be reduced, showing that the differentiation has a statistical significance ($P$<0.05).

*RT-PCR method detects the mRNA expression level of BMSCs cell factors SCF, G-CSF, GM-CSF*

From **Figure 4**, it can be seen that RNA sample drawn after electrophoresis appear as three lines of complete belts, indicating that the total RNA drawing is relatively complete. From **Figure 2**, it can be seen that after the RT-PCR reaction is finished, the amplification curve of a target gene and inner reference gene can be started from the maximum baseline, appearing as an exponential growth phase and platform period, which indicates that the amplification system is suitable for the reaction, and the reaction condition setting up is accurate, with the same gene parallel, and good repeatability. It can be seen from the dissolution curve that the target gene and inner reference gene appear as a single peak, with a sharp form, indicating that the specificity of the amplification products is high, without specificity products, and a primer dimer, so the result is reliable.

It can be seen from **Table 5** that PM2.5 with low concentration (10, 20 μg/ml) stimulates mRNA expression of BMSCs cell factors SCF, G-CSF, GM-CSF, while PM2.5 with high concentration (50, 100, 200 μg/ml) inhibits mRNA expression of BMSCs cell factors SCF, G-CSF, GM-CSF. Compare 10, 20 μg/ml group with control group, with the increasing concentration of PM2.5, mRNA expression level of BMSCs cell factors SCF, G-CSF, GM-CSF is gradually increased, and the fore the differentiation has a statistical significance ($P$<0.05). On the other hand compare 50, 100, 200 μg/ml group with the control group, and with the increasing concentration of PM2.5, mRNA expression level of BMSCs cell factors SCF, G-CSF, GM-CSF is gradually reduced, so the differentiation has a statistical significance ($P$<0.05) (**Figure 2**).

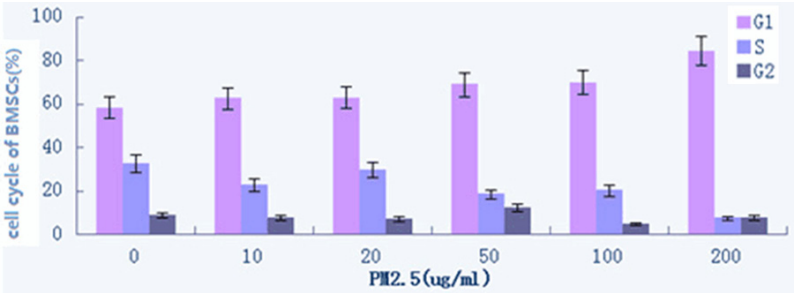


**Figure 3.** The effect of PM2.5 on cell cycle of SD rat's BMSCs.

**Table 4.** The impact of PM2.5 on BMSCs cell factors SCF, G-CSF, GM-CSF secretion quantity ($\bar{x} \pm s$, n=6)

| PM2.5 (μg/ml) | SCF(pg/ml) | G-CSF (pg/ml) | GM-CSF (pg/ml) |
|---|---|---|---|
| control group | 595.20±31.22$^{\Delta}$ | 63.28±4.13$^{\Delta}$ | 10.29±1.17$^{\Delta}$ |
| 10 | 633.64±25.09$^{\Delta}$ | 74.23±4.56$^{*,\Delta,\#}$ | 12.06±1.03$^{*,\Delta,\#}$ |
| 20 | 767.96±64.16$^{*,\#}$ | 93.50±4.22$^{*,\#}$ | 14.89±0.72$^{*,\#}$ |
| 50 | 588.30±16.33$^{\Delta,\#}$ | 60.24±5.01$^{\Delta,\#}$ | 9.16±0.45$^{*,\Delta,\#}$ |
| 100 | 187.49±25.27$^{*,\Delta,\#}$ | 47.77±4.77$^{*,\Delta,\#}$ | 8.14±0.34$^{*,\Delta,\#}$ |
| 200 | 121.45±14.33$^{*,\Delta,\#}$ | 31.20±5.67$^{*,\Delta,\#}$ | 6.47±0.54$^{*,\Delta,\#}$ |

Note: $^{*}P<0.05$, express compared with the control group; $^{\Delta}P<0.05$, express compared with 20 μg/ml concentration group. $^{\#}P<0.05$, compared with the previous group.

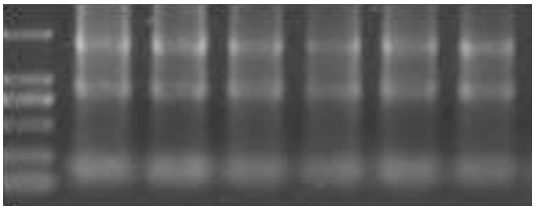

**Figure 4.** The quality of RNA detection electrophoregram.

Marker size: up to bottom: 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp, 100 bp.

Lane order: Marker, negative control group, 10 μg/ml group, 20 μg/ml group, 50 μg/ml group, 100 μg/ml group, 200 μg/ml group.

## Conclusion

PM2.5 contains a wealth of pathogenic substances, and even contains a large number of carcinogens and can lead to the occurrence of malignant tumors [10]. After being inhaled by the human body, it can enter into the blood, and thereafter is widely distributed in various organs through blood circulation [11], resulting in the occurrence of diseases in various systems. The research indicates that PM2.5 organic substances or compounds can cause DNA adducts and DNA oxidation damage [4, 12]. If the body is damaged by DNA, when the damage is not fully repaired, it is easy to cause cancer, abnormality and the occurrence of mutations, so that the organization or cells may eventually turn to malignant proliferation. BMSCs are the most important part of the hematopoietic microenvironment, and as such the basis for the survival of hematopoietic stem cells. The survival environment of a hematopoietic stem cell has changed, the growth bone marrow stromal cells shall be damaged, among which the function of secretion cell factors shall be impacted, to stimulate the growth of hematopoietic stem cells. If a clone appears in the process of reproduction and apoptosis, it shall finally result in leukemia and other malignant diseases of the blood system [13, 14]. We can observe from the experiment that PM2.5 with low concentration demonstrates a stimulation function on BMSCs reproduction. With the increasing concentration under a high concentration, viewed under an inverted microscope, the number of adherent cells can be seen too gradually decrease, cell connection is loose, and the amount of cell debris and floating dead cells also is increased, indicating that PM2.5 shall have a toxic function on BMSCs resulting in cell damage, to reduce the survival rate and inhibit BMSCs reproduction. Such inhibition phenomena of low dose promoting high dose is considered to have a relationship with hormesis effect. Hormesis effect refers to the stimulation of toxic matters on a low dose of substrate and inhibition phenomena of high dose [15]. It can be seen that adverse effect of PM2.5 on BMSCs can be shown in high dose, while PM2.5 of high dose shall be toxic to BMSCs.

BMSCs are the stem cells with multiple directions of differentiation potential. Under the function of osteoblast inducing medium, it can

**Table 5.** The impact of PM2.5 with different concentrations on cell cycle of BMSCs after 48 h, ($\bar{x}$ ± s, n=3)

| PM2.5 (ug/ml) | Cell cycle (%) | | |
|---|---|---|---|
| | G0/G1 | S | G2 |
| Control group | 58.40±0.02 | 32.68±3.09 | 8.91±3.06 |
| 10 | 62.49±0.49 | 22.78±1.45 | 7.56±8.18 |
| 20 | 63.06±14.3 | 29.79±6.93 | 7.19±7.47 |
| 50 | 69.12±4.56 | 18.43±9.01 | 12.45±13.57 |
| 100 | 69.86±5.03 | 20.21±3.06 | 4.92±1.02 |
| 200 | 84.34±3.62▲ | 7.52±8.11▲ | 7.63±6.02 |

Note: ▲$P$<0.05, express compared with the control group.

be transferred into osteoblast cell differentiation. Alkaline phosphatase is a marker enzyme of osteoblast cells secretion, which is an important indicator that bone marrow stromal cells are successfully transferred into differentiation for osteoblast cells [16, 17]. In the process of BMSCs osteoblast differentiation, with an increasing degree of osteoblast differentiation, alkaline phosphatase activity shall be increased. The research shows that: under the same concentration, with an increasing functional time, PM2.5 with low concentration can promote the production of ALP activity, while PM2.5 with high concentration shall inhibit the production of ALP activity, which is consistent with the reproduction result of BMSCs, illustrating that PM2.5 with low concentration can stimulate the increasing amount of cells after BMSCs reproduction, resulting in an increasing secretion of ALP activity in order to promote the reproduction and stimulated its differentiation. Compared with osteoblast differentiation of negative control group, with an increasing concentration of PM2.5 solution, ALP activity shall be reduced, indicating that ALP secretion shall be inhibited, to show the trends of BMSCs transferring to osteoblast cell differentiation, and indicate that PM2.5 with high concentration inhibits a normal differentiation level of bone marrow stromal cells or resulting in abnormal differentiation of cells. Altogether this points out that the reproduction differentiation function of PM2.5 on BMSCs has a toxic influence due to the normal reproduction differentiation function of BMSCs. It is inhibited, except for abnormal reproduction and differentiation appearing in the process of BMSCs reproduction and differentiation.

The research adopts a PM2.5 solution with different concentrations on BMSCs for 48 h, and the result shows that with increasing drug concentration, the cell apoptosis rate shall be increased, to show the direct cell toxicity or inducing apoptosis function of PM2.5 on BMSCs. It has been found that apoptosis of the inducing organic cell of PM2.5 has multiple ways, for instance, after rat alveolar epithelial cells were exposed to fine particulate matter, the expression level of cell cycle and related genes to apoptosis shall be increased, appearing as cell damage [18]. Small glial cells or lung cancer cell lines of atmospheric particulate matter can be applied to stimulate MAPK apoptosis route, and arouse apoptosis [19, 20]. When the cell is stimulated by fine matter, the content of p53 and Bax shall be increased, with mutual function [21], to promote Bax to be disclosed from Bax/Bcl-xl complex. This arouses the oligomer and glycosylation of Bax protein, in order to increase the mitochondrial outer membrane permeability, finally resulting in apoptosis [22]. Our research indicates that when PM2.5 reach high concentration of 200 ug/ml, the toxic BMSCs cell of G0/G1 period, compare S period and control group, the cell cycle has obviously been prolonged, indicating that BMSCs cell cycle reproduction is obviously inhibited, and PM2.5 can disturb the cell cycle, and may cause the cell to display abnormal reproduction in the differentiation process, so that there is the possibility for BMSCs cells to transfer into a malignant clone.

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## Disclosure of conflict of interest

None.

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## References

[1] Kelly FJ, Fussell JC. Air pollution and public health: emerging hazards and improved under

standing of risk. Environ Geochem Health 2015; [Epub ahead of print].
[2] Habert C, Garnier R. Health effects of diesel exhaust: a state of the art. Rev Mal Respir 2015; 32: 138-54.
[3] Rice MB, Ljungman PL, Wilker EH, Dorans KS, Gold DR, Schwartz J, Koutrakis P, Washko GR, O'Connor GT, Mittleman MA. Long-term exposure to traffic emissions and fine particulate matter and lung function decline in the Framingham heart study. Am J Respir Crit Care Med 2015; 191: 656-64.
[4] Silva da Silva C, Rossato JM, Vaz Rocha JA, Vargas VM. Characterization of an area of reference for inhalable particulate matter (PM2.5) associated with genetic biomonitoring in children. Mutat Res Genet Toxicol Environ Mutagen 2015; 778: 44-55.
[5] Jones RR, Hogrefe C, Fitzgerald EF, Hwang SA, Özkaynak H, Garcia VC, Lin S. Respiratory hospitalizations in association with fine PM and its components in New York State. J Air Waste Manag Assoc 2015; 65: 559-69.
[6] Feng ZW, Liang CM. The impact of cell survival rate and IL-10 level of PM2.5 of a 549 in cooking oil fume. Chinese Journal of industrial medicine. 2011; 24: 104-106.
[7] Bernardo ME, Fibbe WE. Mesenchymal stromal cells and hematopoietic stem cell transplantation. Immunol Lett 2015; [Epub ahead of print].
[8] Payushina V. Localization and functions of mesenchymal stromal cells in vivo. Zh Obshch Biol 2015; 76: 161-72.
[9] Zhou BO, Ding L, Morrison SJ. Hematopoietic stem and progenitor cells regulate the regeneration of their niche by secreting Angiopoietin-1. Elife 2015; 4: e05521.
[10] Valavanidis A, Fiotakis K, Vlachogianni T. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. J Environ Sci Health C 2008; 26: 339-362.
[11] Brunekreef B, Holgate ST. Air pollution and health. Lancet 2002; 360: 1233-1242.
[12] Topinka J, Rossner P Jr, Milcova A, Schmuczerova J, Svecova V, Sram RJ. DNA adducts and oxidative DNA damage induced by organic extracts from PM2.5 in an acellular assay. Toxicol Lett 2011; 202: 186-192.
[13] Xu G, Zhang Y, Zhang L, Ren G, Shi Y. Bone marrow stromal cells induce apoptosis of lymphoma cells in the presence of IFNgamma and TNF by producing nitric oxide. Biochem Biophys Res Commun 2008; 375: 666-670.
[14] Dacalescu A, Zlei M, Aanei C, Ungureanu D, Tepeluş M, Burcoveanu C, Dănăilă C. Flow cytometry-based quantification of cytokine levels in AML patients plasma and cell culture supernatants. Rev Med Chir Soc Med Nat Iasi 2008; 112: 196-202.
[15] Toseph VR. Hormesis and toxicological risk assessment. Toxicol Sci 2003; 71: 134-136.
[16] Wu Qinmin. The function of in vitro induction of osteopontin of rat bone marrow stromal stem cells into osteoblast cells differentiation. Shanxi Medical University 2010; 9-10.
[17] Chen Bin. The impact and mechanism of three seven total saponins of bone marrow stromal cells on osteoblast destined differentiation. Medical College of Shantou University 2010; 37-39.
[18] Omura S, Koike E, Kobayashi T. Microarray analysis of gene expression in rat alveolar epithelial cells exposed to fractionated organic extracts of diesel exhaust particles. Toxicology 2009; 262: 65-72.
[19] Sama P, Long TC, Hester S, Tajuba J, Parker J, Chen LC, Veronesi B. The cellular and genomic response of an immortalized microglia cell line (BV2) to concentrated ambient particulate matter. Inhal Toxicol 2007; 19: 1079-1087.
[20] Deng X, Rui W, Zhang F, Ding W. PM2.5 induces Nrf2-mediated defense mechanisms against oxidative stress by activating PIK3/AKT signaling pathway in human lung alveolar epithelial A549 cells. Cell Biol Toxicol 2013; 29: 143-57.
[21] Yun YP, Lee JY, Ahn EK, Lee KH, Yoon HK, Lim Y. Diesel exhaust particles induce apoptosis via p53 and Mdm2 in J77A.1 macrophage cell line. Toxicol In Vitro 2009; 23: 21-28.
[22] Xu Z, Xu X, Zhong M. Ambient particulate air pollution induce oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. Part Fibre Toxicol 2011; 8: 20.