**Abstract**

# Process of Harvesting Stem Cells from Hair (Video Lecture)

# 모낭 줄기세포 추출의 실제 (동영상)

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The process of using hair follicle-induced stem cells for regeneration, particularly in the context of hair loss, involves harnessing the remarkable regenerative capacity of various stem cell populations found within the hair follicle. Here's a breakdown of the key aspects.

Process of Harvesting Stem Cells from Hair Follicle

Harvesting stem cells from hair follicles is a complex process primarily conducted in research settings and specialized clinics, as it's not yet a widely approved or standardized clinical procedure for hair regeneration. The goal is to isolate specific stem cell populations, mainly Hair Follicle Stem Cells (HFSCs) from the bulge region and/or Dermal Papilla Cells (DPCs) from the base of the follicle.

Here's a general outline of the process, which can vary depending on the specific cell type being harvested and the research protocol:

I. Obtaining Hair Follicle Tissue:

Donor Site Selection: Typically, hair follicles are harvested from the occipital (back of the head) or temporal (sides of the head) regions of the scalp, as these areas are generally more resistant to hair loss and provide healthy follicles.

Punch Biopsy or Follicular Unit Extraction (FUE):

Punch Biopsy: This is a common method in research. A small circular punch instrument is used to remove a cylindrical sample of skin containing several hair follicles. This is done under local anesthesia. The size of the biopsy can vary (e.g., 2-4 mm).

Follicular Unit Extraction (FUE) or Modified FUE: While traditional FUE extracts entire follicular units for transplantation, modified FUE techniques are being explored where only a portion of the hair follicle (containing stem cells) is harvested, allowing the donor hair to potentially regrow. This approach aims to minimize donor site scarring and hair thinning.

Tissue Processing: The harvested skin sample is immediately placed in a sterile transport medium (e.g., cell culture medium with antibiotics) to maintain cell viability and prevent contamination.

II. Isolation of Specific Stem Cell Populations:

Once the hair follicle tissue is obtained, the next critical step is to separate the desired stem cells from the surrounding tissue. This usually involves a combination of mechanical and enzymatic methods:

Cleaning and Trimming: The skin biopsy is thoroughly rinsed with sterile solutions (e.g., Phosphate Buffered Saline with antibiotics) to remove any contaminants. Excess adipose (fat) and connective tissue surrounding the hair follicles are carefully trimmed away using micro-scissors and forceps under a dissecting microscope. The goal is to expose the individual hair follicles.

Hair Follicle Dissection (for DPCs):

For isolating Dermal Papilla Cells (DPCs), individual hair follicles are often meticulously dissected from the skin tissue.

The hair follicle is secured, and using fine scissors, a transection is made just above the dermal papilla (the bulb-like structure at the very base of the follicle). This isolates the "end bulb" containing the dermal papilla.

The end bulb is then inverted to expose the dermal papilla, which is a small, distinct cluster of cells. The dermal papilla is carefully separated from the surrounding epithelial cells.

Enzymatic Digestion (for HFSCs and general dissociation):

Dispase/Collagenase Digestion: The isolated hair follicles or trimmed skin pieces are subjected to enzymatic digestion. Enzymes like collagenase (e.g., collagenase type I or IV) and dispase are commonly used.

Collagenase breaks down the extracellular matrix, helping to release intact hair follicles from the surrounding dermis or to isolate DPCs.

Dispase is often used to separate the epidermis from the dermis, and sometimes to further dissociate epithelial components.

The tissue is incubated in the enzyme solution at a specific temperature (e.g., 37°C) for a defined period, with occasional agitation.

Secondary Enzymolysis: Some protocols use a two-step enzymatic digestion (e.g., pancreatin substitute followed by collagenase IV) to optimize cell yield and viability.

Mechanical Dissociation: After enzymatic digestion, mechanical methods like gentle pipetting or trituration are used to further break down the tissue into a single-cell suspension.

Filtering: The cell suspension is passed through a cell strainer (e.g., 40-70 µm mesh) to remove any undigested tissue clumps and obtain a uniform single-cell suspension.

Centrifugation: The filtered cell suspension is centrifuged at a low speed to pellet the cells. The supernatant (enzyme solution and debris) is discarded.

Washing: The cell pellet is washed multiple times with sterile buffer (e.g., PBS) to remove residual enzymes and contaminants.

III. Cell Culture and Expansion (Optional but common):

Resuspension: The harvested cells are resuspended in a specialized cell culture medium (e.g., DMEM/F12 supplemented with growth factors like EGF, bFGF, and fetal bovine serum).

Plating: The cell suspension is then plated into tissue culture dishes.

Selective Growth: Different media and culture conditions can selectively promote the growth of specific cell types (e.g., epithelial cells, mesenchymal cells).

Expansion: HFSCs and DPCs can be expanded in vitro to obtain a larger number of cells, which is crucial for therapeutic applications. DPCs are known to be easier to expand than HFSCs.

Cryopreservation: Once sufficient cells are obtained, they can be cryopreserved (frozen) for long-term storage.

IV. Characterization and Purity Assessment:

Microscopic Observation: Cells are regularly observed under a microscope to assess their morphology and growth.

Flow Cytometry (FACS): This technique is used to identify and quantify specific stem cell populations based on the expression of cell surface markers (e.g., KRT15, CD34, CD200 for HFSCs; Alkaline Phosphatase for DPCs). This helps to ensure the purity and identity of the harvested cells.

Immunocytochemistry/Immunofluorescence: Staining for specific markers can also confirm the identity of the cells.