Effect of Extracellular Matrix Elasticity Upon Stem Cell Differentiation

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Introduction

Hair-follicle-bulge-derived stem cells (HFBSCs) provide a potential source of spiral ganglion neurons (SGN) to treat sensorineural hearing loss, but are new in the field of inner ear regenerative therapy[1]. To induce differentiation of stem cells into appropriate cell types after intracochlear implantation, insight into the factors dictating their fate is essential. Stem cell fate is regulated by the complex interaction between soluble factors and extracellular matrix. Reports show that high-elasticity matrices (mimicking soft brain matrix) induce neurogenic lineage commitment, whereas low-elasticity matrices (mimicking rigid bone matrix) lead to osteogenic differentiation[2,3].

Objective: To investigate the role of soluble factors and extracellular matrix on differentiation of HFBSCs.

Conclusion

Our experiments confirm that elasticity of the extracellular matrix dictates cellular morphology, the types of cellular contact and, to a lesser degree, cellular immunophenotype. In general, the neural crest marker nestin and the glial markers S100b and SOX9 were more prominently expressed in cells cultured on high-elasticity hydrogel-based matrices. After 14 days of culturing on PuraMatrix™ HFBSCs differentiated into cells with an immunophenotype corresponding to mature glial cells. In contrast, HFBSCs differentiated into alkaline phosphatase-producing osteoblast-like cells when cultured on a low-elasticity, collagen-based matrix. We therefore recommend encapsulation of stem cells within high-elasticity hydrogels, prior to cochlear grafting and subsequent SGN repair.

Experimental Design

Specimens: Hair follicles were dissected from the whisker pads of surplus C57BL/6 mice (n=6) obtained from the LUMC Central Animal Facility. Their use was approved by the LUMC Animal Experiments Committee (DEC permit 10172).

Cultures: Hair follicle bulges (HFBs) were isolated from mouse whisker pads according to a protocol previously described by Sieber-Blum et al.[4].

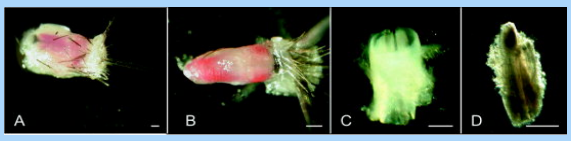

Figure 1. ***A:*** Hair follicles were dissected out of the whisker pads. ***B:*** The follicle was transected below and above the bulge region. ***C:*** A longitudinal incision was made in the capsule. ***D:*** The bulge was rolled out of the capsule. Scale bar: 100 µm.

HFBs were transferred to 12-well cell culture plates pre-coated with 0.15% PDL (Sigma-Aldrich, USA). A single HFB was placed in each well and plates were incubated for 1 hour. Subsequently, 750 µl of freshly-prepared basal growth medium (BGM) was added and the cultures were left in the incubator for 72 hours. HFBs were removed on the third day after outgrowth of cells. Cells were cultured until subconfluency, passaged in PDL-coated dishes and expanded until a yield of >1x10^6 stem cells.

Cryopreservation: 1x10^6 stem cells in 1 ml 10% DMSO in FBS.

Differentiation: Stem cells were thawed and plated at ~10^3 cells/cm^2. Cells were cultured on a low-elasticity matrix (0.01% collagen I) or a high-elasticity matrix (0.15% PuraMatrix™ hydrogel) using four different differentiation media: basal growth medium (BGM), α-MEM with chicken embryo extract (α-MEMC), stem cell medium according to Engler et al.[2] (SCME), and osteogenic medium (OM).

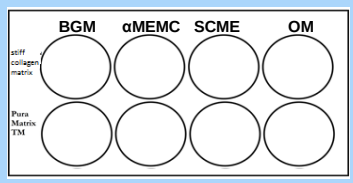

Figure 2. 12-wells plate with either collagen or PuraMatrix™ coating and four different media

Immunostaining: After 8 or 14 days of culture, the cells were fixed and immunostained for nestin (neural crest marker), SOX9 and S100b (immature glial cell markers), and Krox20 and laminin (mature glial cell markers). Digital images were taken with an Olympus IX70 inverted microscope and signal-to-noise ratios (S/N) were determined using LAS AF software. S/N>2 was considered positive.

Alkaline phosphatase: Osteogenic differentiation was established by alkaline phosphatase staining.

Composition of Media

BGM

DMEM/F12	Biochrom AG	
Glutamax	Gibco	1%
Antibiotic-antimycotic solution	Sigma-Aldrich	5%
Fetal bovine serum		10%
B27 (without vitamin A)	Gibco	2%
N2max	R&D Systems	1%
Fibroblast Growth Factor	R&D Systems	20 ng/ml
Epidermal Growth Factor	R&D Systems	20 ng/ml

α-MEMC

α-MEM	Biochrom AG	
Glutamax	Gibco	1%
Antibiotic-antimycotic solution	Sigma-Aldrich	10 µl/ml
Fetal bovine serum		10%
Chicken embryo extract	Seralab	5%

SCME

DMEM/F12	Biochrom AG	
Glutamax	Gibco	1%
Antibiotic-antimycotic solution	Sigma-Aldrich	5%
Fetal bovine serum		10%
B27 (without vitamin A)	Gibco	2%
N2max	R&D Systems	1%
Fibroblast Growth Factor	R&D Systems	20 ng/ml
Epidermal Growth Factor	R&D Systems	20 ng/ml

OM

α-MEM	Biochrom AG	
Glutamax	Gibco	1%
Antibiotic-antimycotic solution	Sigma-Aldrich	10 µl/ml
Fetal bovine serum		10%
Ascorbic acid	Sigma-Aldrich	50 µg/ml
BMP-6	R&D systems	100 ng/ml

Results

PuraMatrix™ High-Elasticity Matrix

Morphology

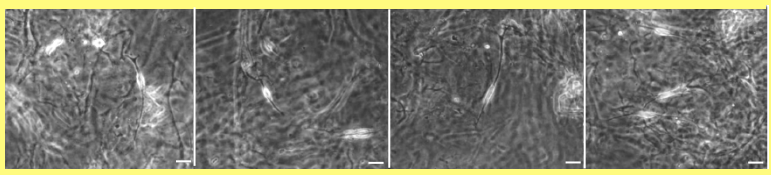

Figure 3. HFBSCs cultured on PuraMatrix™. After 8 days, most cells had a bipolar appearance with long projections and oval-shaped soma. Cells preferentially formed networks. Scale bar**:** 10 µm.

Collagen Low-Elasticity Matrix

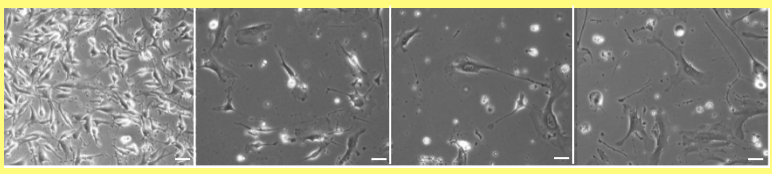

Figure 4. HFBSCs cultured on collagen. After 8 days cells demonstrated a multipolar appearance with short projections and flattened and large soma. Scale bar: 10 µm.

Immunostaining

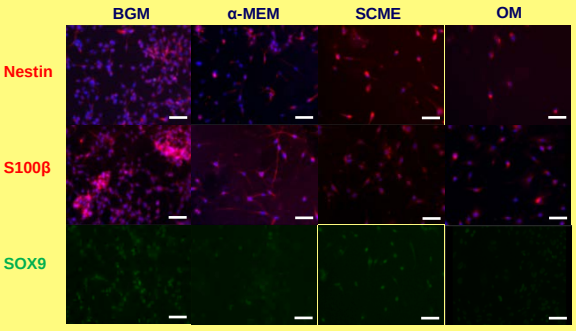

Figure 5. HFBSCs cultured on PuraMatrix™ differentiated into nestin-, S100β- and SOX9-positive cells (S/N>2). SOX9 staining was weak in α-MEM and OM (S/N=2). Nuclei are stained blue (DAPI). Scale bar: 20 µm.

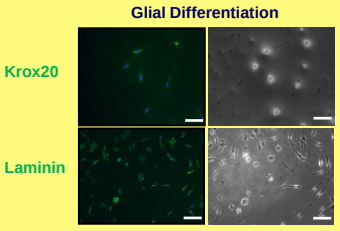

Figure 6. HFBSCs cultured on PuraMatrix™ in BGM without FBS differentiated into Krox20- and laminin-positive cells (S/N>2), suggesting glial differentiation. Nuclei are stained blue with DAPI. Scale bar: 20 µm.

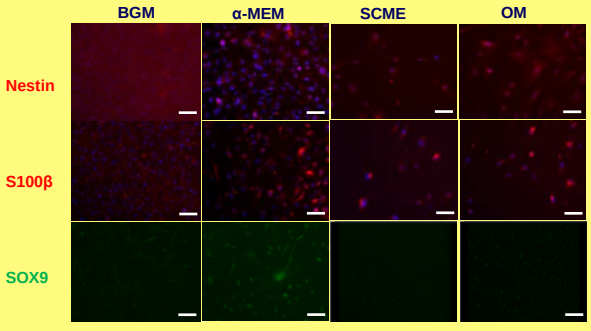

Figure 7. HFBSCs cultured on collagen differentiated into cells that were weakly positive for nestin (S/N≤2). Cells in BGM stained positive for S100β. SOX9 staining was positive in α-MEM. DAPI-stained nuclei are blue. Scale bar: 20 µm.

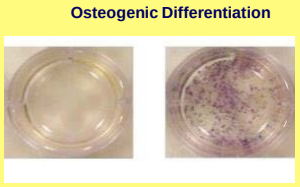

Figure 8. HFBSCs were cultured for 14 days in wells coated with low-elasticity matrix (collagen) with (right panel) or without (left panel) BMP-6. Alkaline phosphatase staining showed that HFBSCs can differentiate into osteoblast-like cells in the presence of BMP-6.

Acknowledgements

The authors wish to thank Fleur ten Tije, Simon de Groot and Ierry-Ann Lourens for technical support.

References

[1] Huisman MA, Rivolta MN (2012) Neural crest stem cells and their potential application in a therapy for deafness. *Front Biosci (Schol Ed)* 4:121-132.

[2] Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126: 677-689.

[3] Even-Ram S, Artym V, Yamada KM (2006) Matrix control of stem cell fate. *Cell* 126: 645-647.

[4] Sieber-Blum M, Grim M (2004) The adult hair follicle: Cradle for pluripotent neural crest stem cells. *Birth Defects Res* (Part C) 72:162-172.