# New Concept of Tissue Aging Based on New Hair Research

## Mystery of hair thinning by aging

MANY THEORIES HAVE been proposed to explain the aging of our bodies since the 19th century, yet aging is still a mysterious phenomenon. Hair thinning is one of the most prominent aging phenotypes in mammals. Hair is generated in a mini-organ called a hair follicle. Stem cells called hair follicle stem cells (HFSCs) divide to renew themselves to keep generating their progeny which grow hair. However, hair follicles gradually decrease in number after repetition of hair cycles, which thins a person's hair during aging. We have studied the common aging phenomenon for the last 10 years and finally succeeded in partially solving the mystery of aging by using stem cell tracing technologies.

## Fate tracing of hair follicle stem cells

Hair follicles repeat their cyclic regeneration and regression forming a hair cycle through their cyclic activation and inactivation of HFSCs. A detailed analysis of HFSCs in aged mice and human scalp skin revealed that the stem cells decrease in number by aging in a scattered manner on the skin. As neither a significant increase of signs for cell death nor cellular senescence has been found in those stem cells during aging, we hypothesized that the fate of HFSCs may change during aging. We thus applied the fate tracing technique of genetic Cre-loxP recombination system onto the analysis of HFSCs in mice to analyze the dynamics and fate of aged stem cells.

HFSCs normally reside in a specific area called the hair follicle bulge and provide their progeny to the hair follicle bulb to grow hair. In aged mice, genetically tagged stem cells in the bulge unexpectedly migrate up toward the epidermis. It turns out that those aged stem cells actually change their fate to the differentiated epidermal keratinocytes to be shed off as dandruff from the skin surface. Those affected hair follicles are indeed miniaturized by loss of the stem cells and eventually disappear from the skin.

## Stem cell-centric aging program

How is the fate of stem cells changed by aging? The answer to this question comes from patients of progeroid syndroms (genomic instability syndrome), who age much faster than usual, and their mouse models. Premature hair thinning in many of those patients occurs due to impaired DNA damage repair. Similarly, we found that HFSCs in aged mice accumulate genomic damage. We thus hypothesized that the fate change of stem cells may be driven by

**Emi Nishimura**
Professor,
MD, PhD,
Stem cell biology,
Medical Research Institute,
Tokyo Medical and Dental University, TMDU

excessive DNA damage response in those stem cells during aging. Indeed, we found that XVII collagen (COL17A1), which we previously demonstrated to be expressed by HFSCs and essential for their maintenance, is degraded in HFSCs in response to DNA damage as well as by aging. Also the sustained maintenance of this collagen in stem cells in mice significantly delayed hair follicle aging and hair thinning during physiological aging. As far as we know, this is the first research to reveal the existence of such a tissue aging program despite the existence of many theories of aging. Importantly, the program is driven by stem cell aging. We believe that this study may open up a new venue for treatment of common hair thinning as well as for intractable alopecia caused by cancer therapy such as radiotherapy and chemotherapy.

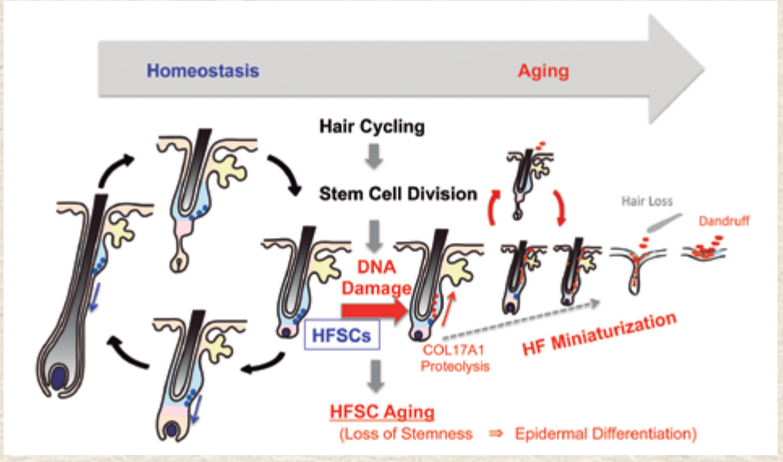


***Fig.1:*** Mechanism of hair follicle aging and hair thinning (Matsumura H et al. Science, 351 (6273) :575, 2016)





# Injected mix of bone-augmenting agents causes new bone growth in mouse jaws

**RESEARCHERS CENTERED AT** Tokyo Medical and Dental University (TMDU) deliver a protein/peptide combination using an injectable gel carrier to promote bone formation in mouse jawbones

The part of the jawbone containing tooth sockets is known as alveolar bone, and its loss over time or following dental disease may ultimately result in tooth loss. While dentures can be used as a tooth replacement, the mechanical stimuli under the dentures causes further bone loss. An alternative and more permanent solution is strongly hoped for. Recombinant human bone morphogenetic protein 2 (BMP-2) has been used to stimulate osteogenesis (bone formation) in humans, but high doses can cause inflammation and are recently reported to increase the risk of cancer. Therefore, agents such as peptide drugs for accelerating bone augmentation need to be developed, even in the presence of lower doses of BMP-2. Additionally, there are no known means of stimulating local bone augmentation without performing surgery.

The peptide OP3-4 has been shown to inhibit bone decay and stimulate the differentiation of cells (osteoblasts) that form bone. Now, an international team centered at Tokyo Medical and Dental University has injected a gelatin-based gel carrying OP3-4 and BMP-2 into mice jawbones to trigger local augmentation of bone around the injection site. The study was recently reported in the Journal of Dental Research.

Use of this injectable gelatin-based gel to carry the agents avoids the need for surgical implantation and resulted in no swelling or other such complications in the experimental mice. The researchers observed a region of increased bone mass around the BMP-2 + OP3-4 injection site that was larger than that seen in mice injected with BMP-2 alone, or with other controls. This mass also had a significantly higher bone mineral content and density (Fig. 1).

Microscopic examination confirmed the deposition of calcified tissue (mineralization) and the intensive bone formation in the BMP-2 + OP3-4-treated mice (Fig. 2).

"Mineralization of the outer region evidently took place before that of the inner region," lead author Tomoki Uehara (section of Pediatric dentistry in TMDU) says (Fig. 2). "We speculate that the size of the new bone is determined before calcification starts, and that OP3-4 plays an important role in making a regeneration site at the early stage of bone formation."

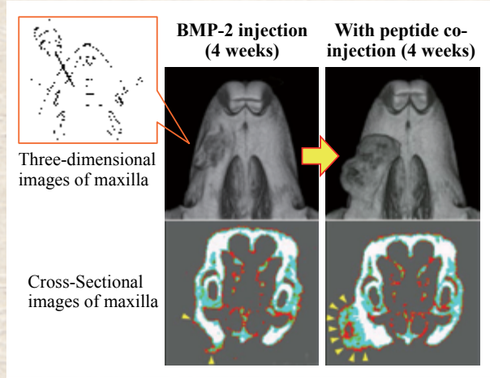


***Fig.1:*** **Peptide co-injection thickens mouse bone.** Yellow arrowheads (lower panels) indicate newly formed bone.

**Kazuhiro AOKI**
DDS, PhD,
Professor
Department of Basic Oral Health Engineering,Graduate School of Medical and Dental Sciences,Tokyo Medical and Dental University,TMDU

Corresponding author Kazuhiro Aoki adds: "OP3-4 further enhanced the number of bone-forming cells induced by BMP-2 treatment, and also increased the expression of genetic markers of bone formation."

The article "Delivery of RANKL-Binding Peptide OP3-4 Promotes BMP-2-Induced Maxillary Bone Regeneration" was published in the Journal of Dental Research (Uehara et al, J Dent Res, 95: 665-72, 2016)

**Summary Text:** A Tokyo Medical and Dental University (TMDU)-centered research team combined a protein that stimulates bone formation with a peptide that promotes osteoblast differentiation, and delivered them into mouse jawbones by injection within a gelatin carrier. The technique induced formation of new bone, suggesting its potential as a non-invasive means of replacing lost jawbone.

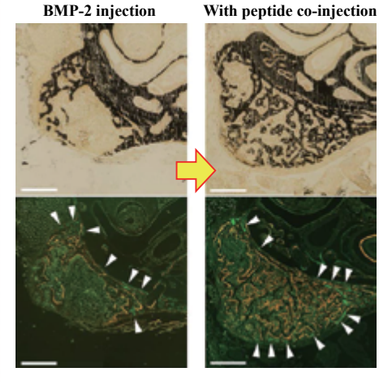


***Fig.2:*** **Microscopic views of newly formed bone**
Black area mainly shows newly formed bone (upper panels). Green lines (white arrowheads) and yellow area indicate bone formation activity on day 12 and 26 after injections, respectively. Bar represents 0.5 mm.

# Role of Sox17 Protein in Embryo Implantation Key to Mouse Fertility

**RESEARCH CENTERED AT** Tokyo Medical and Dental University (TMDU) identifies a novel role for the Sox17 protein in uterine receptivity and mouse embryo implantation

Tokyo–Assisted reproductive technology is commonly used to treat human infertility, but the rate of successful pregnancies is still low. One reason for this is the failure of embryos to implant in the uterus. Embryonic implantation is a complex process that must occur within a short window of time when the lining of the uterus is receptive. Although signaling pathways and hormones from the ovary are known to be necessary for pregnancy to occur, the molecular mechanisms underlying this are unclear. An international team led by Tokyo Medical and Dental University (TMDU) has now revealed that expression of the protein Sox17 is required for uterine receptivity and embryo implantation in mice. The study was reported in Scientific Reports.

Implantation in the uterus occurs on the fifth day of embryo development in mice, and is essential for progression beyond the blastocyst stage. The maternal hormones progesterone and estrogen regulate uterine receptivity in both humans and mice, while the Sox17 protein is known to be expressed during implantation in the uterine lining, with possible roles in progesterone mediation and blastocyst attachment.

TMDU-led researchers confirmed this expression of Sox17 in mice, and also detected slightly lower levels of Sox17 expression in the oviduct and blood vessels. Female mice carrying a mutation in one copy of the Sox17 gene had

**Masami Kanai-Azuma**
DVM, PhD, Professor
Department of Experimental Animal Model for Human Disease
Graduate School of Medical and Dental Sciences,Tokyo Medical and Dental University ,TMDU

lower fertility levels than control mice. "Sox17 heterozygosity had no adverse effect on ovulation, fertilization, or the morphology of the uterus," first author Yoshikazu Hirate says. "However, we observed defective implantation in these females, resulting from fewer implantation sites."

The team predicted that the reduced implantation occurred because of a shortage of Sox17 gene product, so-called haploinsufficiency, although some mutant females were unaffected and had normal litter sizes.

"This suggests that a protein similar to Sox17, such as the related Sox7 or Sox18, is compensating for its absence in these cases," corresponding author Masami Kanai-Azuma says. "However, Sox17 appears to be the key player among Sox-F proteins in embryonic implantation."

The article "Mouse Sox17 haploinsufficiency leads to female subfertility due to impaired implantation" was published in Scientific Reports at DOI: 10.1083/srep24171

**Summary Text**: Tokyo Medical and Dental University (TMDU) researchers discovered the importance of Sox17 protein expression in female mouse fertility. Mice with only one functional Sox17 gene had lower levels of embryonic implantation than controls, causing reduced fertility. This understanding of the role of Sox17 in implantation may help improve human infertility treatment.

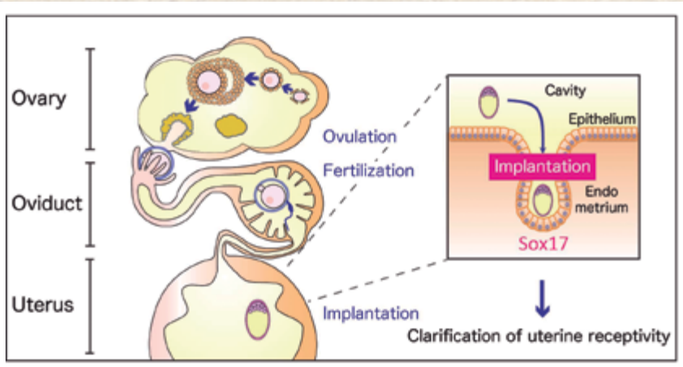


***Fig.1:*** **Uterine Sox17 expression is necessary for embryo implantation.**

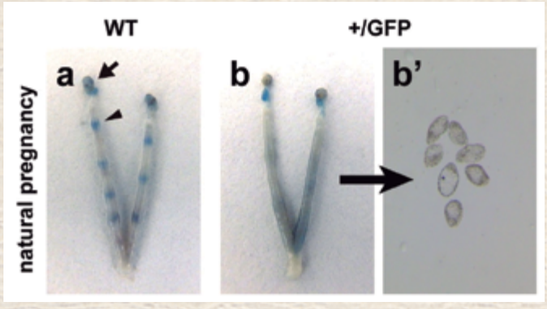


***Fig2:*** **Blue dye staining showing implantation sites in WT and *Sox17*$^{+/GFP}$ uteri at 5 days after natural mating. Arrows and arrowheads show the ovaries and implantation sites, respectively. (b') Unimplanted normal blastocysts recovered from *Sox17*$^{+/GFP}$ uterus by flushing.**





# Breakthrough for bone regeneration via double-cell-layered tissue engineering technique

**TOKYO, JAPAN -VARIOUS** technologies have been developed to introduce laboratory-grown bone-forming cells into bone defects to promote their repair. However, these have many limitations as the conditions of the cells and their surroundings do not accurately mimic those typically found in the body. This means they cannot optimally promote bone formation. A research team at Tokyo Medical and Dental University (TMDU) has now made a major advance in overcoming these difficulties by developing a technique for producing double-layered cell constructs that can be transplanted onto bone defects. The technique increases the speed of bone repair and the flexibility and durability of the constructs make them ideal for many surgical applications.

Cells with various functions can now be cultured in the laboratory and then introduced into the body to treat different medical conditions. However, as individual cells can spread away from the site of injury, they need to be held in place on a scaffold, which is then transplanted into the body. Substantial progress has already been made in this sort of tissue engineering. When the body repairs broken or damaged bones, it employs a complex system of molecular signals and cells, including osteoblasts that build up the calcium matrix on which bone is based. To speed up the repair of bone defects by artificial means or enable recovery from severe injuries, tissue engineering approaches thus need to mimic this complex system.

"After establishing our double-layered cell transfer technology, we used it to apply different combinations of cells related to bone formation to defects in mouse skulls," first author Keiko Akazawa says. "We found that osteoblasts together with stem cells from tooth-supporting ligament were particularly more effective at promoting bone repair than

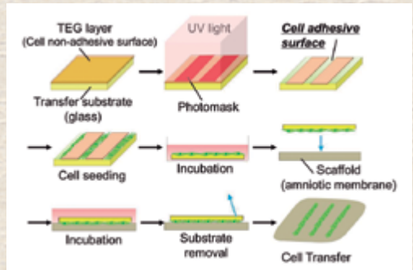


***Fig.1:*** (A) We coated the surface of glass substrate with tetraethylene glycol (TEG, brown) and the layer was partially degraded by UV irradiation to prepare hydrophilic cell adhesive surface (green). Cells to be transferred were poured onto the substrate and incubated to allow the cells to adhere to the substrate surface. Transfer substrate with cells was then placed onto the scaffold (amnion) in the direction of cell surface down. Cells were further cultured and transfer substrate was carefully removed subsequently. Cells were transferred onto scaffold surface. (B) Cells of the first layer (green) were seeded on the transfer substrate and cultured. Then, the cells of the second layer (red) were seeded onto the cells of the first layer. After incubation, transfer substrate bearing two layers of cells was placed onto the amnion to make direct contact between cells and scaffold surface. Double cell layers were transferred onto the scaffold material after the removal of the transfer base.

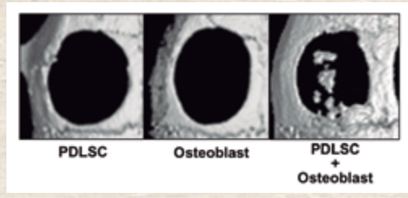


***Fig.2:*** Micro CT images of bone defects 4 weeks after the transplantation of cell-transferred amnion. In the single cell transplantation (mesenchymal stem cells from periodontal ligament (PDLSC) or osteoblast), bone healing was limited while new bone-like tissue formation was observed in bone defects transplanted with double-layered cell-transferred amnion (PDLSC+Osteoblast).

**Kengo Iwasaki**
DDS, PhD, Junior Associate Professor
Department of Nanomedicine (DNP), Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University,TMDU

equivalent scaffolds containing only a single cell layer."

The double-layered cell constructs were also tested for their stability and flexibility. The cells remained attached despite folding the constructs or trimming them to fit the shape of a particular defect. Coauthor Kengo Iwasaki says: "The durability of these new constructs makes them particularly suitable for surgical applications. We have high expectations for their use in regenerative medicine for treating a range of defects using different cell layer combinations."

The article "Double-layered cell transfer technology for bone regeneration" was published in Scientific Reports at DOI: 10.1038/srep33286.

**Summary Text**: Tokyo Medical and Dental University (TMDU) researchers developed a technique for attaching two distinct layers of cells on top of each other on an amnion-based scaffold. When osteoblasts and mesenchymal stem cells were used to form the layers, the cell constructs more effectively promoted bone regeneration after implantation onto skull defects in mice, compared with their single-cell-layer equivalents. This new approach has a range of potential applications for tissue engineering in the field of regenerative medicine.

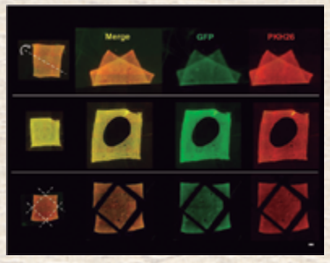


***Fig.3:*** Fluorescence microscopic images of amnion holding double-layered cells after deformation (top), holing (middle) and trimming (bottom) of the membrane. Despite deformations and trimming of cell-transferred amnion, cells stably adhered onto the scaffold material. Green (GFP): First layer cells, Red (PKH26): Second layer cells. Bar = 1 mm