Purification and Neuroglial Differentiation of Multipotent Hair-Follicle-Bulge-Derived Stem Cells

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Introduction

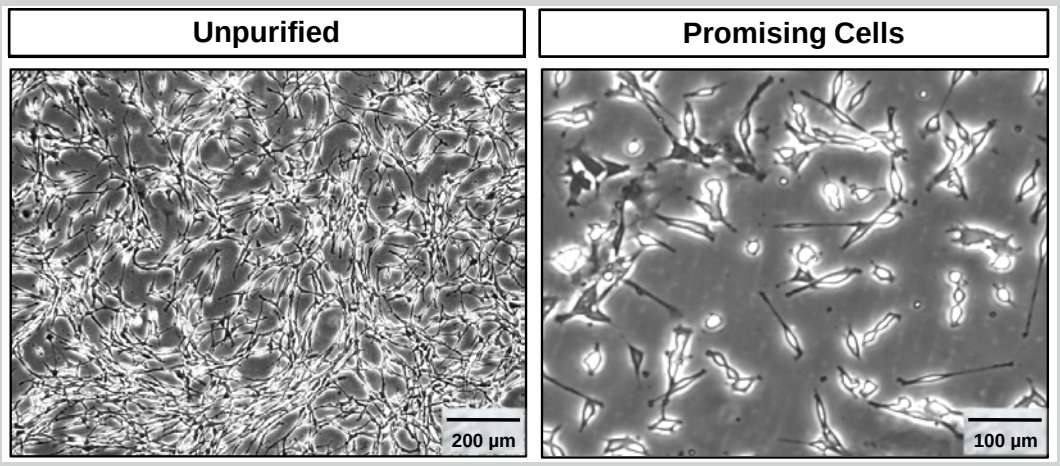
The advantage of adult tissue as a source for stem cells is that large numbers of autologous stem cells can be acquired. However, stem cell cultures from tissues are often contaminated by fibroblasts, and this will obscure the actual number of effective stem cells. Furthermore, these contaminating cells may induce inadvertent formation of extracellular matrix after engraftment, which can have potentially dangerous consequences[1]. For this reason, it is essential to eliminate fibroblasts from tissue-derived stem cells prior to transplantation. We hypothesize that neural crest stem cells derived from the hair follicle bulge (HFBSCs) can be discriminated from non-neuronal cells by their innate sphere-forming capacity[2,3]. In order to test this hypothesis, we have subjected cultures of HFBSCs to the neurosphere-forming assay.

Design of the Experiment

HFBSCs derived from hair follicles were seeded at a density of $1x10^5$ cells/ml unto three different substrates: (1) wells coated with poly-D-lysine (PDL), (2) wells with a PDL-coated cover glass (GC), and (3) wells without a substrate. Two different media were used for the neurosphere-forming assay: (1) stem cell medium (SCM) containing 10% fetal bovine serum (FBS), and (2) serum-free sphere-inducing medium (SIM). Cells that adhered to the bottom and sphere-derived cells were compared with the original population by means of morphological assessment. Sphere-derived stem cells seeded on CGs separated themselves from other cells by migrating underneath the CG. Then, cAMP medium was added to the culture and cells were left for 14 days followed by fixation. The expression patterns of the cells underneath the CG were investigated by means of immunohistochemistry using a selected panel of neural (GAP43) and glial (S100β, GFAP, and MPZ) markers (See also our poster titled *Human dermal fibroblasts demonstrate immunostaining from neuron- and glia-specific proteins*).

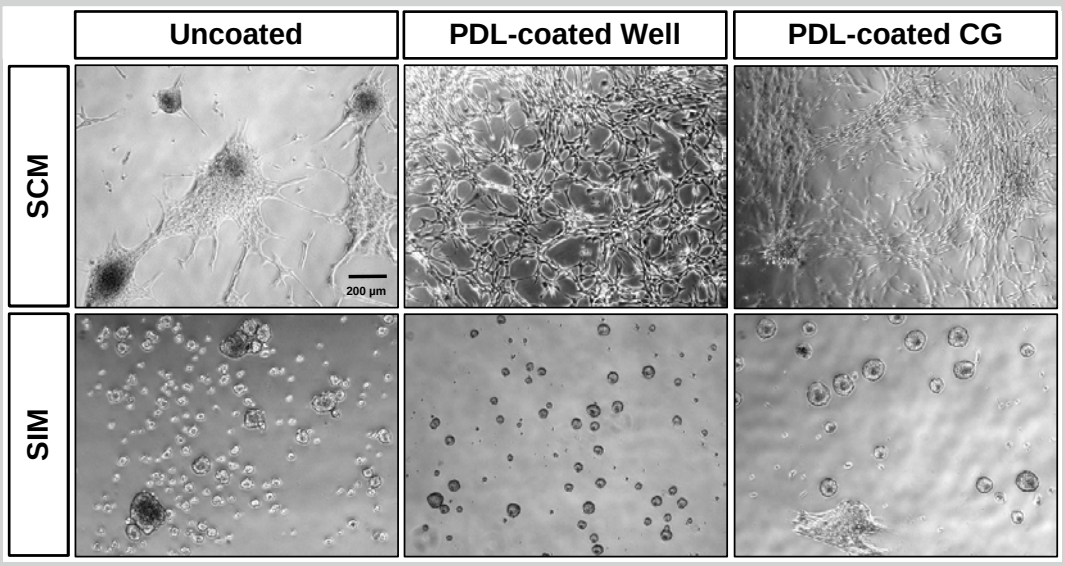
Media	DMEM/F-12	FBS	B27 w/o vitamin A	B27 with vitamin A	N2 plus	EGF	bFGF	NGF	GDNF	BDNF	GlutaMAX	ABAM
Stem Cell Medium (SCM)	+	10%	2%		1%	20 ng/ml	20 ng/ml				1%	1%
Sphere-Inducing Medium (SIM)	+		2%		1%	20 ng/ml	20 ng/ml				1%	1%
cAMP-Containing Medium	+			2%				100 µg/ml	100 µg/ml	100 µg/ml		

1. Cultures Prior to Purification



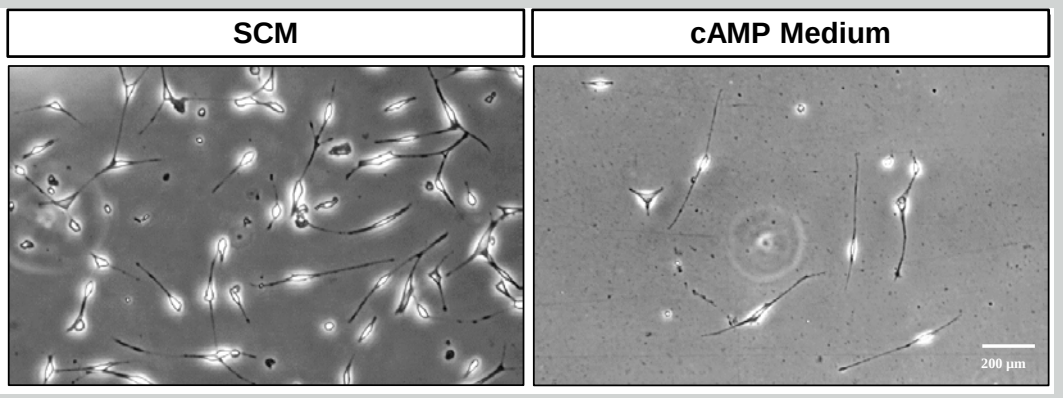
Unpurified cell cultures contain a variety of cell types. HFBSCs (promising cells) can be distinguished upon detailed morphological examination displaying the following characteristics: bipolar, spindle-shaped and slender cells with a compact soma.

2. Sphere Formation under Different Conditions



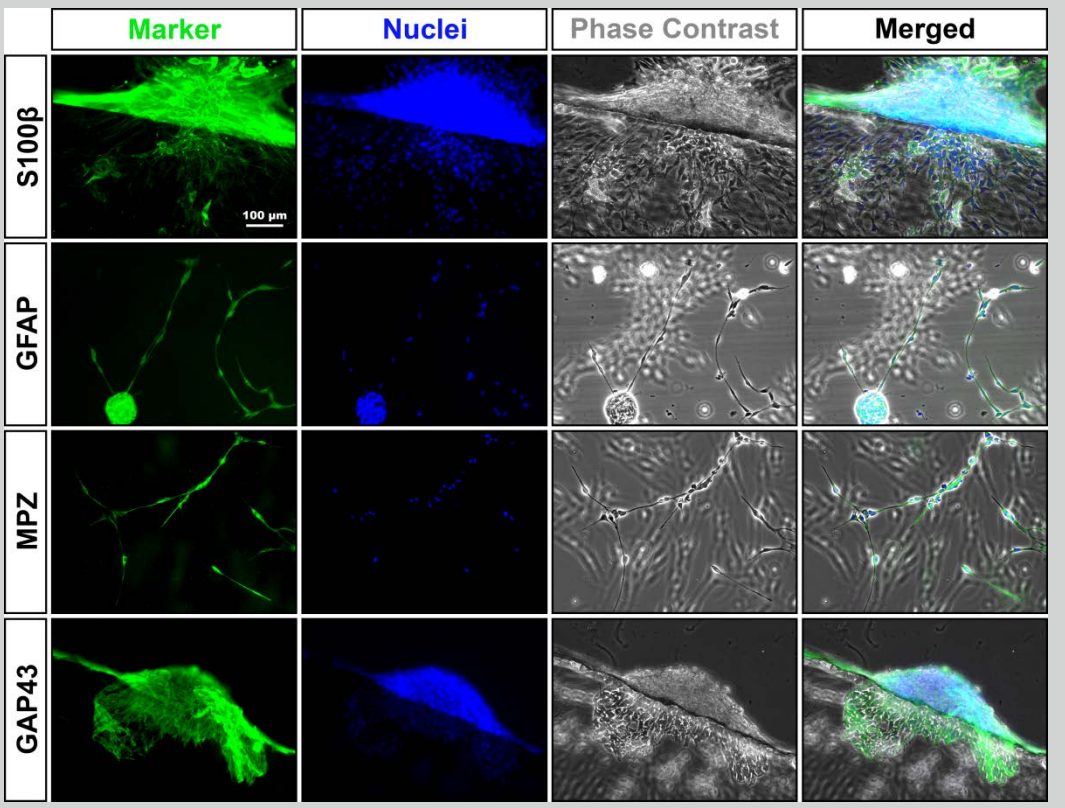
Cells cultured in SCM were all attached to the bottom of the wells, irrespective of the substrates used. When cultured in SIM, all cells in the uncoated wells were afloat and formed floating cell aggregates. When cultured on a PDL-coated substrate in the presence of SIM, spheres were formed and a only a few cells remained attached to the bottom. When cells were cultured on a PDL-coated CG, spheres were formed but many cells also attached to the cover glass.

3. Purified Cells Before and After Differentiation



HFBSCs isolated by means of sphere formation were seeded in SCM for morphological assessment. Subsequently, cells were differentiated using cAMP-containing medium. Cells retained the morphological characteristics of promising cells.

4. Immunohistochemistry of Purified HFBSCs



Spheres, attached to the brim of the CG. Cells detached from the spheres and settled in the shallow space between the bottom and the CG. The cellular outgrowth of the spheres was positive for the glial markers S100β, GFAP, and MPZ and the neural marker GAP43.

References

- Grigoriadis, N., *et al.*, Variable behavior and complications of autologous bone marrow mesenchymal stem cells transplanted in experimental autoimmune encephalomyelitis. *Exp Neurol* 2011; 230(1): 78-89.
- Huisman, M.A. and M.N. Rivolta, Neural crest stem cells and their potential application in a therapy for deafness. *Front Biosci (Schol Ed)* 2012; 4: 121-132.
- Greiner, J.F., *et al.*, Efficient animal-serum free 3D cultivation method for adult human neural crest-derived stem cell therapeutics. *Eur Cells Mater* 2011; 22: 403-419.

Conclusion

We have demonstrated that sphere formation in serum-free medium on PDL-coated cover glasses is a means to purify HFBSCs prior to transplantation. We believe that purification of stem cells is crucial and will contribute to the safety of cell-based therapy, especially with regard to transplantation of cells in delicate areas, such as the brain and the inner ear.