ORIGINAL ARTICLE

# Dynamic analysis of Bone Marrow Mesenchymal Stem Cells migrating to Pancreatic Islets using coculture Microfluidic Chips: An accelerated migrating rate and better survival of Pancreatic Islets were revealed

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**Abstract**

**OBJECTIVES:** A coculture microfluidic chip had been fabricated to investigate the dynamic process of bone marrow mesenchymal stem cells migrating to the pancreatic islets *in vitro*.

**METHODS:** We fabricated a coculture microfluidic chip using standard procedures. On the chip, bone marrow mesenchymal stem cells and pancreatic islets were introduced respectively into two microchambers which could be connected by a traffic tunnel. Migration assay was performed along the tunnel, and the function of pancreatic islets was timely evaluated by analysis of insulin secretion in response to high-glucose-challenge.

**RESULTS:** The results showed that some bioactive factors excreted by freshly isolated pancreatic islets could enhance the migrating rate of BM-MSCs. Besides, pancreatic islets also showed a better survival and function by coculturing with bone marrow mesenchymal stem cells.

**CONCLUSION:** The results demonstrated that bone marrow mesenchymal stem cells had the ability to migrate to pancreatic islets and provide an apparent overall preservation for islet function. This microfluidic device was a potentially useful tool to mimic actual biological processes of bone marrow mesenchymal stem cells migrating to the pancreatic islets *in vitro.*

**Abbreviations:**
BM-MSCs: Bone marrow mesenchymal stem cells

## INTRODUCTION

Regeneration of new pancreatic β cells from stem cells or depression of β cell apoptosis by stem cells is increasingly viewed as a promising therapeutic strategy in the field of diabetic treatment. Bone marrow mesenchymal stem cells (BM-MSCs), which can be isolated from bone tissue and expanded *in vitro* for clinical use, have provided us an ideal cell source for this stem cell-based strategy (Pittenger *et al.* 1999). Recently, Hess (Hess

*et al.* 2003) has showed that transplantation of adult bone marrow derived cells expressing c-kit reduces high glucose level streptozotocin-induced diabetic mice, resulting in a proliferation of recipient pancreatic cells. Increasingly, investigation concerning the process of bone marrow mesenchymal stem cells migrating to pancreatic islets is viewed as an active area.

Cell migration assay is crucial for understanding the underlying mechanism of many physiological and pathological processes including embryonic development, cancer metastasis and immune response. Typical cell migration assay is mostly performed by transwell platform or wound healing assay. However, transwell platform device requires a large amount of cells, thus restricts its application in the field of stem cell research (Sia *et al.* 2003). Moreover, it does not allow visualization of the actual migration paths, providing only an end-point measurement, thus the whole migrating process is unable to be real-timely observed. As far as wound healing assay is concerned, this method also requires hand-operation with low repeatability. Therefore, developing a new platform for observing the dynamic analysis of bone marrow mesenchymal stem cells migrating to pancreatic islets *in vitro* is really worth pursing.

Recently, chip-based cell culture technology have shown significant advance, and several papers concerning the cell-cell interaction assay using microfluidic chip have been published (Chung *et al.* 2005; Nie *et al.* 2007; Kim *et al.* 2006; Walker *et al.* 2005; Tourovskaia *et al.* 2005). However, reconstructing a coculture microfluidic chip preferable for mimicking biological dynamic processes of BM-MSCs migrating to the pancreatic islets *in vitro* has not been reported yet. In this study, utilizing the chip technology, we attempted to fabricate a coculture microfluidic chip for observing two existing possible behaviors by which transplanted BM-MSCs could reduce hyperglycemia in diabetes mellitus models: migrating to the pancreatic islets; supporting pancreatic islets survival. BM-MSCs obtained from diabetic wistar rats were introduced to the microfluidic chip and cocultured with pancreatic islets. Online migration assay of BM-MSCs was performed and real-time functional changes of pancreatic islets were observed on the chip.

## MATERIALS AND METHODS

### Isolation and culture of BM-MSCs

BM-MSCs were separated by density gradient centrifugation from diabetic wistar rats induced by streptozotocin. The protocol had been approved by the Institutional Review Board of Shandong University. BM-MSCs were cultured in RPMI1640 (Gibco) supplemented with 10% Fetal Bovine Serum (HyClone) at 37 °C in a humidified atmosphere containing 5% $CO_2$. The third passage of BM-MSCs had been identified by flow cytometry analyses.

### Pancreatic islet isolation

Pancreatic islets were isolated from diabetic wistar rats by the collagenase digestion method and purified by a Ficoll density-gradient centrifugation. Subsequently, twenty dithizon staining positive pancreatic islets (average diameter was about 200μm) were handpicked using micropipettes under a stereomicroscope and introduced to the microchambers on the coculture microfluidic chip.

### Fabrication of the microfluidic coculture platform

We fabricated a poly (dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning, Midland, MI)/glass coculture microchip using standard procedures (McDonald *et al.* 2002). The microfluidic chip was designed as follows (Figure 1): There were three similar units on one chip, each unit had an inlet reservoir, an outlet reservoir, a microchamber, a seeding channel and two microchannels. The medium droplets were injected into the inlet reservoir and the waste medium was aspirated from the outlet reservoir.

The dimensions of the inlet and outlet reservoirs were 1cm diameter, cell culture microchamber was 1cm wide, 1cm long and 150 μm deep. The depth of microchamber 1 was 500 μm due to the bigger diameter of pancreatic islet.

Every two microchambers were communicated by a traffic tunnel. The traffic tunnel could be completely intercepted. The intercepting process of the traffic tunnel was presented on Figure 1: applying a controlled force on the top of the traffic tunnel. During this application, the microstructures located at the top of the tunnel came into contact with the glass, thus the tunnel was completely shut off. The intercepting process could also provide a leading edge for migration.

### On-chip migration assay

Our first experimental strategy was to observe whether some bioactive factors excreted by freshly isolated pancreatic islets could enhance the migrating rate of BM-MSCs. The third passage of BM-MSCs at the density of $1\times10^6$cells/ml was introduced into the microchamber 2 through seeding channel. Then the seeding channel was closed down by a plug. BM-MSCs were allowed to reach to a 70% confluence on the surface of microchamber 2 in RPMI-1640 culture medium (Gibco) supplemented with 10% (v/v) fetal calf serum(HyClone). Before migration assay, BM-MSCs would be firstly starved by replacing the medium with serum-free RPMI-1640 medium for 18 h, and the proliferation-independent cell migration was performed under serum-free conditions.

Subsequently, twenty handpicked pancreatic islets were introduced into the microchamber 1, the traffic tunnel 1 was opened. The coculture medium was replaced by serum-free RPMI-1640 medium (Gibco) supplemented with 10 mM Hepes (Sigma), 1 mM sodium pyruvate (Sigma), 2 mM L-glutamine(Gibco)

and 50 μmol/L β-mercaptoethanol (Sigma). Under this condition, BM-MSCs started to migrate towards pancreatic islets through the traffic tunnel 1. The migrating distance of BM-MSCs from the initial leading edge to the site of the migrating cells was real-timely measured and photographed periodically using a light microscope. In each traffic tunnel, three randomly chosen fields were analyzed, and the obtained values were averaged. Proliferation-independent cell migration was observed for 24 h under stopped-flow condition and repeated 6 times. As a control group, twenty explants of skin tissue (the same size as pancreatic islets) from diabetic wistar rats were introduced into microchamber 3 and the migrating cells in the traffic tunnel 2 were also photographed.

## Evaluation of pancreatic islets survival

Our second experimental strategy was to investigate whether BM-MSCs could support pancreatic islets survival and function. In order to analyze the influence of BM-MSCs on pancreatic islets, experiment was performed in two groups: (1) Coculture group: traffic tunnel 1 was opened and BM-MSCs were allowed to migrate into the pancreatic islets; (2) Control group: traffic tunnel 1 was cut-off and twenty hand-picked pancreatic islets were monocultured in the microchamber 1. For coculturing assay, coculture medium was replaced by RPMI-1640 culture medium (Gibco) supplemented with 10% (v/v)heat-inactivated fetal calf serum(HyClone), 10 mM Hepes (Sigma), 1 mM sodium pyruvate (Sigma), 2 mM L-glutamine(Gibco) and 50 μmol/L β-mercaptoethanol (Sigma).

The pancreatic islets were observed daily for morphologic changes. Functional analysis of the islets was performed by evaluating baseline insulin release and insulin secretion in response to a high-glucose challenge using insulin ELISA kit (Beijing Atom HighTech Co.Ltd, Beijing, china). For analysis above, all reagents were added through the inlet reservoirs.

## Statistical analysis

All data are presented as means±SD and analyzed by the Analysis of Variance (ANOVA) followed by a Student's t test. A *p*-value of 0.05 was adopted for determining statistically significant differences. All

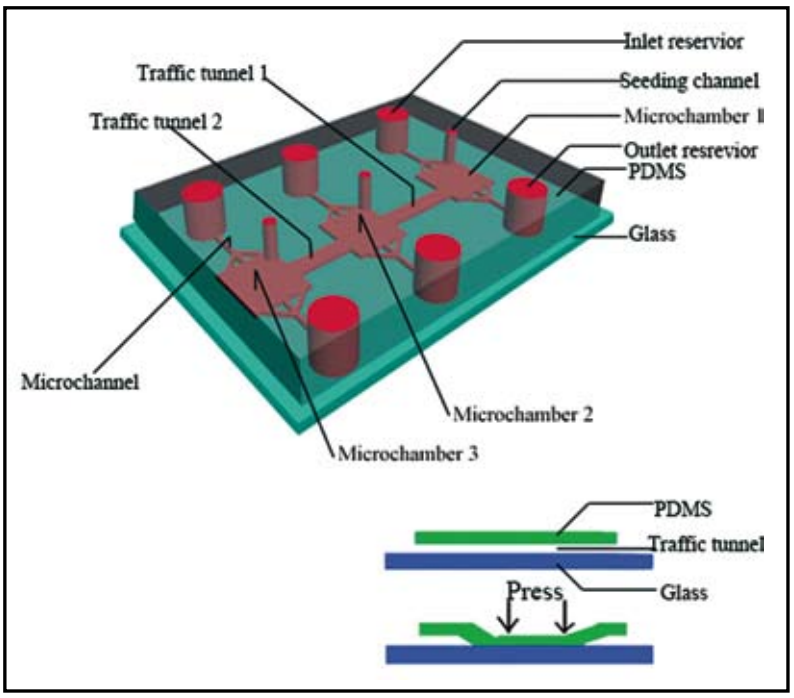


**Figure 1.** Sketch map of the microfluidic chip design: There were three similar units on one chip, each unit had an inlet reservoir, an outlet reservoir, a microchamber, a seeding channel and two microchannels. Every two microchambers were communicated by a traffic tunnel. The traffic tunnel could be completely intercepted by applying a controlled force on the top of the tunnel.

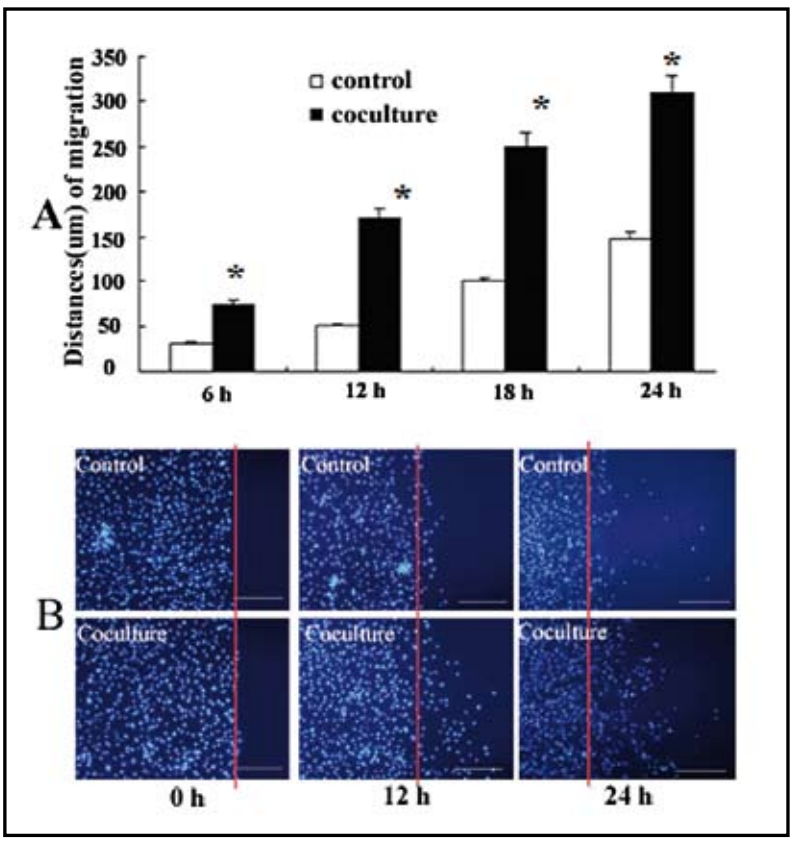


**Figure 2.** Migration on the chip under serum-free condition. (A) The migrating distances (μm) of BM-MSCs was real-timely measured from the leading edge to the migrating site for 24 h. Error bars show the SE (n = 6). *$p < 0.05$ vs. control group. (B) Migrating BM-MSCs inside the traffic tunnel (nuclei labeled by DAPI). Red bar represents the leading edge. Scale bar=200 μm.

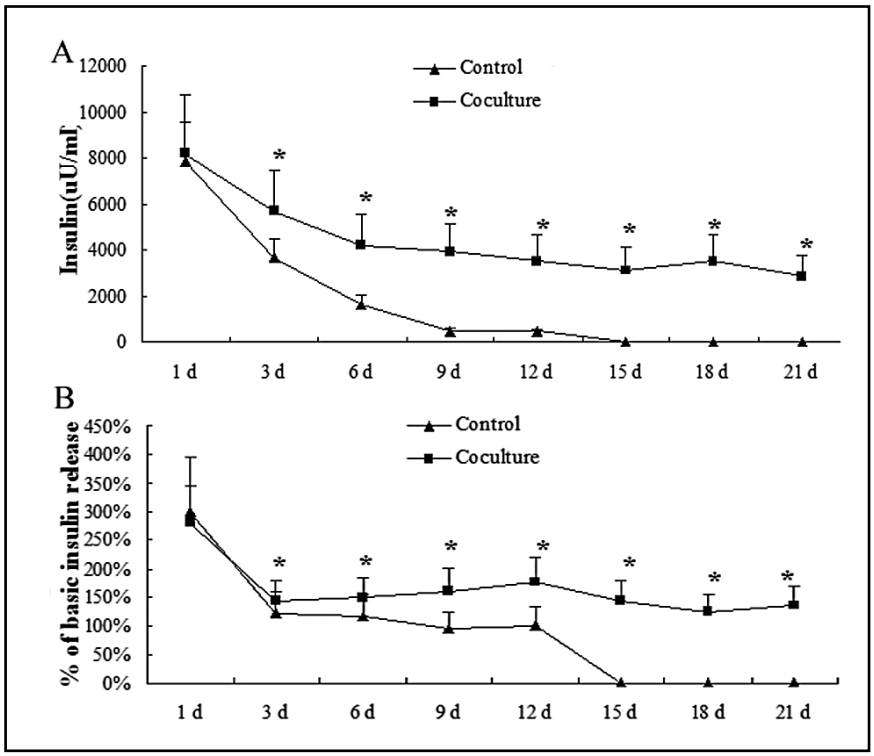


**Figure 3.** BM-MSCs retain pancreatic islet function. (A) Evaluation of baseline insulin release during the three weeks of observation. (B) Insulin secretion in response to a high-glucose challenge. Error bars show the SE (n = 6); *$p < 0.05$ vs. control group.

data represented the results of six independent experiments.

## RESULTS

In this study, BM-MSCs were successfully isolated using routine methods and subsequently used for migration assay. A number of studies had reported that BM-MSCs, which could migrate into the injured region in the myocardial infarction or nerve injury animal models(Barbash *et al.* 2003; Azizi *et al.* 1998), also had the ability to migrate into pancreatic islets and support pancreatic islets survival (Luo *et al.* 2007). Therefore, we tried to fabricate this microfluidic chip for on line observation of BM-MSCs migrating to the pancreatic islets *in vitro.*

### Coculture microchip enabled on line cell migration assay

Cells cultured the microchamber 2 grew to a 70% confluence monolayer three days after cell seeding. For migration assay, when the traffic tunnel was opened, BM-MSCs on the leading edge began to migrate towards pancreatic islets through the traffic tunnel 1. The migrating rate of BM-MSCs inside the traffic tunnel 1 was significantly higher than control group, suggesting a synergistic effect of islets released cytokine on BM-MSCs migration. Further details were shown in Figure 2.

### Better pancreatic islet survival were revealed in coculture condition

When coculture assays were performed with media supplemented with 10% (v/v)heat-inactivated fetal calf serum, cell migration towards the pancreatic islets was significantly higher, compared to experiments performed in the absence of fetal calf serum. Gradually, BM-MSCs migrated into the microchamber 1 and appeared to aggregate around islets and form a monolayer encircling islets, resulting in the formation of a capsule-like three-dimensional structure with apparent overall preservation of islet morphology. Results showed that most of the cells within these three-dimensional structures (average diameter was about 200μm) were positive for insulin staining. More importantly, the evidence for better function protection was illustrated by the observation of insulin release in response to high-glucose challenge (Figure.3).

By contrast, islets in control group underwent a rapidly degenerative process. Most islets lost their morphology with cells spilling out of the islets in a time-dependent manner. After three weeks, stereochemical structure of islets had disaggregated, leaving only a small number of cells. Insulin secretion test failed to reveal evidence of a better maintenance of function (Figure.3).

## DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by the destruction of pancreatic islet. Recently, animal and clinical studies have suggested that transplanted BM-MSCs may home to pancreas and contribute to the repair of impaired pancreatic islets, which has generated a great deal of excitement as a new therapeutic strategy. Accordingly, investigation referring to the underlying mechanism of BM-MSCs migrating to pancreas has attracted more and more attention.

In our study, migration assay performed on chip showed an accelerated migrating rate of BM-MSCs towards pancreatic islets, in agreement with Sordi (Sordi *et al*. 2005), who reported that BM-MSCs could express some chemokine receptors capable of promoting migration to pancreatic islets. In future study, if we add a specific antibody onto the traffic tunnel to block one kind of chemokine receptors, by analyzing of the promoted or inhibited migrating rate, it will be possible for us to identify the most important chemokine that controls BM-MSCs migration and to promote a greater understanding of the molecular, biological and physiological characteristics of this potentially highly useful stem cell type.

Pancreatic islets only culture failed to reveal evidence of long term survival (Paraskevas *et al*. 2000). Researches had found that MSCs naturally produce a variety of cytokines to promote the survival of surrounding cells. In our study, islet cells soon became disintegrated in monoculture condition. By contrast, by coculturing with pancreatic islets, BM-MSCs could provide a supportive nurturing cytokines for pancreatic islet cells' longevity, supplying an apparent overall preservation for islet morphology maintenance and function (Chang *et al*. 2007).

PDMS based microfluidic devices offers many advantages, such as being inexpensive, easy to fabricate, small cell consumption and persist perfusion of culture medium. Besides, this coculture microchip had two additional merits: (1) We fabricated a traffic tunnel to feed through two separate cells cultured in two microchambers, which could be shut off or opened easily according to experimental request. Due to the transparent characteristics of PDMS, online observation of migrating process was feasible. (2)Using conventional coculture device transwell, migration assay was performed by different cells cultured in different cell culture plates, resulting the discrepancy derived from different cultivation environments. In our study, we precisely observed the two different migrating paths of the same cells cultured in same microchamber, aiming to eliminate the discrepancy derived from different cultivation environments or cells.

Taken together, in the present study we described an experimental coculture microfluidic chip to investigate the dynamic process of BM-MSCs migrating to the pancreatic islets *in vitro*. These BM-MSCs, in addition to displaying an accelerated migrating rate towards pancreatic islets, also shared the most important characteristic of increasing islet survival and function. Microfluidic chip provided rapid optimization of cell state using relatively small numbers of cells. This coculture microfluidic chip had a potential for the applications of reliable cell-based migration assays.

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