Literature review of 3D-bio printed hair follicles and the proposal for a permanent hair system on the scalp

By

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Bachelor of Engineering, Mahatma Gandhi University, Kottayam, India, 2013

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Abstract

Modern hair restoration surgery helps restore hair loss or bald areas, which requires a substantial number of hair follicles from the donor area. However, in some cases, people do not have sufficient donor hair follicles for transplant surgery due to diseases, genetics, aging, other biological and environmental issues, and so on. This problem can be addressed using 3D bioprinter technology to cultivate artificial hair follicles.

This project report meticulously reviews six different methods for artificially cultivating hair follicles (HF) using bio cells and a cell-transforming environment created using 3D bioprinting technology. The six methods were 3D-bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle regeneration, tissue engineering of human hair follicles using a biomimetic developmental approach, bead-jet printing enabled sparse mesenchymal stem cell patterning augments skeletal muscle and hair follicle regeneration, robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces hair follicle-inclusive skin regeneration, bioprinting of hair follicle germs for hair regenerative medicine, and using bioprinting, and spheroid culture to create a skin model with sweat glands and hair follicles.

The main disadvantages of these experimental methods are their complexity, the significantly low number of hair follicles generated, and the fact that it will take time to get approval for human trials for these new technologies. The report also proposes a procedure to overcome the disadvantages of artificially grown HF by developing a permanent hair system on the scalp.

Keywords: 3D bioprinting of hair follicles, permanent toupee, bioink, biocell, stem cell.

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List of Acronyms

HFG	Hair follicle gram
HFU	Hair follicle-like unit
HF	Hair follicle
HT	Hair transplant
AGA	Androgenetic alopecia
FUT	Follicular unit transplantation
FUE	Follicular unit extraction
FU	Follicular unit
FB	Fibroblast
HUVEC	Human umbilical vein endothelial cell
DPC	Dermal papilla cell
EPC	Epidermal cell
KC	Keratinocyte
MSC	Mesenchymal stem cell
HDB	High cell density bead
LDB	Low cell density bead
HDG	High cell density bulk gel
CTM	Cascade tubing microfluids
EV	Extracellular vesicle
NTA	Nanoparticle tracking analysis
GelMA	Gelatin methacrylate
Epi-SC	Epidermal stem cell
SKP	Skin-derived precursor
ECM	Extracellular matrix
DOF	Degree of freedom
HMG	Hair microgel
gHMG	Guided-hair microgel
PDMS	Polydimethylsiloxane
SG	Sweat gland
PBS	Phosphate-buffered saline

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Firstly, I am expressing my sincere gratitude to **Dr. Yang Shi,** a decent and professional scholar, for accepting me into his research group. Dr. Yang Shi became my mentor during my MEng study and helped me achieve my academic goals. He also gave me many tips that laid the foundation of this thesis. During the project research period, Dr. Shi helped me overcome the obstacles to the project goals.

Finally, I thank my parents, **Mr. Issac Mathew**, **Mrs. Jessy Issac**, and my friend, **Ms. Ekta Bangar**, for their love and support.

1 Background Information

The focus of this project is to analyze six different scientific research papers on cultivating using 3D-bioprinting technology and stem cells and propose a permanent toupee surgical procedure on the human skin with the help of 3D-bioprinting and its application.
My experience of premature hair loss at the age of 26 due to a family history of baldness is the primary motivation for working on this project. It was an emotionally challenging situation that had a consequential impact on one's self-confidence. In this instance, hair transplantation was an available option. So, with the help of hair transplant experts, the hair was transplanted from the donor area (back side of the head) to the bald area (front side of the head). Hair grew on the scalp after six months of the successful transplantation.
After this procedure, I have deliberated on people who do not have donor area hair follicles, such as cancer patients, skin disease patients, fully bald people, and people with less donor area, as it will be difficult or impossible for them to grow natural hair on their scalp. So, this project examines six different methods to produce artificial hair follicles using 3D-bioprinting and stem cells and discusses the best method. Also, the project proposes a technique called permanent toupee surgical procedure to reduce the disadvantages of the hair grafting method.
The six methods to grow hair follicles artificially using 3D-bioprinting are as follows:

I. 3D-bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle Regeneration: 3D-bioprinting uses Fibroblasts (FBs), human umbilical vein endothelial cells (HUVECs), dermal papilla cells (DPCs), and epidermal cells (EPCs) to generate the hair follicle (HF) artificially in a controlled environment created by 3D-bioprinting.
II. Tissue engineering of human hair follicles using a biomimetic developmental approach: A 3D printer created a microwell to create a microenvironment for developing Hair follicles (HF). The dermal papilla cell (DPC) and Keratinocyte (KC) cells were used in this procedure in the hair follicle unit (HFU).
III. Bead-jet printing enabled sparse mesenchymal stem cell patterning to augment skeletal muscle and hair follicle regeneration. In this procedure, a bed jet bioprinter is used to create a hair follicle (HF).
IV. Robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces hair follicle-inclusive skin regeneration: the Epidermal stem cell (Epi-SCs) and Skin-derived precursors (SKPs) cells are inserted into a Gelatin methacrylate (GelMa) bioink to create the required hydrogel, which is then placed in a live experimental body to cultivate hair follicle (HF) using a robotic arm.
V. Bio-printing hair follicle germs for hair regenerative medicine: A 3D bioprinter was used to Combine Mesenchymal stem cells (MSCs) and epithelial cells to create a hair follicle gram (HFG). In the experiment, two types of hydrogels were made: hair microgels (HMGs) and Guided hair microgels (gHMGs).

VI. Using bioprinting and spheroid culture to create a skin model with sweat glands and hair follicles (HF), Fibroblasts (Fbs), Keratinocytes (KCs), and Mesenchymal stem cells (MSCs) were grown in a controlled and designed environment using 3D bioprinting to cultivate hair follicles (HF).

The literature review section (section 2) provides a detailed review of the above methods.
The artificially grown HF process is complex, time-consuming, and costly. Also, the number of hair follicles generated is significantly low, and it will take time to get approval for human trials for these new technologies. Finally, the hair grafting procedure is painful. In the proposal section, a permanent hair system on the scalp is introduced. The permanent hair system eliminates the disadvantage of the hair grafting procedure. Section 3 gives more information about the permanent hair system. The following section provides basic information about hair structure, the reasons and types of hair loss, and the current hair transplantation procedure.

1.1 Hair Structure

Hair follicles are structures within the human skin that grow hair. They are tube or pore-like structures containing a sebaceous gland stimulated by hormones such as androgens and providing lipid-rich sebum that protects the hair and provides a hydrophobic protection layer and arrector pili muscle, helps the hair strand to erect during the cold weather, commonly known as goosebumps. The hair follicle originates from the first and second layers of the skin, such as the epidermis and dermis. Still, the hair follicle-holding part is extended to the third layer of the skin-subcutaneous tissue. The hair has three main segments: the infundibulum, the isthmus, and the lower follicle or inferior segment, which contains the bulb. The infundibulum portion includes the upper section of the hair follicle, beginning from the dermis layer to the sebaceous duct. The isthmus section starts between the sebaceous duct opening and the bulge. The bulge contains the arrector pili muscle and different epidermal stem cells. The inferior segment area begins from the bulge to the base of the hair follicle. The bulb is the core of the hair follicles and contains the follicular matrix surrounding the sides and top of the dermal papilla. This matrix interaction helps to form hair shafts and grow [20][23]. The structure of hair is shown in Figure 1.

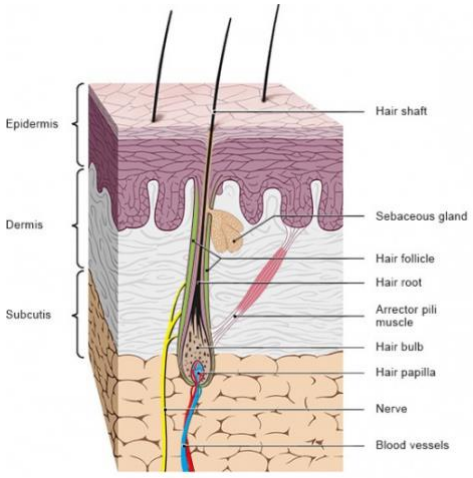

Figure 1: Structure of a hair [20].

1.2 Hair growth cycle

The hair follicle growth cycle contains three phases: the anagen phase, the catagen phase, and the telogen phase [20]. Ninety percent of the total growth time is consumed in the anagen phase, which lasts between two and seven years. Hair growth begins at the root, such as the dermal papilla, which contains blood supply and nutrition for growing hair [19][23]. The catagen phase, also known as the transition or regression phase, is the second and shortest period of the hair growth cycle and may only last several weeks. In this phase, the hair detaches from the blood supply, forming a club hair with a white, complex node on end. The telogen phase is the last stage of the hair cycle, also known as the resting phase. The hair sheds or falls out of your hair follicle so the anagen phase can begin again with new hair [22][19].

1.3 Reason for baldness or hair loss

A healthy average adult can lose about 70 to 100 hairs on their head per day. But because new hair grows, the loss is replaceable and unnoticed. If the hair loss increases and the hair roots are damaged during the growth phase or if many hairs go into the resting phase simultaneously, the balding stage of an adult starts, also known as alopecia. The alopecia is mainly due to drugs, diet, hormone imbalances, altered mitotic activity, growth cycle abnormalities, and some unknown reasons [21][19].
Most baldness in adults is not irreversible, and the most common hair loss treatment practice is the hair transplant surgical procedure [21].

1.4 Types of baldness or hair loss

Male hair loss or baldness, also known as Androgenetic alopecia (AGA), has different levels of baldness, known as the Hamilton-Norwood classification. Figure 2 shows the classification. Female baldness is classified into three: Ludwing pattern, androgenic pattern, and diffuse unpatterned alopecia [20].

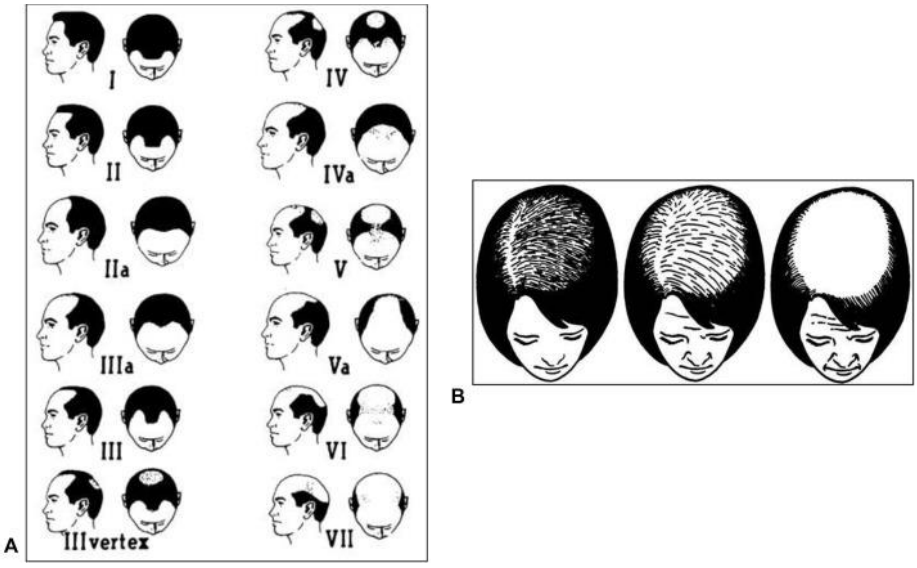

Figure 2: The Norwood and Ludwig scales of hair loss [21].

The figure 2, section A, Male androgenetic alopecia according to the Norwood scale and section B, Female pattern hair loss according to the Ludwig scale. The current methodology for treating baldness or hair loss is to harvest the hair follicle from the donor area and transplant it to the hair loss area. This procedure is commonly known as hair transplantation or hair grafting method.

1.5 Hair transplantation procedure

The current and prominent methodology for treating baldness or hair loss is to harvest the hair follicle from the donor area and transplant it to the hair loss area.
There are four main steps for the hair transplant procedure:

- Anesthesia.
- Donor harvesting techniques.
- Mark the recipient area and transplant hair follicles.
- Postoperative care and follow-up.

1.5.1 Anesthesia

The hair transplantation procedure runs under local anesthesia, which is commonly used with lidocaine and bupivacaine. This step minimizes the pain of harvesting the hair follicles from the donor area using small-calibre syringes (1-3 mL Luer lock), 30-gauge needles, and blunt microcannulas [20].

1.5.2 Donor harvesting technique.

Most hair transplant surgeons harvest naturally occurring hair follicles from the patient's donor area. Two techniques are used to harvest the hair follicle: strip harvesting or follicular unit transplantation (FUT) and follicular unit extraction (FUE) or punch method or punch biopsy method. In this section, the report discusses the donor area specifications and harvesting techniques and reviews these techniques [20].

a) Donor area specifications

The donor area, defined in hair transplant surgery, is the area that harvests healthy hair follicles. Most of the standard donor area lies in the occipital scalp, with an approximate width of 5 to 6 cm (Figure 3). Hair density is another category used to determine the donor area. The recommended hair follicle density is 40 FU/cm^2 (Follicular Unit). The surgeons use a formula to calculate the donor site harvesting area [24].

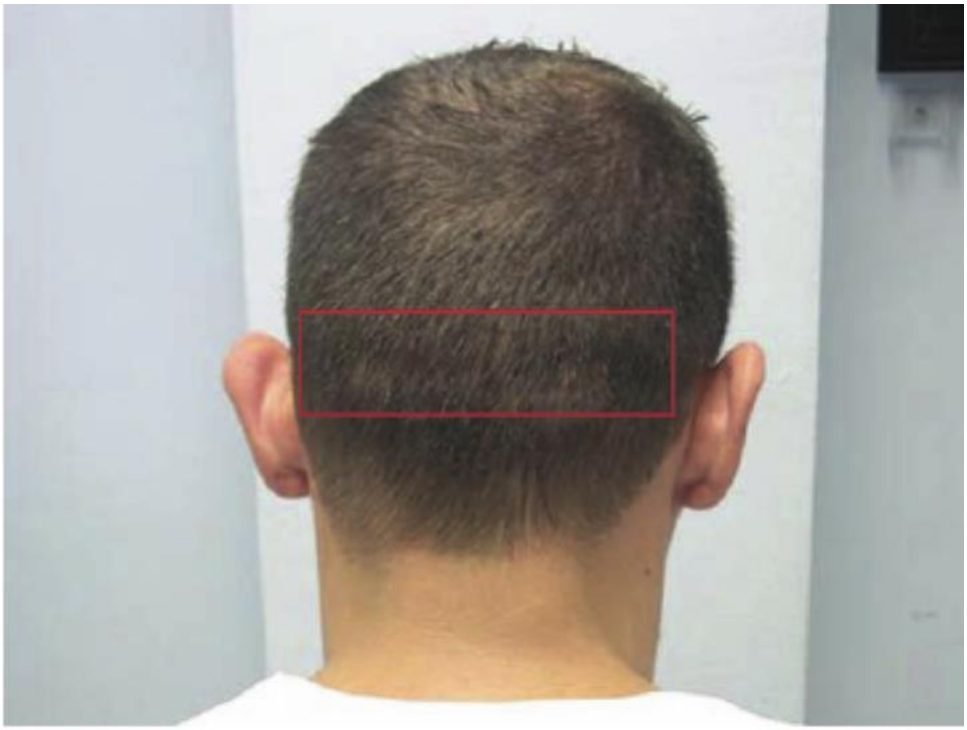

Figure 3: Marking the hair follicle at the donor area [24].

Number of follicular units desired to harvest = Follicular unit density X area of donor strip (cm^2).
Table 1 shows an example: the Follicular unit density is $85FU/cm^2$.
The following paragraph explains the techniques used to harvest hair follicles [24].

#FU	Length of Strip(cm) (Table consider strip width is 1 cm)
500	6
700	8
900	10.6
1000	12
1200	14
1400	16.5
1600	19
1800	21
2000	23.5

Table 1: Length of the donor strip according to the number of follicular units desired to harvest.

b) Follicular unit extraction (FUE) or punch method or punch biopsy method

This method originated in Japan and is currently used in hair transplantation procedures worldwide. It extracts the hair follicles from the donor area individually through tiny punches with approximate punches as 4mm. The described area can be harvested in two ways: by using smaller punches and, second, by finely dissecting the large hair-covered skin cylinders into smaller grafts. Figure 4 shows the Follicular unit extraction (FUE) area [24].

Figure 4: A, preparing the scalp for FUE, Harvesting the HF from the donor area [24].

c) Strip harvesting or follicular unit transplantation (FUT)

Most hair transplantation is based on strip harvesting or follicular unit transplantation (FUT). This is mainly because the success rate of the transplantation is very high compared to Follicular unit extraction. Follicular unit transplantation (FUT) removes a complete hair follicle strip from the donor area, such as the occipitoparietal region of the back of the head (Figure 5). The harvested graft is placed in the direction of growth, and the donor site wound area can be closed with sutures or staples [24].

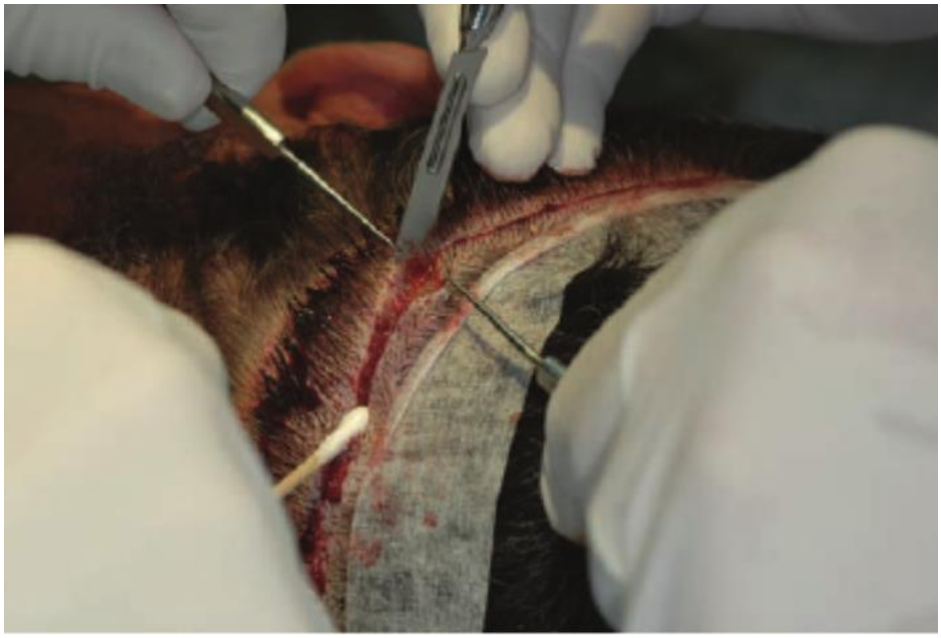

Figure 5: FUT method, Harvesting HF as a strip [24].

d) Review of the harvesting methods

Follicular unit extraction (FUE) advantages

- The method minimizes the trauma to the patients.
- The harvesting procedure is undemanding due to its simplicity (Not a surgical procedure)

Follicular unit extraction (FUE) disadvantages

- The procedure has a risk of several tissue traumas in the donor area if the surgery is not performed well.
- Extensive scarring.
- Low graft yield.
- Graft loss due to iatrogenic destruction of neighbouring hair follicles.
- Graft loss due to iatrogenic destruction of telogen hair follicles.
- Because multiple procedures take place, the tissue can be damaged and irreversible.

Follicular unit transplantation (FUT) advantages

- The cost is less compared to Follicular unit extraction (FUE) due to the rate of hair follicle harvesting.
- The success rate is higher due to the higher number of Follicular units.
- The harvesting phase is shorter compared to FUE.

Follicular unit transplantation (FUT) disadvantage

- The procedure requires several steps and is more prolonged. Due to the above problem, the survival rate of the Follicular unit is lower because of the graft's dehydration.
- Because of the lengthy procedure, storage time exposes the grafts to an increased risk of hypoxic cell damage and necrosis.
- The doner area wound can create extensive scarring [24]

1.5.3 Mark the recipient area and transplant hair follicles

The recipient area needs to be given local anesthesia, and the recipient is marked to match with other hair around the baldness area. The usual transplant rate is 25-45 FU grafts per cm^2 in the recipient area. The transplanted hair follicles are implanted at the same angle and direction as the non-transplanted hair. Second, the single-hair FUs should be positioned in the anterior-most part of the hairline to create a transition zone [20].

1.5.4 Postoperative care and follow-up

After the implantation, hair starts growing in two to three months, and getting complete results takes six to twelve weeks. The patient was advised to sleep with head elevation and a short course of oral prednisone. The recipient area needs to be hydrated using the physiologic saline every 2-3 hours for the first 2-3 days and start with regular activities after the physiologic saline hydration ends [20]. Figure 6 shows the before and after the surgical procedure.

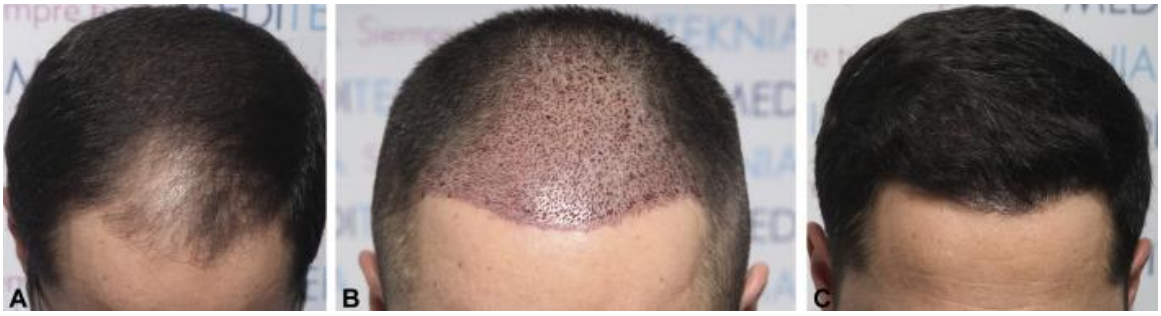

Figure 6: A, before HT. B, after the HT procedure. C, after six months of HT [20].

The current hair grafting method requires grafting the patient's donor hair follicle. However, the hair transplantation procedure will be hard and impossible to implement if the patients do not have natural hair follicles. Further, there are a few downsides to using current hair transplantation, such as high chances for infection in the donor area, hair loss in the donor area, and a more painful procedure in general.
The project is to focus HF artificially using the 3D-bioprinting technology and to propose a permanent toupee.

2 Literature Review

2.1 3D-Bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle Regeneration.

D. Kang's team conducted this study in 2021 in China. The hair follicle was regenerated in an HF microenvironment in vivo. A 3D bioprinter constructed a microenvironment, and the bioink used in this procedure is gelatin/alginate hydrogel to print a multilayer composite scaffold that contained Fibroblasts (FBs), human umbilical vein endothelial cells (HUVECs), dermal papilla cells (DPCs),

and epidermal cells (EPCs). The 3D bioprinting prints different composite scaffold layers that mimic skin properties.
The fibroblast is a type of cell that helps form connective tissue. These cells support and connect other tissues or organs in the body. The fibroblasts secrete collagen proteins that help to maintain the framework of tissue [4]. The human umbilical vein endothelial cells (HUVECs) are widely used as the primary source of endothelial cells in vitro studies. The cell can be collected using the umbilical cord, a waste after childbirth [2]. Dermal papilla cells (DPCs) are a group of mesenchymal cells found underneath the skin [3]. Epidermal cells (EPCs) are found in the skin's innermost layer, which protects the body. The epidermis contains melanocytes that produce melanoma and Langerhans cells, which help the immune system [1].
The nude mice used for experimental purposes were BALB/cAJcl-nu and newborn C57B/6 mice. The cells used in this study are mainly from the 4-6-week-old mice—the epidermal cells (EPSc) and fibroblasts from the reborn C57BL/6 and cultured [1]. The following are the steps for the testing procedure and the discussion of the results.

2.1.1 Preparation of Boink

The bio-ink used in this project was alginate, and gelatine was dissolved using a magnetic hotplate at room temperature, 100 rpm, for two hours. The ratio of the solution is 1:1 [1].

2.1.2 3D printing of GAH scaffolds

The 3D-printed scaffold mimics the properties of the skin. The printer used rapid prototype technology and visual machine software for design and printing. The multilayer scaffold is a pre-designed square shape with 10mm × 10mm × 2mm dimensions.
The multiplayer composite scaffold has 3 layers – lower, middle and upper layers. The lower layer is considered the dermis layer, the middle layer is regarded as the HF appendant, and the upper layer mimics the epidermis layer [1].

- Construction of lower layer: The dermis layer printed the GAH-encapsulating FBs that were 1×10^7/mL and HUVECs that were 1×10^7/mL. The GAH containing 1×10^6mL DPCs was dot-printed into the cavities [1].
- Construction of the middle layer**:** The bio-printing of the intermediate layer is the same as a lower layer. A various diameters 3D-dot pattern was introduced before the upper layer to evaluate the distribution of DPC clumps. The dot diameters were 50–100 μm, 100–150 μm, 150–200 μm, and >200 μm [1].
- Construction of upper layer: The upper layer contains GAH with 5×10^6/mL EPCs. The EPCs were distributed around the hole area in the upper layer, and other cells were distributed in the grid area. The cell can be intact in the bio-ink medium.

After printing the artificial scaffold, it was transferred to a cell culture medium for two weeks.
After the 7th day, Figure 7 [1] shows An HF structure created by DPCs and EPCS. In Figure 7, Section (a) takes the DPCs, HUVECs, EPCs, and FBs from the subject mice and cultures them;

Section (b) is the 3D-bio-printed scaffold; and finally, section (c) transplants the 3D-bio-printed scaffold to the nude mice.

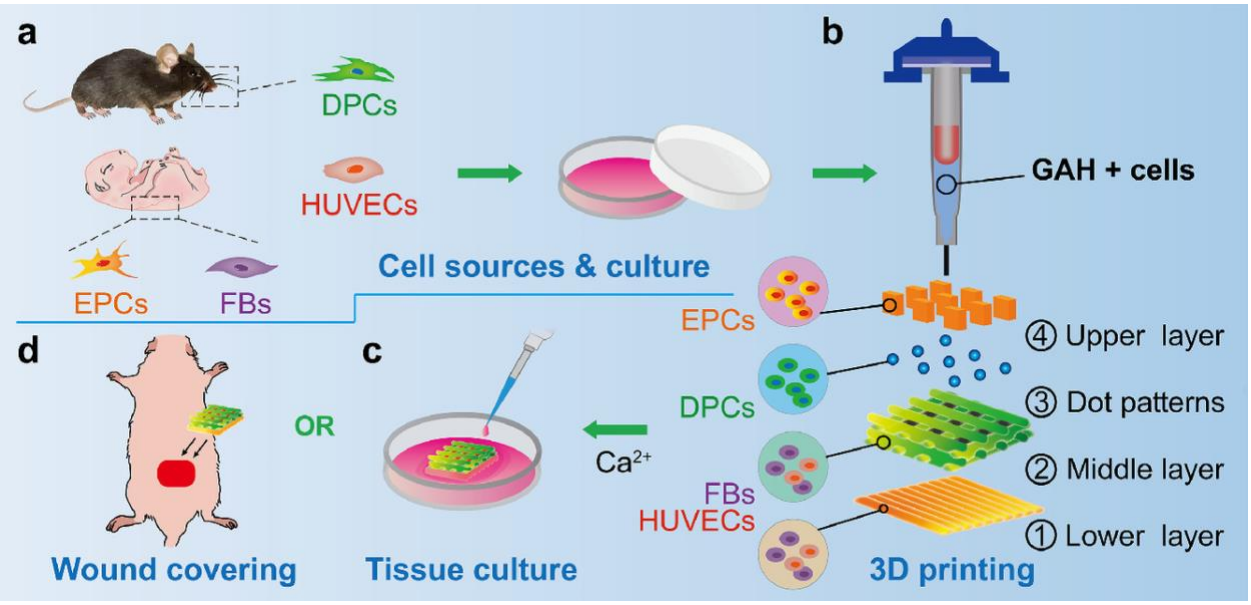

Figure 7: 3D-bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle regeneration steps [1].

2.1.3 Results in Vitro and Vivo study

In the Vitro and Vivo studies, GAH containing 20% (w/v) gelatin and 3% (w/v) alginate showed the highest performance, such as suitable cytocompatibility performance, mechanical properties and printability. With the increment of passage number in 2D culture, the DPSc showed fewer biological characteristics, such as aggregation and growth, the expression of cell-specific markers, and the ability to induce HF regeneration [1].

2.1.4 Hair growth results after transplantation

Figure 8 shows the results after the mimicked scaffold was printed and cultured for two weeks in Petri dishes. The scaffolds are carefully implanted on nude mice, and the results are in six to eight weeks. Figure 8 shows the different groups of hair regeneration; the results show no differences. Figure 8, section (a) shows a 3D-bio printed multilayer composite scaffold for HF reconstruction in vivo; in section (b), One square centimetre of skin was removed from the dorsal nude mice; and finally, in section (c,d) A silicone chamber was inserted beneath the skin on the dorsum of the mouse [1].

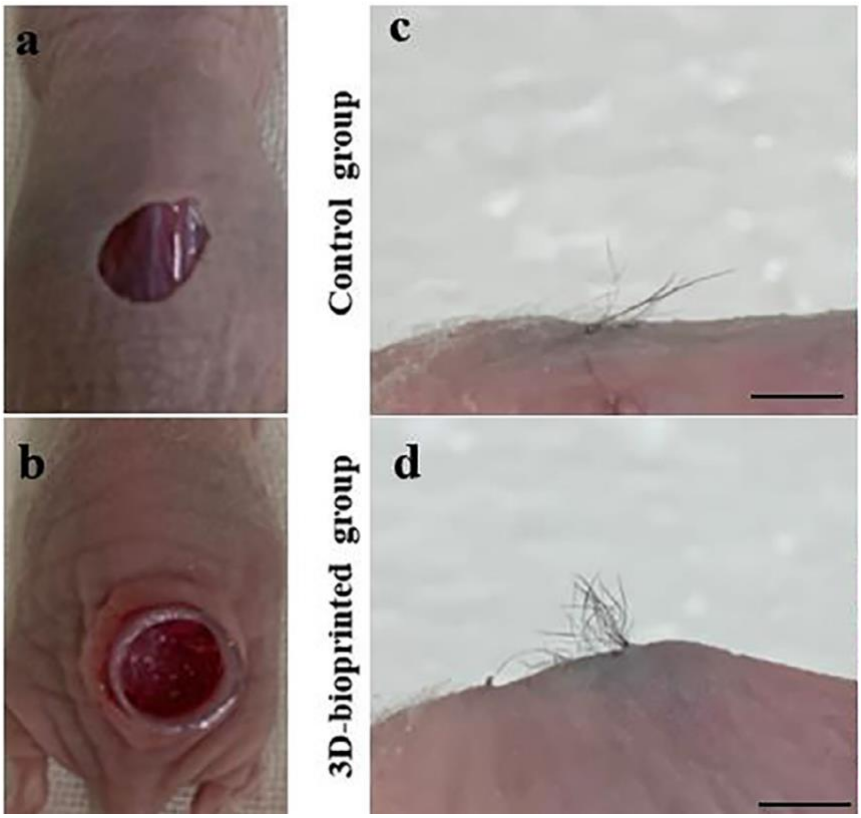

Figure 8: Hair growth results after the procedure [1].

2.2 Tissue engineering of human hair follicles using a biomimetic developmental approach.

This study was conducted by Dr. Angela Christiano's team at Columbia University in 2018. Here, researchers grow the hair follicle in 3D-printed moulds. The primary cells used in this study were dermal papilla cells (DPCs), highly specialized mesenchymal cells that are indispensable for HF morphogenesis and cycling, and these cells are used for seeding. DPCs were isolated from discarded scrap tissue from hair restoration surgery in this study. Another cell used in this study is dermal fibroblasts. Fibroblasts are taken from mesenchymal progenitors and can be isolated from lung tissue and other tissues. For this study, isolated from human foreskin and cultured in CnT-07 and Dulbecco modified Eagle medium with 10% Fetal Bovine Serum up to P.5. For the comparative study, the researcher took GFP-tagged HUVECs (Angio-proteomie) cutured. After the culture of hair cells, it was crafted on 8-10 weeks old male immunodeficient nude mice on the skin. A 0.8 cm^2 skin was removed from the dorsal anteroposterior midline surface to graft the cultured HFs. The following are the steps to produce HF in laboratory conditions and the experimental results [5].

2.2.1 3D printing of spatial arrangements and the creation of microwells using FB

The microwell arrangements are printed in microfabricated plastic moulds that resemble the shape of HF extensions. The measurements were 4mm long and 500 μm in length for HF-shaped extensions. The moulds are placed in type 1 collagen to create biobased microbeads containing fibroblasts. The 3D-printed moulds can control the depth and size of the HF-shaped extensions.

The scientist made 19 and 81 HF per cm^2 in this study and high- and low-mold density microwells [5]. Figure 9 shows the procedure and in sections (a) a CAD design of HF moulds, (b) a 3D printed moulds, and finally, section (c) the preparation of microwells.

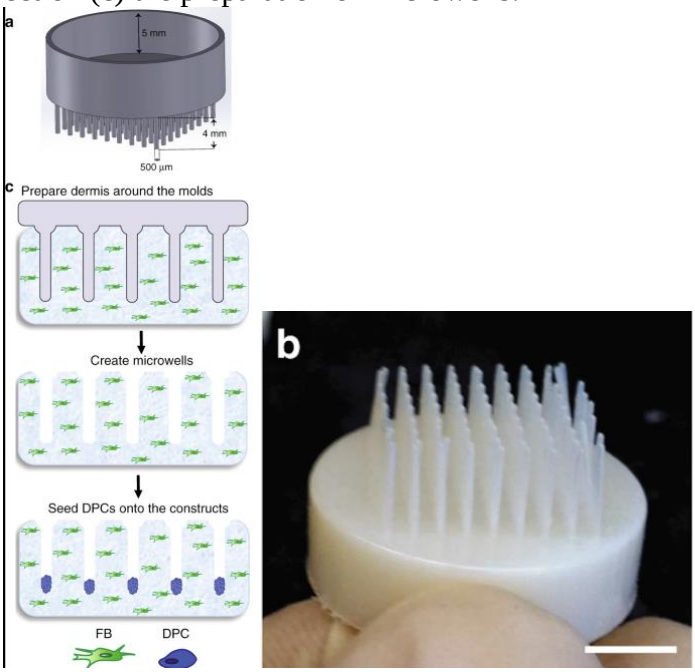

Figure 9: Steps for the tissue engineering of human HF using a biomimetic developmental approach method [5].

2.2.2 Seeding of DPCs in the FB microwells

The researcher seeded the keratinocytes (KCs) over the dermal constructs and cells to settle down and fill with DPC aggregates, forming an HF regenerative system or a hair follicle-like unit (HFU). The Figure 10 shows ALP's sustained activity in DPC after adding KCs. After a few days, the HF cell grew in an experimental environment, hair follicles were generated, and hair fibre formed from the system [5]. The figure 10 shows the induction of DPCs in microwells.

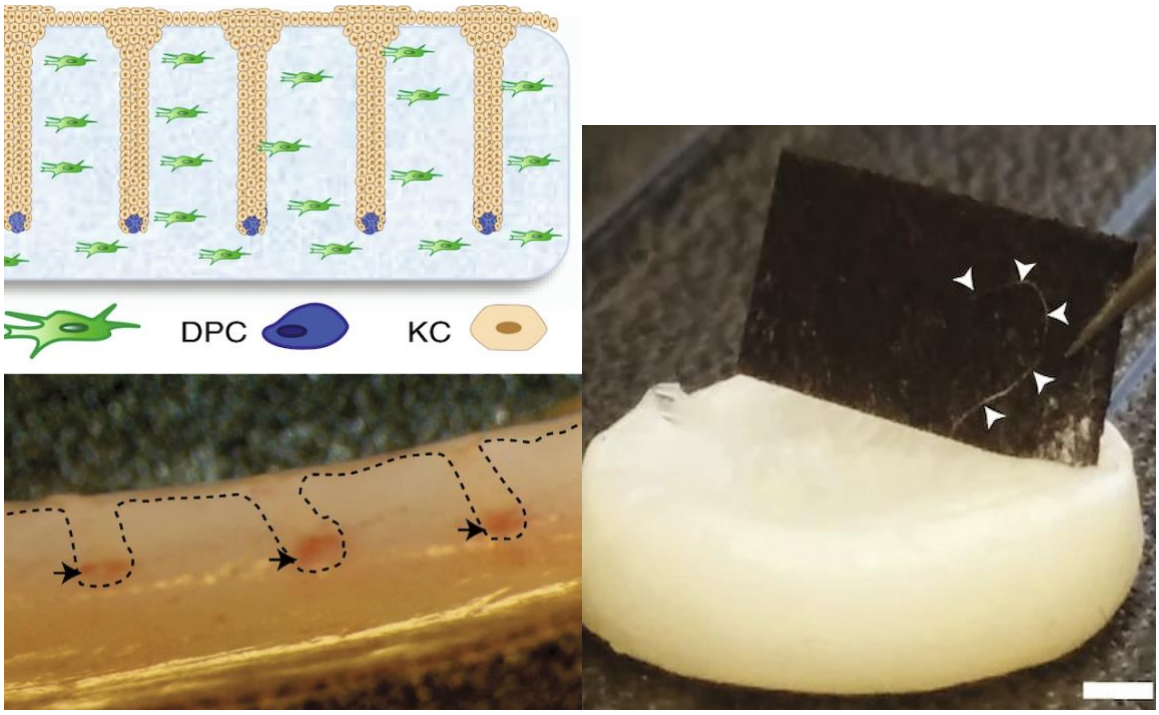

Figure 10: DPC injection on microwells and induction hair follicles generated in the cultured medium [5].

2.2.3 Induction of HFs in HSCs grafted onto mice.

After the cultivation of HF, it was grafted onto nude mice at a high follicle density of 255 HF per cm^2, and scientists analyzed the hair regeneration after four to five weeks. In this research, scientists used ten mice per condition, and seven survived. Of those seven mice, four mice generated successful human HFs. The experiment shows immunostaining with a human-specific nuclear antibody differentiated from human KCs and DPCs. The results were also compared with natural human hair fibre, which closely resembles the morphology of human hair. Also, this cell culture restored 22% of the hair inductive DCS gene signature [5]. Figure 11 indicates the hair growth in a mouse, as shown in the section above.

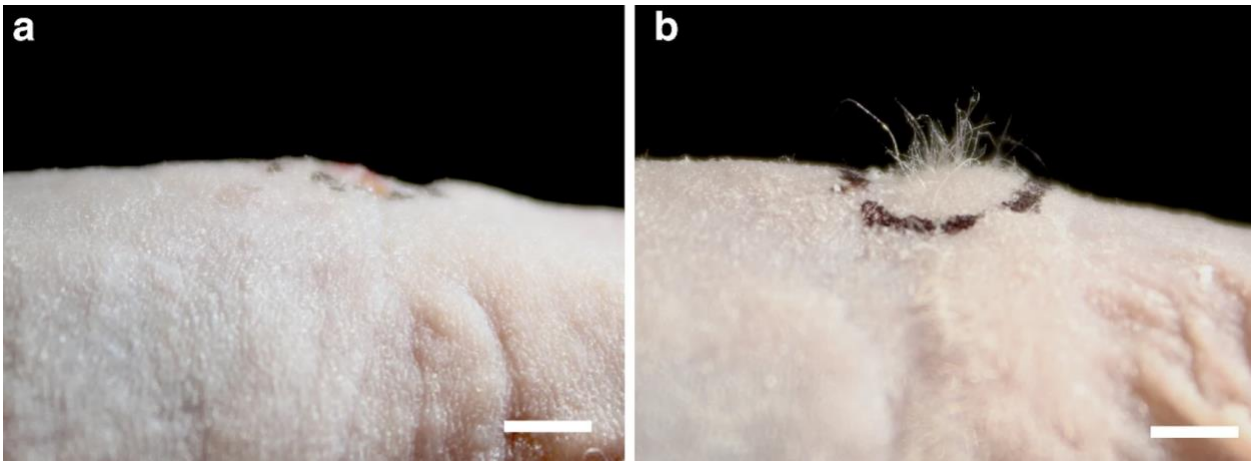

Figure 11: HF growth after the transplant [5].

2.3 Bead-jet printing enabled sparse mesenchymal stem cell patterning augments skeletal muscle and hair follicle regeneration.

This study was conducted in 2022 by Yuanxiong Cao's research team from China. Mesenchymal stem cell (MSCs) transplantation is the primary key to skin repair for traumatic injuries. However, after the transplantation, the performance of MSCs is reduced to the hostile environment within damaged and diseased tissues, producing limited retention and survival of injected cells [6]. In this study, the researcher formulates a bio-ink that preserves the quality and performance of MSCs after transplantation using a "bed-jet" bioprinter. The bioprinter makes MSCs-laden Matrigel beads that augment MSC function, increasing MSC proliferation, migration, and extracellular vesicle production after the graft. To conduct this experiment, scientists collected and cultured MSCs from human umbilical cords and grafted them onto the back of a mouse.
The study also compared the results of high-cell-density beads (HDB), low-cell-density beads (LDB), or cast high-cell-density bulk gel (HDG) encapsulation of the equivalent number of MSCs [6].

2.3.1 Bead-jet printing of MSCs- laden Matrigel beads

The study uses a customized bead-jet printer. It had two modules: a bead forming module and a bead printing or jetting module. The two modules operate in synchronization. Matrigel was used to produce microbeds. The Matrigel liquid was loaded into a 1 mL syringe at a 4-degree angle and connected to a fluid injection pump to create Matrigel beads. The flow rate was 20L/min. The HFE 7000 oil was loaded in a 10 ml syringe connected to a fluid injection pump at a 300 litres/min flow rate. The Matrigel solution is injected into the cascade tubing microfluids (CTM) and formed into Matrigel droplets using the oil shearing effect, the capillary effect, and the tubing boundary condition. The Matrigel droplets were produced and collected in a long connecting tubing via one three-way PDMS cube and then gelled at 37 degrees Celsius for 30 minutes. The tubing outlet was connected to a three-port connector with a PDMS shape, with a side channel width of 1 mm open to the compression air at a five-psi pressure. The microbeads were ejected through a vertical channel onto the wound of the mouse. The bed-jet printer is controlled by a robotic arm, which has a motion of the XYZ direction. The stem cell is delivered in three different ways: high cell density beads (HDB) printed in sparse patterns, low cell density beads (LDB) printed in dense patterns, and high cell density bulk gel (HDG). The number of cells remains constant. The figure 12 shows the bioprinting of cells and grafting onto the mice. The figure 12 section (a) is a high-throughput intra-operative formulation and printing of MSCs-laden Matrigel beads, and section (b) is a parallel comparison of printed high cell density beads (HDB) and low cell density beads (LDB) or cast high cell density bulk gel (HDG) and the space was printed with acellular beads or cast with acellular bulk gel, and finally, section (c) is a bead-jet printing augment stem cell retention, reduces stem cell consumption, and increases volumetric injury recovery in muscle and skin regeneration [6].

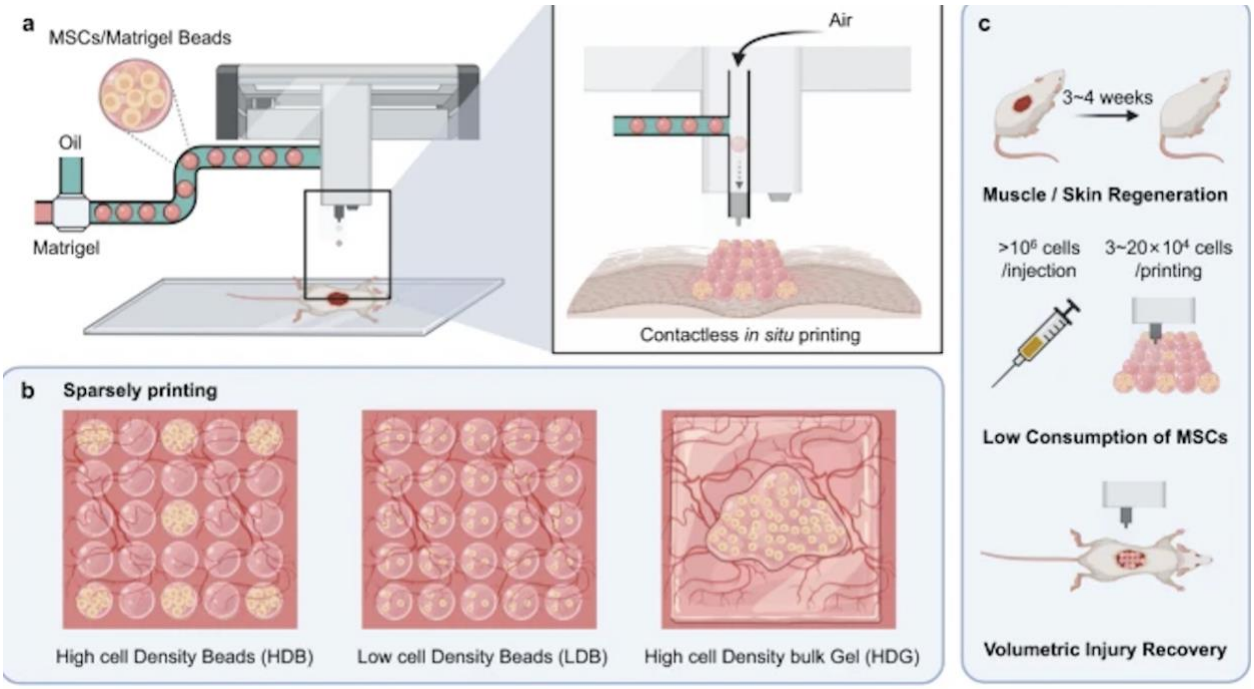

Figure 12: Bead-jet printing of MSCs-laden Matrigel beads for hair follicle regeneration procedure by steps [6].

2.3.2 Performance analysis of MSCs beds after transplantation

In this study, three types of beads were created to understand the material's different conditions and behaviour. The main tests characterized MSCs cultured in vitro in Matrigel HBD, LBD, and HBD groups. The number of cells was kept constant during the test. From the LDH-Cy Quant assay, the HBD MSCs' growth rate of 1500 cells per microbead was higher than that of the other two groups. The Alamar blue assay was to test the metabolic activity of the grafted cells. After being cut for seven days, the number of cells at the HBD MSCs was much higher. Those tests showed that the growth of MSCs before and after transplantation was at a higher rate than the LBD and HDB MSCs beads. The metabolic activity of the MSCs was examined from the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. In this assay, the metabolic activity in HBD was higher than in the other two groups. The extracellular vesicle (EVs) production of Matrigel HDG, LDB, and HDB groups was examined by nanoparticle tracking analysis (NTA), and it revealed that the Matrigel HDB-MSCs had the highest production of EVs.

2.3.3 Hair-follicle generation analysis of grafted area

The above paragraph shows that the HBD-MSCs promote the regeneration of skin cells and EVs signal, including promoting cell proliferation and resolving inflammation. The hair follicle-like structures were only observed in the HBD-MSCs group. Also, the Matrigel HBD-MSCs accelerated the formation of hair follicles and other related glands [6].

2.4 Robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces hair follicle-inclusive skin regeneration.

This study was conducted in 2022 by Chen Haiyan and his researchers group from China. In this study, the researcher used an in situ bioprinter to produce a stem cell with hydrogels and deliver it to the back skin of mice using a robot-assisted arm. The bio-ink used in this experiment was a biocompatible, photosensitive hydrogel material comprising gelatin methacrylate (GelMA). The cells used in this study were a combination of epidermal stem cells (Epi-SCs) and skin-derived precursors (SKPs). The epidermal stem cell (Epi-SCs) is a skin stem cell that can convert into a hair follicle (HF), sebaceous gland and erector pili muscle. The skin-derived precursors (SKPs) are multipotent cells [9] [8].
This experiment used 57BL/B6 and BALB/c nu/ni mice. A symmetrical skin wound was created on the back of the mice. The in situ bioprinting procedure was completed at the back of the mice.
The following report shows the importance of bioink and its properties, robot-assisted in situ bioprinting and its process, the biocompatibility of hydrogel after transplantation, and the analysis of hair follicles after transplantation [7].

2.4.1 Importance of bio-ink and its properties

In this study, the researcher used Gelatin methacrylate (GelMA). It is a biologically photosensitive hydrogel material with high biocompatibility that can mimic the properties of extracellular matrix (ECM) and promote nutrient permeability, cell growth and differentiation. The GelMA bio-ink supports the development of stem cells within the gel and helps regenerate hair follicles. The stem cells were cultured in the GelMA. The GelMA is a transparent solution; the hydrogel concentration can reduce the transparency. The GelMA is formed very quickly using a blue light. The higher the concentration is, the smoother the hydrogel surface is. The scientists tested the survival of SKP in the GelMA hydrogel. According to the research, the SKP cultured within the hydrogel very effectively [7].

2.4.2 Robot-assisted in situ bioprinting and its process

In situ bioprinting is also referred to as "in vivo" bioprinting. The GelMA hydrogel was prepared and printed using a 3D single nozzle bioprinter. The GelMA solution was transferred to a 1-ml syringe and frozen at 4 degrees for 5 min before printing. After that, the hydrogel was passed through a 0.26-mm nozzle, eight on the printing platform and the printer GelMA hydrogen; scaffolds were irradiated with 405-nm UV light for 10 to 30 seconds. The Robert arm has a degree of freedom or multi-degree freedom (DOF) mechanism that provides a fast, precise and complex structure. The GelMA was printed on the back wound of the mice [7]. The figure 13 shows the process of preparation of bio ink and the in situ bioprinting method.

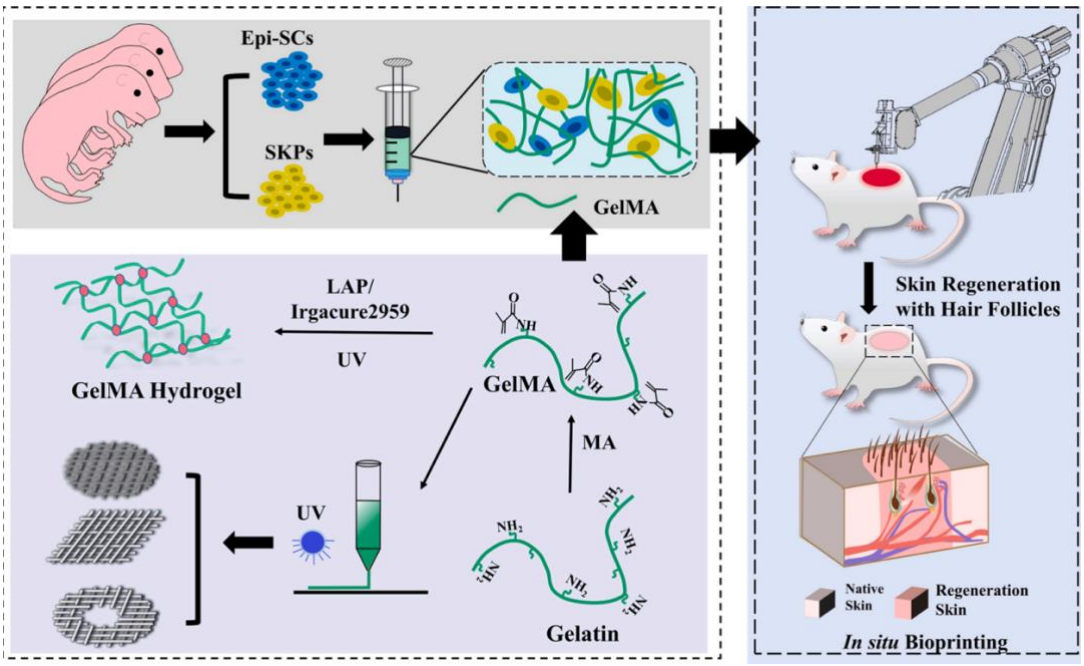

Figure 13: Procedure steps of the robot-assisted in situ bioprinting of GelMA with stem cells induced HF regeneration [7].

2.4.3 Biocompatibility of GelMA hydrogel

When a researcher considers biomedical applications, biocompatibility is an essential factor. The black hair shaft generated at the back of the mouse is maintained for ten months after transplantation. The results show that the hair shaft remained after ten months, and the rendered skin was healthier than the regular skin of the mice [7].

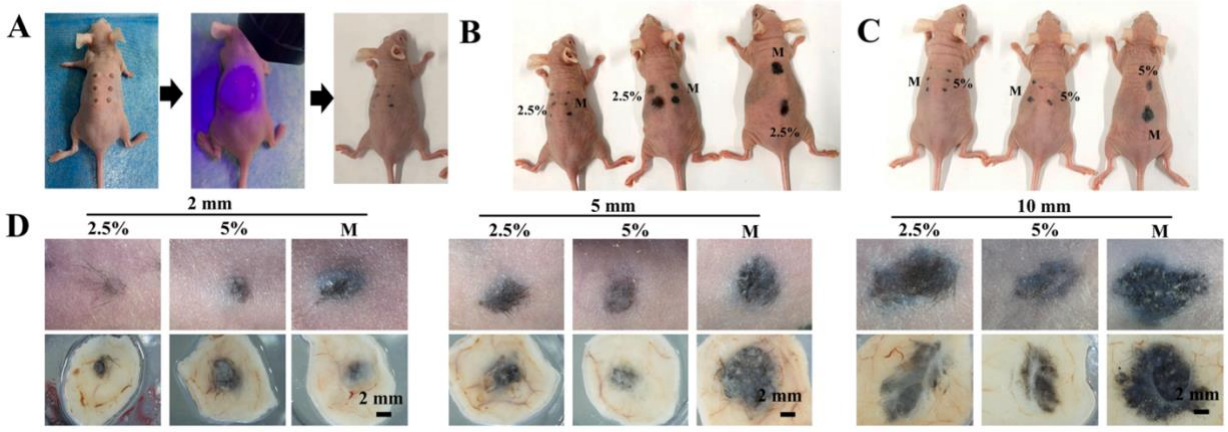

Figure 14: GF regeneration using the GelMA hydrogel and stem cell [7].

2.4.4 Analysis of hair follicles after transplantation

The hydrogel is biocompatible, and the gel is compact for culturing the Epi-Scs and SKPs. After four weeks of grafting, the hair was regenerated and observed through a microscope. The researcher also observed the hair regeneration of hair follicles in the Matrigel bio-ink. The results showed no significant differences in the hair growth of the two mediums. The regenerated skin

had an epidermis, dermis, and density of hair follicles, blood vessels, and sebaceous glands, the same as regular skin features. Figure 14 shows the skin and hair regeneration after four weeks of surgery in the different wounds with different scales of Epi-SCs and SKPs. Figure 14 section: (a) The process images of GelMA hydrogels and stem cells induced hair follicle reconstitution model and section (b and c) Representative images of skin regenerated four weeks after transplantation of Epi-SCs and SKPs in 2.5%, 5% GelMA hydrogel, Matrigel of 2 mm, 5 mm, 10mm diameter wound.

2.5 Bio-printing of hair follicle germs for hair regenerative medicine.

This study was conducted in 2023 by Japanese scientist Ayaka Nanmo and his team. The method is to make highly hair-inductive grafts using 3D bioprinting. The hair follicle germ (HFG) graft is prepared by compartmentalizing the mesenchymal stem cell (MSCs) and the epithelial cell. These bioengineered HFGs efficiently regenerate hair follicles and repeat the hair cycle naturally.
The mesenchymal stem cells (MSCs) are multipotent stem cells that can be derived from bone cells (osteoblasts), cartilage cells (chondrocytes), muscle cells (myocytes) and fat cells [11]. Epithelium tissue is made up of epithelial cells, a type of body tissue covering all internal and external surfaces of the human body [12].
To conduct this experiment, scientists took C53BL/6 pregnant mice for the stem cell and five-week-old ICR-nude mice for grafting the HFGs.
The HFGs were converted to hair microgels (HMGs) using a collagen solution to bioprint the graft [10].
This study created and used two types of hair microgels: regular hair microgels (HMGs) and guide-inserted hair microgels (gHMGs). The following are the main steps used in this experiment.

2.5.1 Preparation of hair microgel (HMGs)

The mesenchymal and epithelial cells were harvested from the pregnant mouse embryos. After this procedure, scientists cultured the cells. After that, the cells were suspended separately into a collagen solution at $5x0^6$cells/ml using a 3D-bioprinter and the collagen gel constructs were suspended in DMEM/KG2 and then cultured on a non-cell-adhesive flat-bottom dish for three days. Because of the upregulation of gene expression, microgel contracts and concentrates the density of collagen and cells in the HMGs [10].

2.5.2 Preparation of guide-inserted hair microgel (gHMGs)

The MGs have the same HMGs, but the HMGs were in polydimethylsiloxane (PDMS) flame, had an aligned nylon structure, and were the lid of a culture dish. The inner dimensions of the flame were 3 cm in length, 3 cm in width, and 5 mm in height. The gHMS was transferred to a non-cell-adhesive dish and cultured for three days [10].

2.5.3 Transplantation of HMGs and gHMGs

The HMGs and gHMGs were transplanted individually on the ICR-nude mice onto a shallow stab wound prepared using a 20G ophthalmic V-lance surgical knife. The HMGs graft orientation was uncontrollable during the transplantation. For the gHMGs, the researcher planted that the epithelial

cell aggregate side was closer to the skin surface. This technique is mainly used to prevent hair outgrowth [10]. Figure 15 shows the schematic representation of hair beds and hair follicle germs.

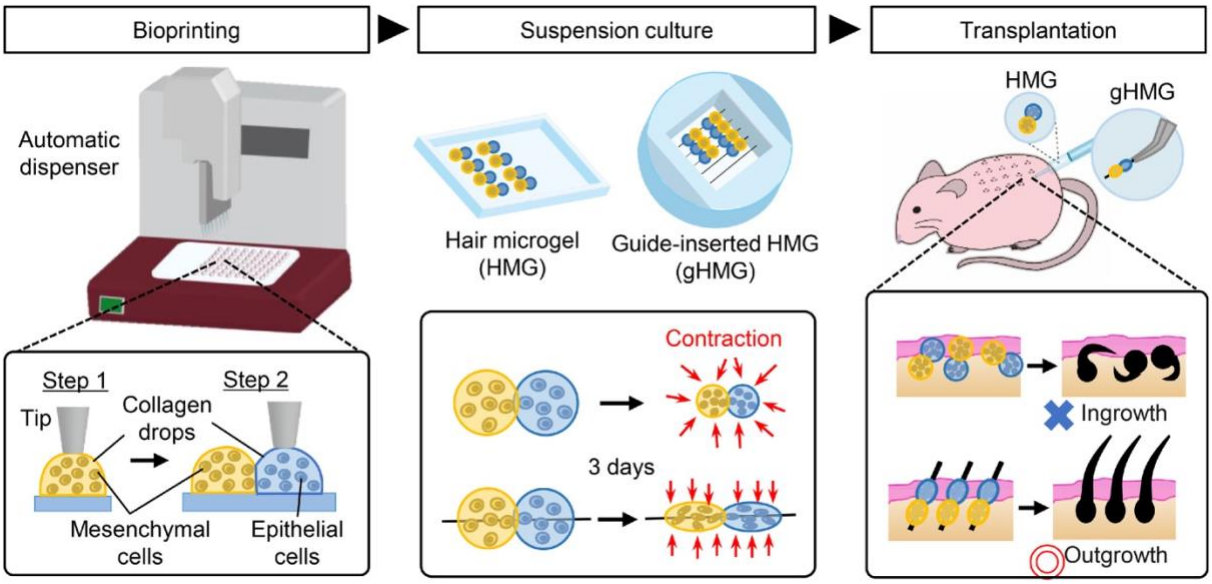

Figure 15 Bioprinting of hair microgels (HMGs) and guide-inserted HMGs (gHMGs [10]).

2.5.4 Hair regeneration activity of HMGs

The hair regeneration of GHGs is compared to HFGs without collagen. The HFGs and HMGs were transplanted 30 grafts per pocket on the back skin of the nude mice. After three weeks, an accumulation of black hair was observed in the transplanted areas. After the analysis, the HMGs were 2.4-fold more significant than the HFGs. Also, after the transplantation, the collagen in the HMGs increases cell migration and morphogenesis and improves the supply of oxygen and nutrition from the host tissue. The main drawback of this method was that some of the hair that developed in the mice grew inside the skin tissue. After analysis of the 88 Wnt-signalling related genes using RT2 profiler PCR, the HMGs at three-day culture 6 and 4 genes out of 88 genses. They were upregulated and downregulated [10].

2.5.5 Hair regeneration activity of gHMG

In this study, the researcher compared three different oriented cell graft transplants. They were random order HMGs (RDM), gHMGs epithelial cell aggregate side (forward direction or FWD) and gHMGs mesenchymal cell aggregate side (reverse direction or REV) close to the skin surface. The surgical sutures were placed in a square frame at regular intervals to get the orientation. The collagen drops containing mesenchymal and epithelial cells were then placed onto the sutures, whereas the cells were separated. The MGs contract after three days and the graft is placed on the mice's back skin. The gHMGs were transplanted in the order RDM, FWD, and REV, which were close to the skin surface. A surgical tape was used in this procedure for seven days until the wound healed. The growth of the FWD gHMGs was two to three times more than REV gHMGs and RDM gHMGs. The results indicated that the orientation of gHMGs and the location of the graft are essential in hair growth [10]. Figure 16 shows the hair growth of 3 different transplants.

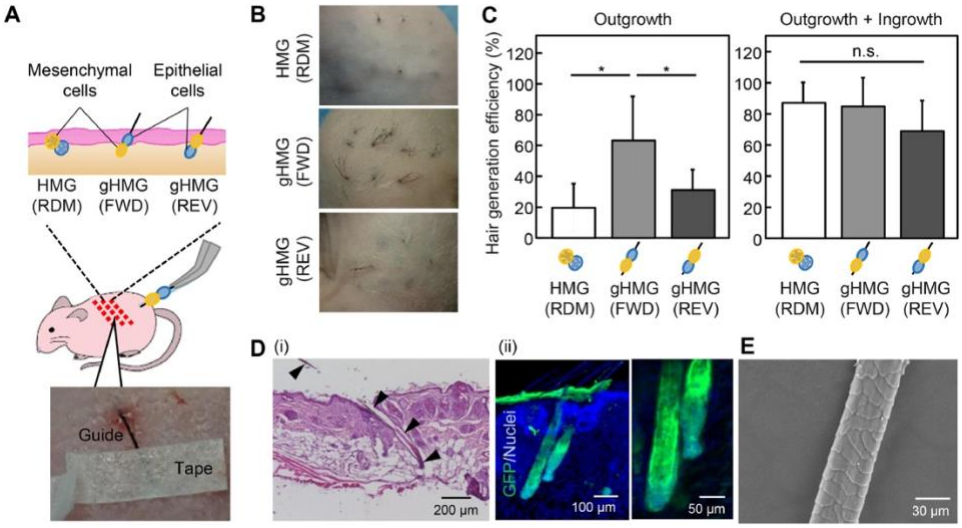

Figure 16: 3 different graft growth and its results[10].

2.6 Using bioprinting and spheroid culture to create a skin model with sweat glands and hair follicles.

The study was conducted in 2021 by Yijie Zhang's group from China. In this study, the researcher designed a new model for forming sweat glands (SGs) and hair follicles (HFs) in vitro. The SG and HF were cultured in a 3D-bioprinted skin construct. The primary steps are the following: first bioprinting of a mesenchymal stem cell (MSC)- laden scaffold and SG induction, simultaneously the separation of keratinocytes (KCs) and fibroblasts (Fbs) combined and produce droplet culture to form HF spheroids. The final stage is to culture the HF spheroids onto the SG scaffold. Keratinocytes (KCs) are the most eminent cells within the epidermis. The KCs can be found in the four layers of the skin. Fibroblasts are a particular type of cell that helps form connective tissue. The fibrous cellular material that supports and connects other tissues or organs in a body.
In this experiment, C57BL/6 J mice were used to collect KCs and Fbs from the back skin. The MSCs were collected from human tissue and cultured in a proliferation culture medium.
The paragraphs below explain this project's main stages and the discussion of the results [13].

2.6.1 Preparation of MSC-laden by 3D-bioprinting scaffold and SG induction

The bio-ink used in this experiment combines type B gelatin and sodium alginate with a ratio of 1:3 dissolved in phosphate-buffered saline (PBS). PBS solution is used for dilutions and washing cell suspensions and is an excellent additive to cell culture media. After the formation of bio-ink, add 200 μL 5 × 106 single-cell suspension of MSCs, followed by gelatin solution. An extrusion-based 3D-bioprinting is used to print scaffolds that mimic the properties of skin tissue. After forming the scaffold bed, it was cultured into an SG induction culture medium. The medium was replaced every three days. Finally, after seven days, the scaffolds are ready for HF spheroids [13].

2.6.2 HF spheroid formation and seeding on SG scaffolds

After collecting KCs and FBs from the mice, they were mixed in a ratio of 1:5-9 and rinsed two times with phosphate-buffered saline (PBS). Then, the mixture was seeded into an HF-induction culture medium at a rate of 20 µL droplet with a 5×10 five-cell count. The cells were sedimented to the bottom of a droplet and produced a spheroid after 24 hours of culture. After three days of induction, the HF spheroids were manually collected and seeded onto SG scaffolds. The cell was again sedimented after 1 hour. The cell mixture was incubated at 37 c for 15 minutes and washed with PBS three times to avoid light. Figure 17 shows the process of regeneration of hair follicles. Section (a) is a schematic diagram showing the procedure to establish 3D skin constructs in vitro, section (b) is the time points used in inducing SGs and HFs separately and SG–HF co-culture, section (c) is the 3D-bioprinting of SG scaffold. Brightfield imaging of HF spheroid in droplet culture 10 minutes, section (d) and 60 minutes, section (f) after seeding (scale bar, 300 µm) and finally, section (e) is the gross imaging of HF seeding on SG scaffold (black arrow shows a gross view of HF spheroids seeded on SG scaffolds) [13].

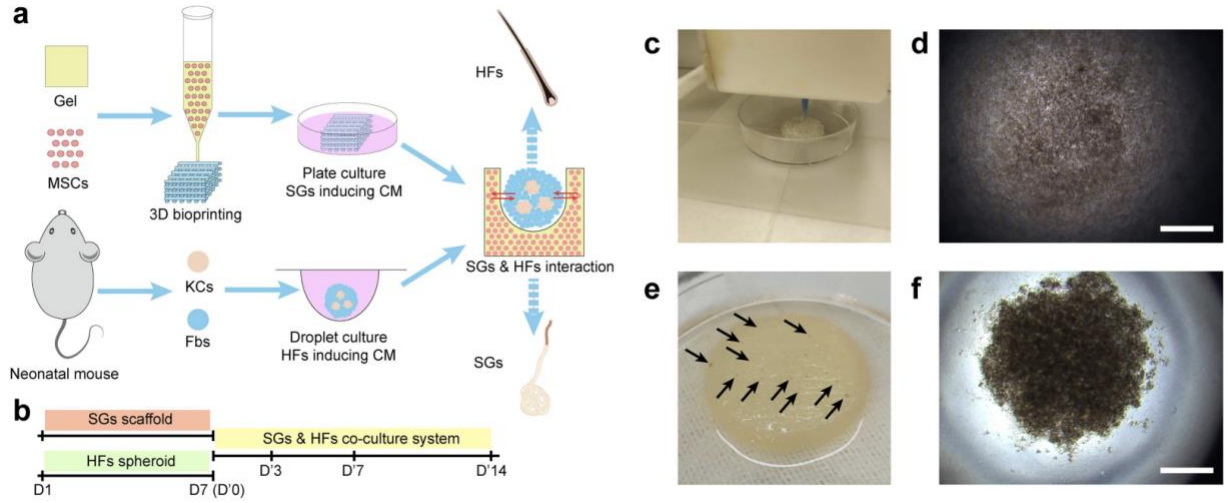

Figure 17: Regeneration of HF using spheroid culture [13].

2.6.3 HF regeneration results study

In this study, the SG and HF cultured in vitro 3D model. After seven days of HF culture in 3D-bio-printed scaffolds, the HF and SG were grown in the vitro model. A KRT17 is a structural marker protein generated, while alkaline phosphate is the functional protein expressed in the hair shaft. Also, when in vitro 3D skin builds, the HF contributes Krt 17, cdh3 and Alpl in the hair progenitor, contributing to the development and saturation of HFs. Although the creation of HF after seven days is perfect, there might be various results in live animals [13].

The six methods reviewed in the above section show different techniques for regenerating HF artificially using a biomolecule in a controlled environment using a 3D bioprinter. The biomolecule used in each method is different. In the first method, the cells used were Fibroblasts (FBs), human umbilical vein endothelial cells (HUVECs), dermal papilla cells (DPCs), epidermal cells (EPCs) and a controlled environment created using the 3Dbio-printer. In the second method, DPC and KC cells were used in this procedure in HFU. A bed jet bioprinter was used in the third method to produce an HFU, and the main biocells used in this project were mesenchymal stem

cells. In the fourth method, the Epi-SCs and SKPs cells are inserted into a GelMa bioink to create the required hydrogel using a robot-assisted in situ bioprinter. The hydrogel is placed on the nude mice wound area to regenerate the HF. The fifth method, the HFG, combines MSCs and epithelial cells using a 3D bioprinter. In the final method, Fbs, KCs, and MSCs stem cells were grown in a controlled and designed environment using a 3D bioprinter to cultivate HF. Table 2 provides information regarding the comparison of six existing methods to create HF using bioprinting.

These six methods are very complex. The HF is cultivated in a controlled environment and uses a complex 3D bioprinter. Also, the artificially cultivated HF is under experimentation and will be highly regulated by regulatory authorities such as the FDA (Food and Drug Administration)'s Center for Devices and Radiological Health (CDRH) and the FDA's Center for Biologics Evaluation and Research (CBER), which might create a considerable time delay to enter the market. Finally, the retention rate of hair grafting could not be promising, and Hair loss can occur at any point in time due to biological and external environmental conditions. Table 2 provides the information about the comparison of six methods.

I propose a permanent toupee method to reduce the disadvantages of the current experimental method for creating HF. The technique proposes acquiring permanent hair on the scalp to reduce the complications of hair grafting and artificial hair growth. This method minimizes the disadvantages of hair grafting (artificial or HFU) from the donor area. Since the procedure is not developing in a live body, the government might minimize the FDA regulations for the proposed method. Section 3 explains more about the proposed method.

Existing methods to create HF	*Research material used (Bio cell and bio ink)*	*Retention rate (after the HT)/ Efficiency*	*Direction control of HF*	*Other applications*	*Animal testing*
3D-Bioprinting of a gelatin-alginate hydrogel for tissue-engineered HF Regeneration [1]	Bio cell-FB, HUVEC, DPC, and EPC. Bio ink-gelatin/alginate hydrogel	Information not available	Direction of HF is controllable. The transplantation method resemblance to FUT	Skin tissue engineering, with the associated appendage	BALB/cAJcl-nu and newborn C57B/6 mice used
Tissue engineering of human HF using a biomimetic developmental approach [5]	Bio cell- FB, DPC and KC. Bio ink-type 1 collagen gel	70% success rate	Direction of HF is not controllable	Only for HT	Immunodeficient nude mice used
Bead-jet printing enabled sparse MSC patterning augments skeletal muscle and HF regeneration [6]	Bio cell- MSC. Bio ink- Matrigel	Less efficient, percentage is not available	Information not available	Volumetric muscle loss (VML) injury treatment	Specification of mice is not mentioned
Robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces HF-inclusive skin regeneration [7]	Bio cell- Epi-SC and SKP. Bio ink-GelMA hydrogel	Information not available	Direction of HF is not controllable	Repairing skin wounds with regenerated appendages application	57BL/B6 and BALB/c nu/ni mice used
Bio-printing of hair follicle germs for hair regenerative medicine [10]	Bio cell- MSC and EPC. Bio ink-collagen	Information not available, but by analysing the results, 85% success rate	Direction of HF is controlled by gHMG	Only for HT	C57BL/6 mice used

Using bioprinting and spheroid culture to create a skin model with sweat glands and hair follicles [13]	Bio cell- MSC, KC and FB. Bio ink- alginate–gelatin	Information not available	The information is not available, but analysing the results the HF direction can be controlled (Because of similar HUT procedure)	Forming sweat glands (SGs) and hair follicles (HFs)	C57BL/6 J mice used

Table 2: Comparison table- Existing methods to create HF through bioprinting methods.

3 Proposed hypothesis- Permeant toupees

To reduce the complexity and safety problems in a hair grafting method, I propose a technique called permanent hair piece for the hair loss area. The process is to fix an artificial hair system that mimics the properties and structure of hair and permanently stays in the bladed area. The structure has two sections: the base and the hair system. The base is a biocompatible material that mimics the structure of a hair bulb and is immersed in the subcutis layer of the skin. From that base, a biofibre is produced using a 3D bioprinter. The 3D bioprinter printed the artificially mimicked hair to a certain length. Since the hair system comes from the skin, it mimics the natural look of a real hair system. Also, the system gives SG and other skin glands space to perform their duties. The following paragraph will explain further information about this method.

3.1 Background Information

The term toupees comes from the French toupee, which means a tuft of hair. Toupees are petite wigs or hairpieces covering the scalp's bald spot. The hair used in the toupee system can be natural or synthetic. The hair is attached to a synthetic material, creating a base for the hairpieces. The following are the steps to make a wig or hairpiece [16].

1. Make head mould- customize the mould according to each individual using a plastic film or other synthetic material.
2. Make hair net- after creating the mould, create a hair net according to the mould.
3. Prepare hair- collect the hair, cut it, and smooth it according to customization.
4. Process hair- after preparing hair, disinfect and colour it according to customization.
5. Grade hair- before coming to an end product, the company checks the QA and QC for the hair.
6. Knot hair: The workers use a hair knitting needle with a small hook to thread hair from one side of the sleeves to the other [17].

The mould can be synthetic or plastic that resembles the customized skin colour.

3.2 Advantages and disadvantages of toupees/wig

The main advantage of a toupee/ wig is that it covers hair loss or bald areas and is realistic. That will boost a person's confidence. The procedure is painless and easy to apply. The results are instantaneous in the hairpiece or wig system. The style in the hairpiece or wig is limitless, such as colour, appearance, and thickness. The procedure is less expensive than a hair transplant surgery [14][15][16].

Even though it has some advantages, the disadvantages of hairpieces or wigs are the main reason for this project hypothesis. The maintenance and care for the hairpiece or wig are adequate. The toupee or wig system destroys the natural hair around the toupee or wig-applied area and prevents natural growth. Wearing the hairpiece or wig for a long time can create skin problems such as rashes, itchiness and discomfort—the hairpiece or wig limits activities such as exercise, swimming or sleeping. In a survey, 49% of people who wear toupees or wigs avoid the activity due to fear

and embarrassment. Placing a hairpiece or wig is time-consuming and requires maintenance and re-application [14][15][16].

3.3 The inspiration behind the hypothesis

I lost my scalp hair and eventually ended up with low self-esteem. For myself, I used the surgical method that grafted the hair follicle from the back of my head and placed it in the bald area. If the person has no hair follicles to transplant, it might be difficult to do hair transplant surgery. Also, when I was researching the 3D-bio-printed hair follicle, the research was in the budding stage, and it needed time to develop to be safe for human use.
The main reason behind the skin irritation caused by using hairpieces or wigs is the synthetic material used to hold the hair material. That material became a barrier between the skin and the outer environment [15].
In my hypothesis, the demerits of hairpieces or wigs will be reduced, and this technology can help many people suffering from hair loss.

3.4 Proposed hypothesis

The proposed hypothesis is to implant permanent hairpieces or wig systems in the bald area of the human skin. So, most of the disadvantages of hairpieces or wig systems are reduced, such as skin damage problems; they do not destroy natural hair growth, can be used for all activities like sports and sleeping, and look absolutely natural.
The first is to build a base for the hair follicle system. We use a thermoplastic polyurethane based on polycaprolactone-block-polytetrahydrofuran-block-polycaprolactone copolymers [18] or any other flexible material. This material is degradable, and the degradability can be controlled. The material is used primarily for soft tissue engineering. The material can be changed according to future research, printability and availability. Fig 18 shows the structure of the base material, which can be 3D printed. To give stability and durability to the hair system, the bulb area or the base area needs a spike formation. This will prevent hair from falling.

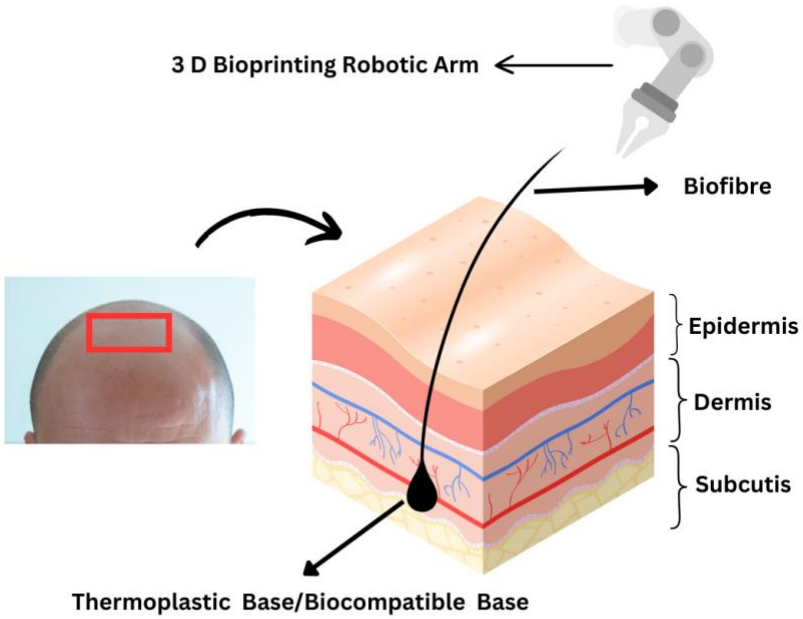

Figure 18: Permanent toupee hair system

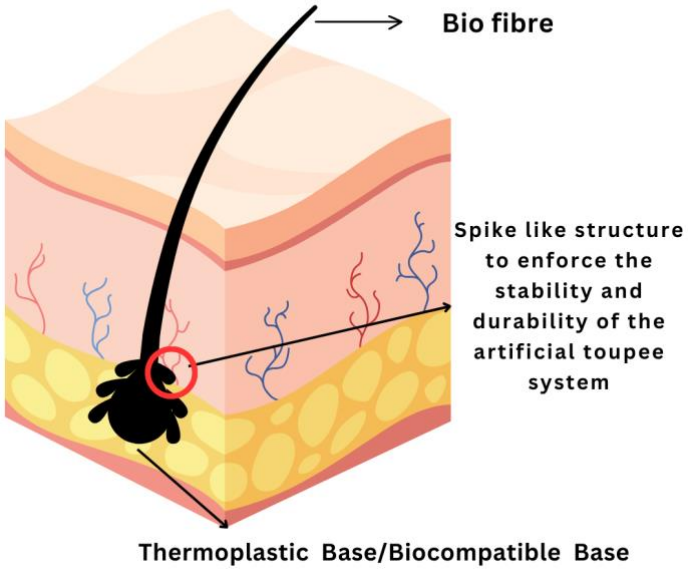

Figure 19: Detailed permeant hair system overview.

The base material is surgically implanted, using an in-situ bioprinter, to deposit the material in the nude area.
The base for the system should be implemented beyond the natural hair system. It attached a biocompatible fibre structure from the base that resembles natural hair. The hair system and bio-fibre can be printed together and surgically implanted. Fill the gap with biocompatible hydrogel to fasten the healing process.

3.5 Vitro and vivo analysis

For this hypothesis, the biocompatibility of materials and how they respond to human tissue should be studied in the lab.
For the vivo test, we can test this procedure on nude mice. The test is not only for biocompatibility but also for daily activity tests such as washing the hair system, response in different temperature systems temperature and the comfortability of the hair system on a new body.

3.6 Advantages of permanent toupee or wig

The permanent hair system will act like natural hair and does not require special attention like a toupee. The person can do regular exercise, such as indoor and outdoor sports. In the permanent system, we do not use synthetic material, so the skin damage will be reduced, and natural hair on the scalp will grow naturally.
The main disadvantage of this procedure is that the hair system cannot be altered once fixed.

4 Discussions and Recommendation

Hair is an extra natural barrier between the skin and the outer environment, such as UV radiation and temperature. It also helps to regulate body temperature [1]. Hair loss is mainly caused by gene type, the occurrence of the external environment, aging, and hormonal imbalances. All the 3D-printed hair follicle generation technology discussed in this project primarily focuses on cultivating hair follicles in an external environment or a live animal.
In the first method, the "3D-bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle regeneration", a multilayer composite scaffold is made using the bioprinter. The bioactive molecules are protected in a hydrogel solution and printed and inserted in a complex scaffold that mimics the properties of the skin. After the printing, the artificial scaffold is cultivated and transferred to a live animal, producing hair follicles. The main biomolecules used in this study were dermal papilla cells (DPCs), human umbilical vein endothelial cells (HUVECs), Fibroblasts (FBs), and epidermal cells (EPCs), and the bio-ink used in this study was based on a gelatin/alginate hydrogel (GAH) [1]. However, this study showed some limitations in hair growth, such as the growth direction needing to be more controllable. Also, the scaffold is complex, so the overall cost and time increase. The artificial scaffold mimics the properties of skin, but it does not interact with the natural area of skin once it is transplanted.
The second method explains the "Tissue engineering of human hair follicles using a biomimetic developmental approach." The HF is created and cultivated in a controlled environment such as a microwell containing hydrogel, FBs, KC, and DPCs. In this method, the scientists created a microenvironment-vasculature and intestinal epithelium to develop an HF. In this method, the

researcher restored 22% of hair. Inductive gene signature and other strategies, such as genetic and microenvironmental reprogramming, have a 70% success rate in HF regeneration. However, the success rate is high; it depends upon the quality of the biomolecule [5].

Further, the third method, "Bead-jet printing enabled sparse mesenchymal stem cell patterning augments skeletal muscle and hair follicle regeneration," research, a micro bed of mesenchymal stem cells (MSCs) in Matrigel bioink induced in a live animal. The study mainly focuses on skeleton muscle regeneration, but after the week of transplantation, a hair follicle-like structure is produced on the mice's body [6]. The insertion of cells was practised in this study by a bioprinter. So, if future studies isolate the genes for hair growth, it can be used for cosmetic purposes.

In the fourth article, "Robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces hair follicle-inclusive skin regeneration," an in-situ printer was used to induce the biomolecule Epi-SCs and SKPs in a GelMA solution. The regenerated skin promotes hair follicle regeneration [6]. The experiment was conducted in a controlled environment, and further study was needed for the human trial.

In the fifth research article, "Using bioprinting and spheroid culture to create a skin model with sweat glands and hair follicles," the KCs and FBs were combined and seeded in an MSCs hydrogel bio-printed environment and cultivated HF [13]. The scientists created an HF, but it lacks the Vitro study. In the future, the study needs to be conducted on live animals, and new hair follicles in the grafted skin need to be analyzed.

The final method, "Bioprinting of hair follicle germs for hair regenerative medicine," is a scientific process that uses mesenchymal cells and epithelial cells combined to regenerate the cell using a guide inserted HMg to control the hair growth direction [10]. In the future, researchers estimate that a combination of type 1 collagen, laminin, and fibronectin can lead to higher hair regeneration [10].

In all scientific research papers, the HF is regenerated in a controlled environment. The HF needs to be fully or partially developed outside the body and grafted onto human tissue for commercial purposes. As a research student, I like the idea of "Bioprinting of hair follicle germs for hair regenerative medicine" and "Using bioprinting and spheroid culture to create a skin model with sweat glands and hair follicles". These two research papers mainly focus on hair regeneration, and "Bioprinting of hair follicle germs for hair regenerative medicine" has extra benefits in guiding hair growth. For commercial purposes, 3D-bio-printing is expensive and time-consuming due to the complexity of the HF regeneration environment.

In the six research papers I analyzed, the stem cell or biomolecule formed a hair follicle in a controlled environment using 3D bioprinting technology. The procedure is complex, and the success rate can vary by individual. Hair loss can also occur after the procedure. In the proposed hypothesis, the researcher can avoid the complexity of the process and time consumption. Since the hair shaft is placed underneath the skin with a strong base, it will not affect the daily purpose. Also, it will not affect the natural growth of other hair cells in the skin. None of the research papers discuss other natural hair growth in the bald area. The permanent toupee hypothesis will help eliminate conventional hair transplants and regular toupee or wig systems. The principal drawback of this hypothesis is that the system is unchangeable once fixed. Also, further research should be conducted about the bio-fibre that mimics the properties of hair and its biocompatibility. The research in the base also needs more research and the use of biocompatible material.

The study is mainly based on scientific papers and hypotheses. This 3D-bioprinting technology is the future for the baldness problem, and the hypothesis is for an affordable procedure for hair cosmetics.

4.1 Future work

If this hypothesis becomes successful, more research is needed on the biofibre and biodegradability of the base material. It also reduces the human interface in the permanent hair transplant procedure. Also, the length of the biofibre needs to be controlled. This means it should be done if the biofibre needs longer after the procedure.

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