

Grade 2,0

Protocol for the experiment
Attachment of bmMSCs to type I collagen

Master Biomedical Technologies, Labwork Implantology

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1 Introduction

Mesenchymal stem cells (MSCs) are multipotent cells found in stem cell niches such as bone marrow stroma, adipose tissue or dental pulp. Components of these niches are predominantly extracellular matrix (ECM) proteins, other niche cells and growth factors, which contribute to the regulation of differentiation, profiling and apoptosis of MSCs.

Due to their differentiation ability, MSCs can be used as a therapeutic agent driving wound healing and tissue regeneration by introducing them into damaged tissue [1]. Depending on the origin of the MSCs, they differ in their properties such as the expression of integrin receptors on the cell surface, which provide for the attachment of the cells to the protein ligands of the ECM [2]. Therefore, studies of integrin protein attachment are important to find suitable carrier materials for the introduction of different MSCs into the ECM that integrate into the target tissue, support MSC integration, and promote cell profiling through cell-matrix interaction. Collagens represent a promising material for this purpose, as they are common components of stem cell niches. In addition to studies of suitable materials for cell attachment, the importance of activation factors is crucial as they lead to activation of MSC and thus expression of integrins and attachment of cells to the ECM [1].

In this experiment, the attachment of bone marrow MSCs (bmMSCs) to type I collagen was investigated, for which spots with different collagen content were applied to a Petri dish and the resulting cell concentration in these areas was examined under the microscope.

2 Methods

The laboratory work was divided into three main steps: preparation of the substratum, preparation of the bmMSCs and attachment of the cells to the substratum.

In the first step, the substratum was prepared. First, a Petri dish was labelled and divided into quadrants by drawing a cross with a Sharpie on the outside of the bottom. In addition, a circle was drawn on the inside with a pap pen, creating an outer circular ring that was protected from liquid. In the area of this circular ring, each quadrant was labelled based on the intended sample. Next, a 1:1000 collagen solution was prepared by mixing 1 µl of type I collagen concentrate with 999 µl of phosphate buffered saline (PBS). From this solution, 100 µl were taken and mixed with 900 µl PBS to obtain a 1:10000 collagen solution. 1 µl of both collagen dilutions, 1 µl of collagen concentrate and 1 µl of the PBS

negative control sample were added to each quadrant. The protein ligand fluids and PBS were allowed to air dry for 15 minutes. Subsequently, the binding sites of the Petri dish surface not coated with the samples were blocked by adding 200 µl of a 1 % bovine serum albumin (BSA)/PBS solution to the centre of the painted cross and spreading it evenly over all quadrants by gently tapping and shaking the Petri dish. The prepared Petri dish was incubated at 37 °C for 60 minutes in a cell incubator.

In the second step of the experiment, bmMSCs were prepared. First, their viability was examined under a microscope and after the cells were found to be healthy, the growth medium was removed and they were rinsed with 10 ml PBS to remove dead cell material. Since the cells were adherent to the vessel wall after incubation, 3 ml of Accutase was added for their gentle proteolysis and the mixture was incubated for 5 minutes at 37 °C in a humidified incubator. A microscope was used to check whether the cells had detached after this time and, if necessary, it was gently tapped against the vessel to loosen them. Proteolysis was stopped by adding 3 ml of growth medium containing 5-10 % plasma. From the cell suspension, 50 ml was placed in a centrifugation tube, the liquid was centrifuged at 4 °C 1500 rpm for 7 minutes and the supernatant was removed after washing with Dulbecco's Modified Eagle's Medium (DMEM). Cells were resuspended in 10 ml medium and centrifuged again at the same settings as before.

In the third step, the cells were placed on the prepared Petri dish for attachment. First, the supernatant was removed from the cells and the Petri dish was rinsed three times with PBS eliminating unbound BSA/PBS. The around 1 Mio. cells were resuspended with 200 µl of the already prepared attachment medium (CAM), which consisted of 4.45 ml DMEM-LG, 500 µl 1 % BSA and 50 µl ion mix containing 1mM CaCl₂, 1 mM MgCl₂ and 0.025 mM MnCl₂. For good mixing of the settled cells with the CAM, the mixture was carefully aspirated and drained several times using the pipette. 200 µl of this mixture was centred on the cross of the Petri dish and gently tapped against the dish so that all quadrants were covered with the cell suspension. The Petri dish was placed in the cell culture incubator for 15 minutes at 37 °C. After the incubation period, it was rinsed three times with PBS to remove all non-adherent cells. Cell density in the negative control area and collagen dilution spots in each of the four quadrants were examined using a microscope in darkfield mode and images were taken for evaluation.

3 Results

Inspection of the MSCs under the microscope prior to their preparation showed that the cells appeared alive and healthy and were therefore suitable for the experiment (see figure 1A).

The resulting microscope images, of the collagen solution spots respectively from each quadrant of the Petri dish can be seen in figures 1B-1E.

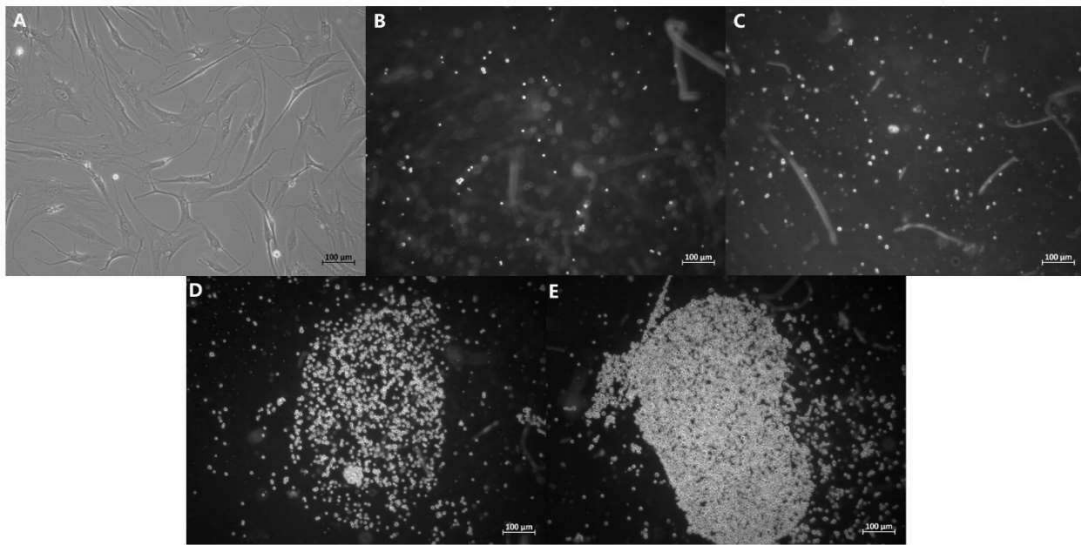


Figure 1: Microscope images of the bmMSCs before preparation (A) and spots of the negative control (B), the 1:10,000 collagen dilution (C), the 1:1,000 collagen dilution (D) and the collagen concentrate (E) after addition of the cells (violet) and subsequent washing.

Figure 1B shows the microscopy image of the negative control PBS spot. There are scattered cells (violet) across the image, but no attachment of these to a specific location can be observed. In comparison, in figure 1C, which shows the spot of the 1:10,000 collagen dilution, a slightly higher cell density of bmMSCs can be seen in the centre in addition to the individual cells distributed across the whole image. A clear difference in cell migration compared to the first two images can be seen in figure 1D. This shows the circular spot of 1:1,000 collagen dilution, where an increased number of cells are accumulated. However, in some cases, larger gaps without cells are visible. Here, as in the images before, single cells can be seen at the edge of the image. The microscope image showing the spot of the collagen concentrate is shown in figure 1E. Here, an almost gapless collection of cells is visible in the area of the oval spot. In contrast to the spot in figure 1D, a clear boundary between areas of high cell density and low cell density can be observed. Around the area of the spot, a slightly higher cell density is found than at the edge of the image.

4 Discussion

The experiment showed that adhesion of the MSCs to the type I collagen occurred, as a higher proportion of collagen in the spots led to higher cell concentrations in these areas. This can be explained by the integrin receptors of the cells that bound to the collagen ligands. The higher the proportion of collagen, the more attachment sites there were for the integrins, allowing more cells to adhere to the substratum. In the image of the negative control PBS spot, there was no accumulation of cells, so non-specific binding can be excluded. Thus, these results are in agreement with those of Warstat et al. that increasing collagen concentrations promotes cell migration [1]. In addition to the accumulation of cells in the areas of the substrate spots, cells were also found in areas of the Petri dish where no collagen was present. The reason for this is thought to be insufficient rinsing of the Petri dish with PBS at the end of the experiment, resulting in cells remaining on the Petri dish. Furthermore, measurement errors and contamination may have occurred during preparation, which could have led to falsification of the results. It is assumed that the attachment of the cells was favoured by activation factors such as the calcium contained in the ion mix, since this led to the activation of the MSCs and the expression of the integrins [3]. In contrast to Warstat et al., the effect of activation factors like calcium and TGF- β was not studied, but this could be investigated in further experiments [1] [3]. Based on these results, it is suggested that type I collagen may be suitable as a carrier material for the incorporation of MSCs into ECMs, although further studies, especially in vivo, are needed to confirm the integration of MSCs through the collagen carrier material.

5 References

- [1] K. Warstat et al., "TGF- β Enhances the Integrin $\alpha 2\beta 1$ -Mediated Attachment," *STEM CELLS AND DEVELOPMENT* 19(5), 2010.
- [2] J. Maerz 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* 7(29), 2016.
- [3] S. Tharmalingam et al., "The Calcium-Sensing Receptor and Integrins in Cellular Differentiation and Migration," *Frontiers in Physiology*, 2016.