

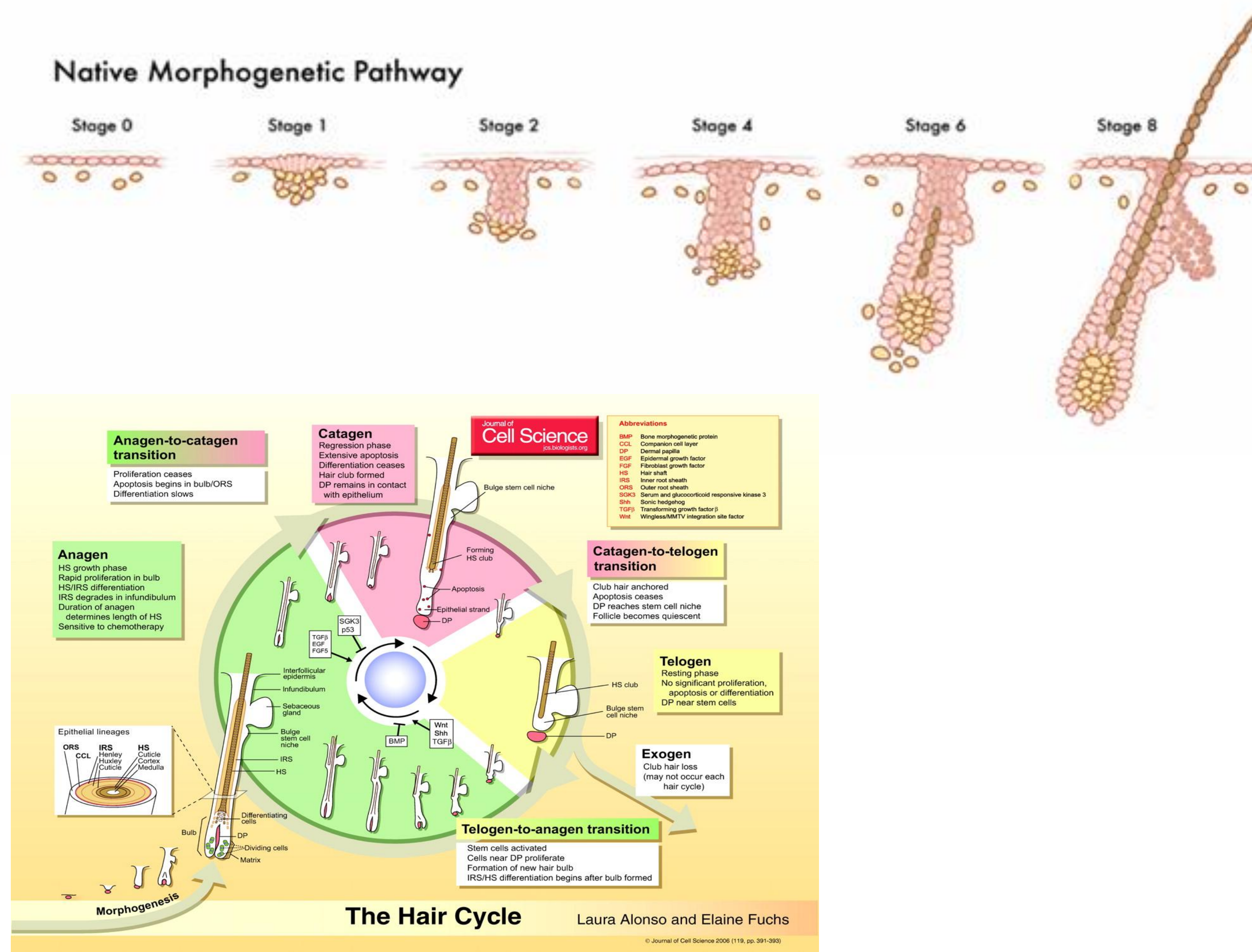
Fibroblasts Derived from Human Scalp are Multipotent and Trichogenic

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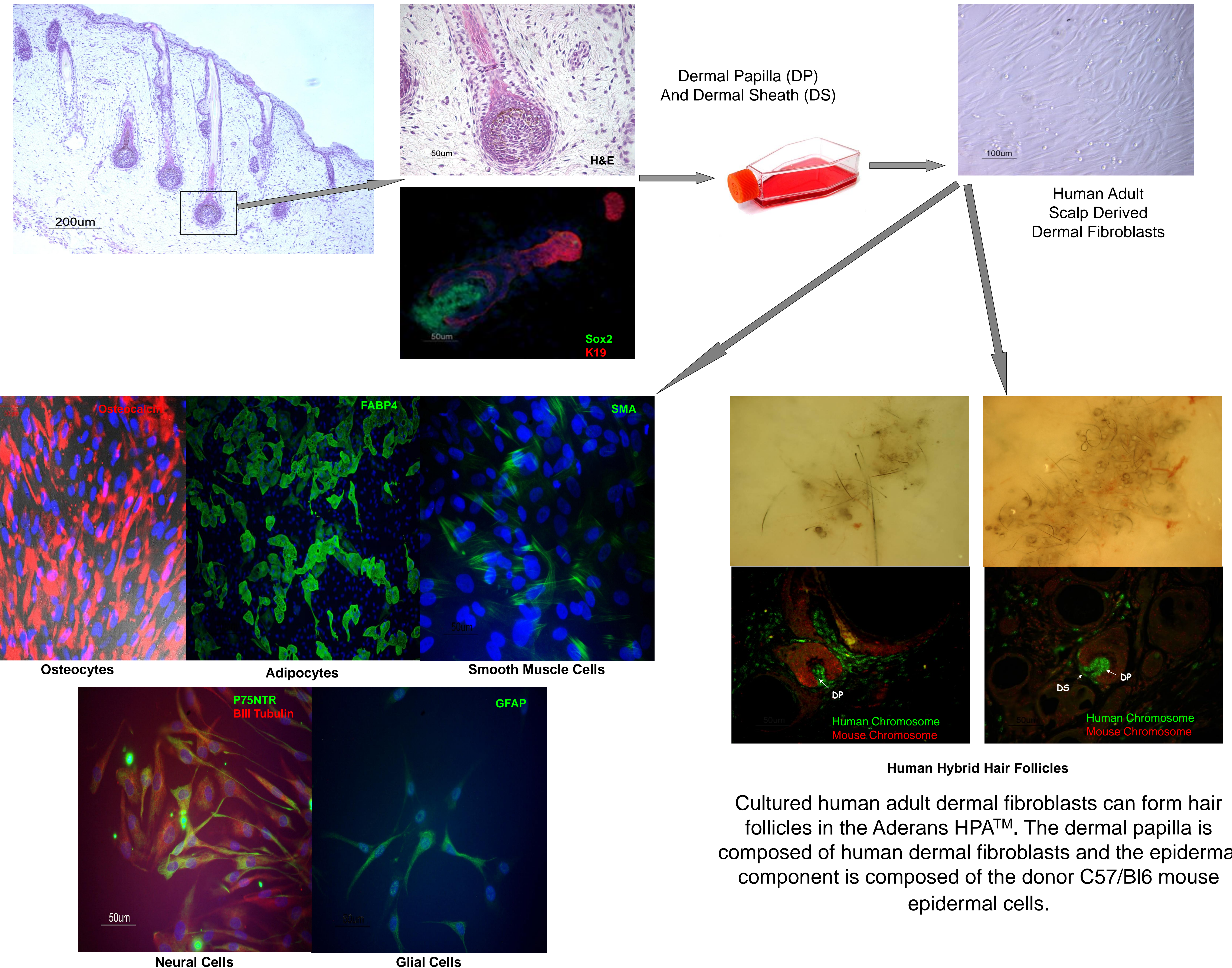
ABSTRACT: The hair follicle is a small but complex mini-organ showing extensive epithelial mesenchymal interactions, organ cycling, and inherent regeneration. It is one of the three current laboratory models - along with the hematopoietic and intestinal systems - for studies of stem cell biology; moreover, because of its cyclic reformation the hair follicle serves as a powerful tool for elucidating the fundamentals of regenerative medicine. Heretofore, because of ease of access and genetics most hair follicle studies have been done with the mouse. Using a mouse model, stem cells have been found and characterized in epithelial and mesenchymal compartments. Under appropriate conditions isolated and dissociated hair follicle-derived cells are capable of regenerating the complete organ system. We are interested in regenerating human hair follicles derived from tissue culture expanded cells for clinical applications. In this study we report the isolation of human adult scalp fibroblasts which have mesenchymal stem cell and trichogenic (ability to produce hair follicles) properties, similar to reported findings using cells derived from mouse truncal skin. Human adult fibroblasts are able to produce hair *in vivo* in a hair formation assay when combined with mouse epidermal cells and are able to differentiate into multiple lineages; adipocytes, neural cells, glial cells, osteocytes, and smooth muscle cells. Human adult fibroblasts also form neurospheres and express Sox2, characteristic of stem cells.

BACKGROUND:

Hair follicles are dynamic organs made up of dermal fibroblasts and epidermal cells. Cross talk between the two cell types is critical for hair follicle formation and cycling. Hair follicles are unique organs containing both a dermal and epidermal stem cell niche. These niches are activated to rebuild the hair follicle after the regression phase (catagen) and resting phase (telogen), resulting in the growth phase (anagen). The epidermal stem cell niche has been localized to the bulge region of the follicle and dermal stem cell niche has been localized to the dermal papilla and dermal sheath region. This study focuses on cells derived from the dermal papilla and sheath regions.



RESULTS:



Cultured human adult dermal fibroblasts can form hair follicles in the Aderans HPA™. The dermal papilla is composed of human dermal fibroblasts and the epidermal component is composed of the donor C57/Bl6 mouse epidermal cells.

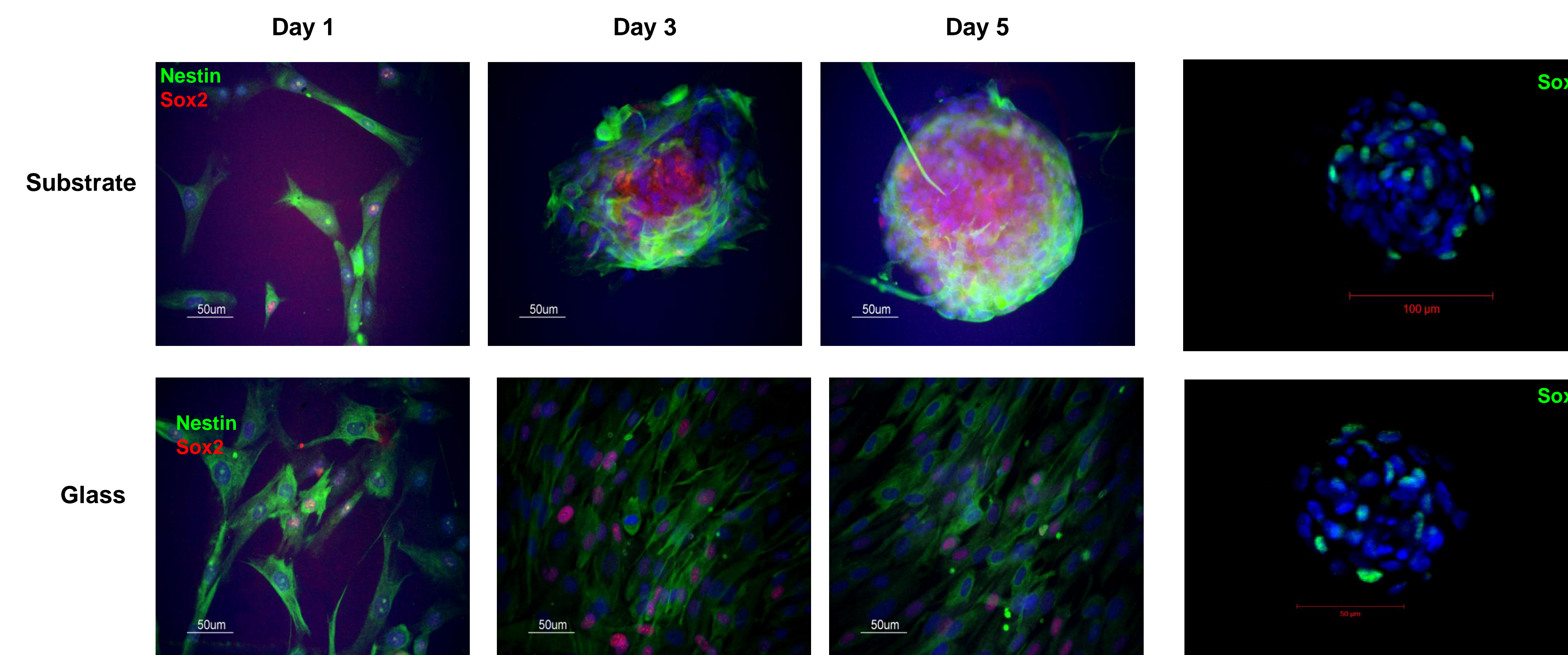
MATERIALS AND METHODS:

Fragments of human occipital full-thickness scalp skin were donated by healthy middle-aged adults undergoing hair restoration procedures following an approved Institutional Review Board protocol. Fibroblasts were isolated by a procedure involving tissue fragmentation, proteolytic enzymes exposure, and tissue culture expansion in a derived medium. Cell differentiation studies were performed using commercial kits: osteocyte and adipocyte (R&D Systems SC-006); smooth muscle cell differentiation (TGF- β induced differentiation); neural differentiation (BDNF, NGF, NT-3, FGF2, Retinoic Acid induced differentiation), and glial differentiation (Neuregulin-1 β and Forskolin induced differentiation). Differentiation of human fibroblasts was determined by immunofluorescent staining; adipocytes (FABP4), neural cells (p75NTR, BIII Tubulin), glial cells (GFAP), osteocytes (osteocalcin), and smooth muscle cells (Smooth Muscle Actin).

Trichogenicity was measured using the Aderans HPA™ (Hair Patch Assay) [Zheng et al. 2005]. Postnatal day 1 C57/Bl6 mouse skins were obtained from (Charles River, Williamston MA) and shipped overnight on ice in dispase. Mouse epidermal cells were isolated according to Zheng et al, 2005. Briefly, the epidermis was removed from the dermis following dispase treatment, and cells dissociated with trypsin. Mouse epidermal cells were combined with human cultured dermal cells and injected into a *nu/nu* mouse.

Neurosphere type structures were formed by culturing passage 1 human adult fibroblasts on substrate coated dishes and stained with Sox2 and Nestin.

Cultured human adult dermal fibroblasts contain stem cells which can differentiate into, osteocytes, adipocytes, smooth muscle cells, neural cells, and glial cells



Human scalp derived adult fibroblasts form neurosphere type structures when cultured on a substrate. Adult fibroblasts express the neuro- precursor marker Nestin, as well as the stem cell marker Sox2.

CONCLUSIONS:

The human scalp contains cells which are naturally multipotent and trichogenic. A population of progenitor cells is available in human skin which has inherent stem cell properties. These properties are present without the need of genetic manipulation.

