Influences of Extracellular Matrix Properties and Flow Shear Stresses on Stem Cell Shape in a Three-Dimensional Dynamic Environment

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Abstract— **Collagen and ceramic matrices are extracellular scaffolds frequently used for bone tissue engineering. Human mesenchymal stem cells from adipose tissue with their unique properties for multipotent differentiation were cultured on a microporous collagen scaffold (Matriderm) and a macroporous ceramic scaffold (Sponceram) under static culture and in a dynamic flow environment in a laminar flow bioreactor. Both matrices differed in respect to cellular distribution after seeding which depended on pore size of the matrix. Cultivation under dynamic flow conditions influenced cellular morphology, collagen fiber alignment and extracellular matrix deposition compared to static culture. The flow rate had an effect on the amount of extracellular matrix formed by mesenchymal stem cells on the ceramic matrix. Further studies are necessary to reveal the ideal matrix and environmental culture conditions for bone tissue engineering.**

Keywords— **laminar flow, bioreactor, fluid shear stress, stem cell, bone tissue engineering.**

I. Introduction

The intercellular substance of bone tissue consists to 50% of minerals, to 25% of organic materials, here mainly collagen (90-95%) and to 25% of hydration water. Ceramic and collagen scaffolds are frequently used as biomaterials together with mesenchymal stem cells to imitate extracellular matrix and initiate osteogenesis for bone tissue engineering. Stem cell shape is influenced by extracellular matrix properties and external stresses (1). Recent studies have shown that exposure to fluid stress can induce osteogenic differentiation of mesenchymal stem cells (2,3). We examined a microporous collagen scaffold and a macroporous ceramic scaffold in respect to the influence of matrix stability, matrix porosity, matrix material properties and fluid shear stresses on stem cell shape of primary human mesenchymal stem cells inside a laminar flow bioreactor.

II. Material and methods

Stem cell isolation and culture: Human mesenchymal stem cells were isolated from abdominal fat of patients undergoing abdominoplasty after obtaining informed consent and approval from the Ethics committee of Hannover Medical School. Fat pads were dissected, digested in collagenase solution and separated by centrifugation according to standard protocols (4). Stem cells were plated on T150 culture flasks and cultured in DMEM-F12 medium (PAA Laboratories, U.S.) supplemented with 5% fetal calf serum, non-essential amino acids, sodium pyruvate and penicillin-streptomycin at 37°C and 5% CO_2. Multipotency of the isolated stem cells was verified by differentiation into the adipogenic, chondrogenic and osteogenic lineage and demonstration of common stem cell surface markers by FACS analysis.

Biomaterials: Sponceram discs (Zellwerk GmbH, Germany) made from zirconium dioxide with a medium pore size of 400-600µm were autoclaved and rinsed in PBS solution before use. Matriderm sterilization (Suwelack, Germany) made from a collagen-elastin matrix with a medium pore size between 20-150µm was obtained as an 1cm thick block and cylindrical discs of 10mm diameter were cut out by a blade after gas sterilization.

Cell-matrix seeding and cultivation: Stem cells of 2nd and 3rd passage were used for experiments. Briefly, cells were rinsed with PBS without magnesium and calcium addition and were detached from culture plates with 0,1% trypsin-EDTA solution. Cells were centrifuged at 300 rpm and suspended in standard culture medium. Cells were seeded onto Sponceram discs at a concentration of 2-6*10^6/disc and onto and into the Matriderm matrices by means of a syringe at a concentration of 4-10*10^6/cylinder. Controls were cultured in 6 well plates under static conditions in a standard incubator (37°C, 5% CO2).

Bioreactor cultivation: For three-dimensional dynamic culturing cell-seeded matrices were inserted within a laminar flow bioreactor (5,6,7). The bioreactor allows flow control through a differential pressure control system with a baseline set at 5 mbar and an integrated bypass system, which can release pressure built up during the cellular growth within the matrix pores. Cultivation was done within an incubator at 37°C, standard culture medium was supplemented with HEPES buffer solution at a concentration of 0.5mM in order to maintain pH balance. Perfusion was done via a roller pump (Ismatec, Germany) at a pump rate of 0.2 to 5ml/min which equivalates a flow velocity of 20 mm/sec to 500mm/sec. when corrected to the open diameter area. Culture period covered 3 days up to 2 months.

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Analysis: Samples were analysed by reflected-light microscopy (Olympus, Germany) using Giemsa staining and life-dead-assay (Invitrogen, US). Matriderm was embedded in paraformaldehyde, processed into sections via a microtome and stained with Hematoxylin and Eosin and Alzarin Red staining. Cell morphology and extracellular matrix deposition was evaluated by raster electron microscopy (REM) (Zeiss, Oberkochen, Germany). For REM analysis, samples were fixated in 5% glutaraldehyde in cacodylate buffer solution and freeze-dried before being sputtered with gold labeling.

III. Results

Matrix porosity affects cell distribution: Microporous collagen matrices (Matriderm) demonstrated an inhomogenous cell distribution when cultured under static as well as dynamic flow conditions. Cross-sections showed cell clusters packed within the collagen fibrous network.

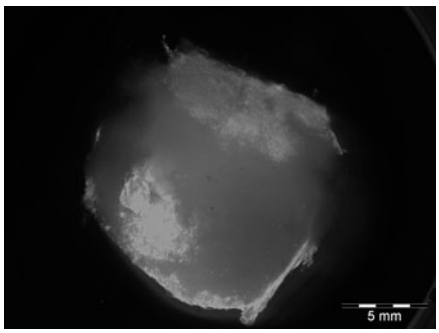

Fig. 1 Inhomogenous cell distribution within microporous collagen scaffold (Matriderm) cultured for 3days under static conditions (fluorescence viability staining)

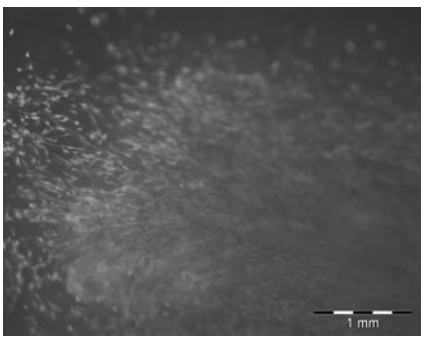

Fig. 2 Bioreactor culture of Matridem for 10 days shows cell alignment in flow direction but no homogenous cell distribution (fluorescence viability staining).

In contrast, macroporous ceramic scaffolds revealed a homogenous cell distribution within the matrix after seeding and cultivation under static conditions or low flow dynamic culture conditions. When using high flow rates in the bioreactor, some cell clustering was observed after a 10 day culture period.

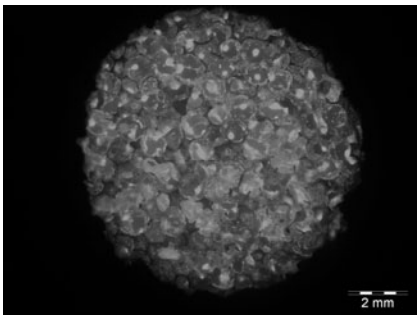

Fig. 3 Sponceram matrix seeded with stem cells and cultured for 3 days under static conditions (fluorescence viability staining)

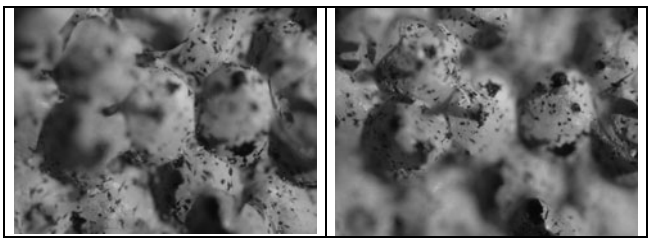

Fig. 4 Homogenous cell distribution in Sponceram matrix after a cultivation period of 10 days under low flow conditions (Giemsa staining)

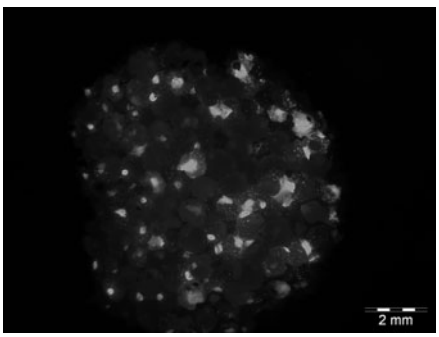

Fig. 5 High flow exposure within the bioreactor leads to cells clusters which are located within the pores after 10 days of culture (fluorescence viability staining)

Fluid shear stress affects collagen fiber organization along the fluid stream line when cultured in the bioreactor, but not under static culture conditions: Cross-sections of Matriderm scaffolds demonstrated alignment of cells within a fibrous network along the fluid stream line, whereas collagen scaffolds cultured under static culture condition showed a less organized matrix.

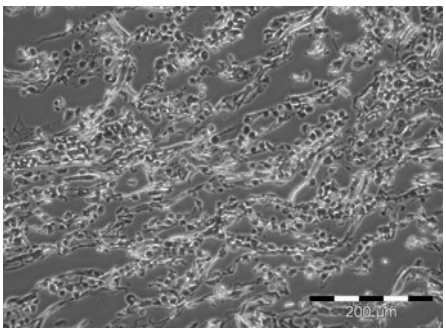

Fig. 6 Cell and collagen fiber alignment along flow direction in Matriderm cultured in the bioreactor for 2 months (Alzarin Red staining)

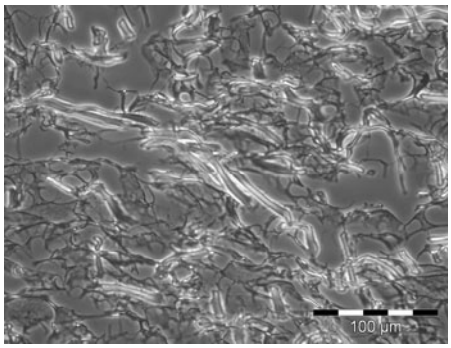

Fig. 7 Unorganized arrangement of collagen fibers in plain Matriderm without cells (Alzarin Red staining)

Cell morphology in macro-porous ceramic scaffolds differ between static and dynamic culture conditions: Morphology of stem cells cultured under static culture conditions showed long cell protrusions and extended cell bodies spanning between pore walls. Stem cell morphology differed from control fibroblast cultures under static conditions. Scaffolds cultured in the bioreactor showed a more flat cell body with radiating protrusions.

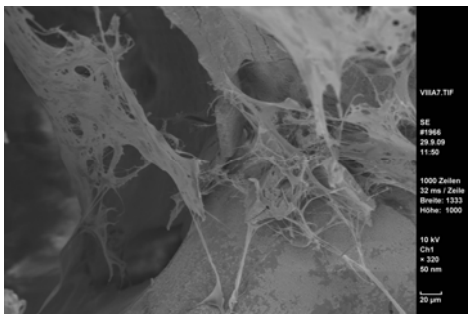

Fig. 8 Thin and dendritical shape of mesenchymal stem cells cultured on Sponceram under static culture conditions (REM)

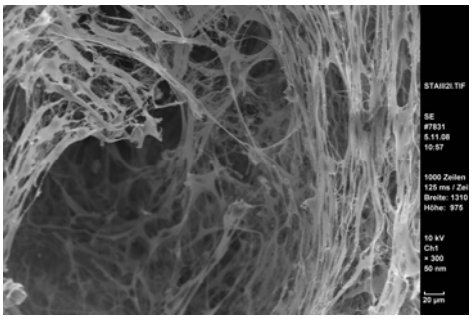

Fig. 9 Dense fibroblast morphology during static growth on Sponceram (REM)

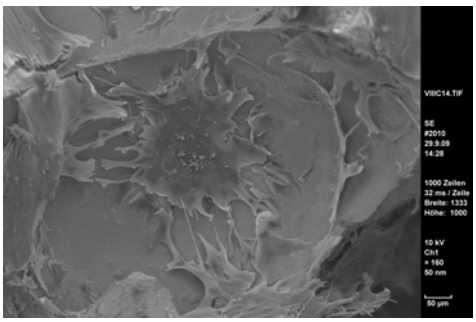

Fig. 10 Flat and star-like morphology of stem cells grown on Sponceram under dynamic flow exposure (REM)

Cell morphology and extracellular matrix formation depend on flow exposure and flow rate: Mesenchymal stem cells cultured onto macroporous ceramic scaffolds (Sponceram) formed extracellular matrix when cultured under dynamic flow conditions. The flow rate affects the amount of extracellular matrix (ECM) deposited by the cells, where the highest and densest amount of ECM was found under high flow rates.

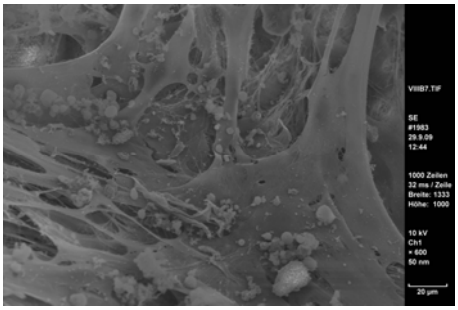

Fig. 11 ECM deposition by stem cells grown on Sponceram under low flow conditions cultured for 3 weeks in the bioreactor (REM)

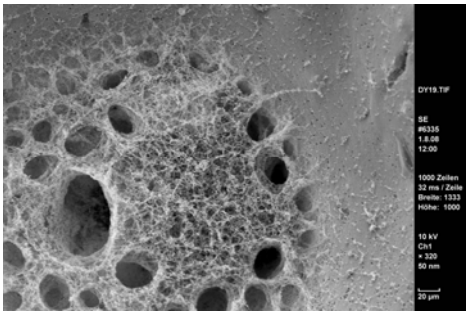

Fig. 12 Fibrous-like ECM formed after 3 weeks of stem cell cultivation on Sponceram under high flow condition in the bioreactor (REM)

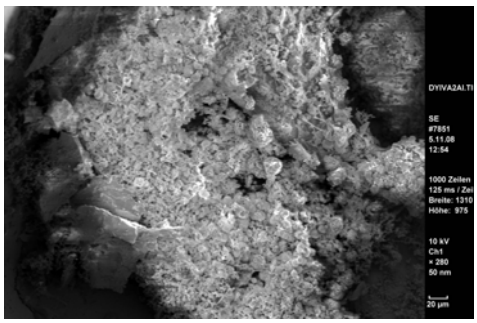

Fig. 13 Dense ECM deposition by stem cells on Sponceram after 6 weeks of high flow culture in the bioreactor (REM)

IV. Discussion

Extracellular matrix properties and external stresses can influence stem cell behaviour (1,7). Our study demonstrates that the two main components of bone extracellular matrix, mineral (here ceramic) and collagen, affect cell morphology, cell growth and cell differentiation diversely due to their unique properties. Recent studies have demonstrated that flow shear stress can induce osteogenic gene markers in mesenchymal stem cells and can induce osteogenic differentiation (2,8). Since high shear stresses can be detrimental to cells and can led to cell death, further studies have to reveal the control pathway of mechanical activation of gene expression within these cells in order to correlate cellular shape and extracellular matrix deposition to the functional state of the cells. Our results may add some information to learn about the optimal external and internal cellular forces required for ostegenic cell stimulation. The ideal biomaterial as well as the best culture environment for bone tissue engineering has still to be defined.

V. Conclusion

Cell shape of primary human adipogenous stem cells is affected by matrix architecture, matrix properties and externally applied flow shear stresses. Further evaluations such as gene expression studies are necessary in order to correlate differences of stem cell shape to their functional state.

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