

Lab Report

“Attachment of mesenchymal stromal cells (MSCs) on collagen substrates”

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1 Introduction

Mesenchymal stromal cells (MSCs) can be differentiated in vitro into osteoblasts, adipocytes, and chondroblasts. Therefore, they provide huge potential as new therapeutic approaches with respect to tissue regeneration [1]. MSCs are mainly derived from bone marrow, but can also be found in adipose tissue, or the placenta [2]. To be able to insert MSCs in the human body, so that they perform the admired function, the cells need to be attached on an appropriate substrate. As it is known that cell-matrix interactions influence the proliferation and differentiation of cells, there is the necessity to evaluate the attachment of MSCs on different surfaces [2]. Generally, MSCs bind to an extracellular matrix (ECM), often at an RGD sequence [3], via integrins expressed by the MSCs [1]. Integrins consist of α and β subunits, where the binding of the β subunit to the RGD leads to a cascade in the inner of the cell [3], inducing among others, migration, growth and differentiation [3]. As collagen I is a protein often present in ECMs, it is of great importance to obtain further knowledge regarding MSCs attachment on collagen I [1]. Thus, the aim of this practical was to investigate the attachment of bone marrow derived (bm) MSCs incubated on collagen spots in a plastic petri dish, where the concentration of the collagen varied.

2 Methods

For substrate preparation, a petri dish was labelled with a circle, having a few millimeters distance to the edge of the petri dish, and a cross inside the circle, so that a circle separated in four equal quarters was obtained. Between the edge of the petri dish and the circle, fat was added with a pen. In the middle of each quarter 1 μ l of either PBS, collagen solution, or collagen solutions diluted by a factor of 1:1000, and 1:10000 were pipetted. Hereby, PBS was taken as negative control for further evaluation. Then, the petri dish was air dried for 15 min at room temperature. Afterwards, the surface of the dish was blocked with 200 μ l of 1 % BSA/PBS to prevent unspecific binding, and the dish was put in an incubator at 37 °C for 15 min.

Fourth passage bmMSCs, obtained from the BG Trauma Center Tübingen, were checked under the microscope whether they adhered on the ground of the cell culture flask. To remove all proteins and dead cells from the flask, the cell medium was aspirated, and the cells were flushed with 5 ml PBS. Next, 3 ml of Accutase were added to the flask for gentle detachment of the MSCs adhered on its ground. Generally, Accutase is an enzyme behaving significantly more gently than e.g. Trypsin that is

usually used for cell detachment. Therefore, it was important that all dead cells and proteins were washed off from the flask, before adding Accutase. The flask was incubated at 37 °C for 5 min in a humidified incubator. After checking whether the cells floated in the flask, thus, if the detachment was successful, the proteolysis induced by the Accutase was stopped by adding 3 ml of growth medium. The cell suspension was pipetted in a 50 ml centrifugation tube, and centrifugated at 4 °C for 7 min at 1500 rpm. Then, the supernatant was aspirated, the remaining cell pellet was resuspended in 10 ml of medium, and again centrifugated for 7 min. The supernatant was aspirated again to remove all proteins.

In the last step of the practical, the attachment of the MSCs on the substrate was observed. Therefore, 200 µl of the cell attachment medium CAM were used to resuspend the MSCs. For homogenous distribution of the cells in the medium, they were pipetted up and down several times. After adding 200 µl of cell suspension on the petri dish, it was incubated at 37 °C for 15 min, to enable the MSCs potential binding on the collagen and PBS. After that, the cells were washed four times with PBS, thereof the first time with 400 µl, and the other three times with 200 µl, to ensure complete removal of non-adherent cells. Finally, under the microscope, using dark field mode, the density of cells adhered on the different spots of the petri dish was investigated.

3 Results

Figure 1 depicts the MSCs attached on the ground of the cell culture flask, that were used in this experiment. By observing the cells under an optical microscope, the typical elongated shape of MSCs could be detected.

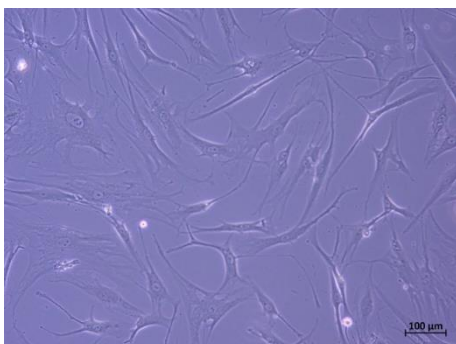


Figure 1: Bone marrow derived MSCs attached on bottom of cell culture flask, observed with an optical microscope

Figure 2 shows the attachment of the MSCs on the different substrates. These results were obtained by another practical group. Dark field microscopy revealed a high density of cell attachment on collagen (A). The collagen diluted by a factor of 1:1000

(B) also provides good attachment for the cells. In C, it can be seen that almost no MSCs attached on collagen diluted with a factor of 1:10000. On the PBS negative control (D), no attached cells can be observed.

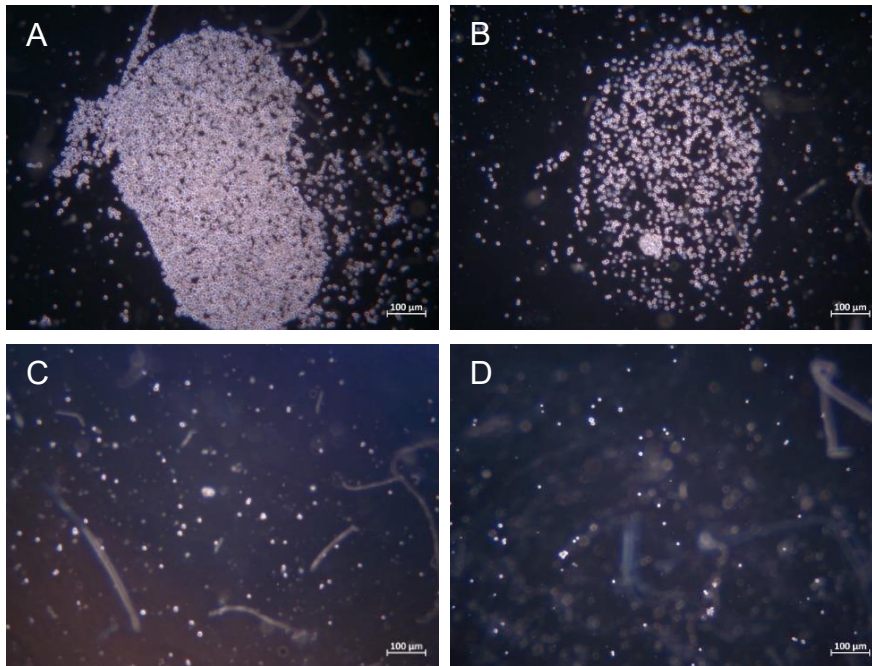


Figure 2: Attachment density of MSCs on A: collagen I, B: collagen I diluted 1:1000, C: collagen I diluted 1:10000, D: PBS negative control

The results obtained by our practical group showed some attachment of MSCs on the collagen, that was comparable to the amount of attached MSCs in Figure 2B (collagen diluted 1:1000). For our collagen diluted with a factor of 1:1000 and 1:10000, as well as for the PBS negative control, no cell attachment could be detected under the microscope at all.

4 Discussion

In this practical, the attachment of bmMSCs on varying collagen concentrations was investigated. Contrary as reported by Warstat et al. [1], in this practical MSCs attachment was only observed on the undiluted collagen I. The two diluted collagen spots (dilution factor 1:1000 and 1:10000) showed no cell attachment at all. Although specific binding of MSCs on a collagen substrate could be demonstrated, the cell density on the undiluted collagen spot was not as high as expected in this practical. The PBS that served as negative control revealed no cell attachment, indicating that there was no unspecific binding of the MSCs. There are several potential sources of error leading to reduced/no binding of MSCs on the collagen. First, there might have been a problem with the collagen, as e.g. being too old, or insufficiently air dried after

having been pipetted on the petri dish. While washing the non-adherent cells off with PBS, it was more PBS used than recommended, what might have potentially led to detachment of some MSCs. Furthermore, there might have been happened some mistakes during the conduction of the experiment. Imaginable are dilution mistakes during preparation of the collagen samples, wrong pipetting, or insufficient washing of dead cells.

An option to enhance the attachment of MSCs on collagen I might be using the growth factor TGF- β 1 as reported by Warstat et al. [1]. This effect was not investigated within this practical.

5 Literature

1. Warstat, K., et al., *TGF- β Enhances the Integrin α 2 β 1-Mediated Attachment of Mesenchymal Stem Cells to Type I Collagen*. Stem Cells and Development, 2010. **19**(5): p. 645-656.
2. Maerz, J.K., et al., *Bone marrow-derived mesenchymal stromal cells differ in their attachment to fibronectin-derived peptides from term placenta-derived mesenchymal stromal cells*. Stem Cell Research & Therapy, 2016. **7**(1): p. 29.
3. Ruoslahti, E., *RGD and other recognition sequences for integrins*. Annu Rev Cell Dev Biol, 1996. **12**: p. 697-715.

I hereby declare that my elaboration was made independently and is not a copy of an existing protocol.

Date: May, 25th 2022

Signature: