Journal of Functional Foods 116 (2024) 106124

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Short communications

Revealing novel insights on how oral supplementation with collagen peptides may prevent hair loss: Lessons from the human hair follicle organ culture

Karin I. Pappelbaum [a], Nicolina Virgilio [b], Lisa Epping [a], Bastiaan van der Steen [b], Francisco Jimenez [c,d], Wolfgang Funk [e], Janne Prawitt [b], Marta Bertolini [a,*]

[a] Monasterium Laboratory Skin and Hair Research Solutions GmbH, a QIMA Life Science Company, Münster, Germany
[b] Rousselot BV, Ghent, Belgium
[c] Mediteknia Hair Transplant Clinic and Hair Lab, Gran Canaria, Canary Islands, Spain
[d] University Fernando Pessoa Canarias, Las Palmas de Gran Canaria, Spain
[e] Clinic for Plastic, Aesthetic and Reconstructive Surgery Dr. Dr. med. Funk, Munich, Germany

ARTICLE INFO

Keywords:
Collagen peptides
Dietary supplementation
Hair growth
Hair shedding
Hair loss
Hair follicle stem cells

ABSTRACT

Dietary supplementation with bioactive collagen peptides (CPs) may be a helpful adjuvant strategy in reducing the excessive hair shedding and thinning associated with aging or patterned hair loss. However, the underlying biological effects of CPs on hair follicle (HF) function have not yet been characterized. Excessive hair loss occurs when HFs exit the growth phase (anagen) prematurely due to, among other factors, impaired epithelial HF stem cell (eHFSC) activities. The aims of this study were to investigate the response of human HFs to peptides from the two most popular CP sources, i.e. marine and bovine collagens, which were previously *in vitro*-digested to mimic gastrointestinal breakdown, in one of the most clinically-relevant *ex vivo* HF models. Both types of collagens reduced the proliferation of pluripotent K15+ eHFSCs and enhanced the generation of K19+ and/or CD34+ stem cell progenies. In addition, bovine CPs significantly increased K15+ cells in the bulge and marine CPs significantly maintained HFs longer in anagen. Our results suggest that both marine and bovine CPs may help to prevent hair loss and maintain healthy hair by preserving eHFSCs and/or improving the generation of SC progenies. This data invites the further exploration of other CPs for the prevention of excessive hair shedding.

1. Introduction

Healthy hair comprises both the hair follicle (HF) in the scalp and the hair shaft. (Bertolini et al., 2018; Schneider et al., 2009). Healthy hair growth, density and fiber quality depends on optimal HF function and low levels of damage to the hair shaft. To achieve healthy hair, both cosmetic (topically applied) and dietary supplements should be used to improve HF performance and hair shaft properties (Bertolini et al., 2018; Lim et al., 2019).

HFs are mini-organs that undergo cyclical growth- (anagen), transition- (catagen), and resting- (telogen) phases, during the so-called hair cycle. The hair cycle is regulated by reciprocal interactions between epithelial and mesenchymal cells of the HF (Schneider et al., 2009). At the onset of anagen, molecular inductive signals from a specialized mesenchyme (dermal papilla (DP) fibroblasts) activate epithelial hair follicle stem cells (eHFSCs) and initiate a new hair cycle (Hsu et al., 2014; Rahmani et al., 2014). In human HFs, eHFSCs are identified as K15+ cells residing in their stem cell niche in the bulge region of the upper HF (Garza et al., 2011; Purba et al., 2017). eHFSCs maintain the capacity for self-renewal and pluripotency throughout life, which is linked to their K15 expression, and they are normally quiescent (non-proliferating) during anagen (Garza et al., 2011). However, during activation, eHFSCs proliferate and generate K19+ and CD34+ cell progeny, which are postulated to differentiate into HF outer root sheath (ORS) keratinocytes and fast-cycling, transit-amplifying cells (TACs). These TACs continue to divide to form a new hair bulb containing the hair matrix (HM) keratinocytes which proliferate, differentiate and synthesize all the different hair keratins required to form the hair shaft and its surrounding structures during anagen (Hsu et al., 2011, 2014; Inoue et al., 2009; Purba et al., 2014, 2017).

* Corresponding author at: Monasterium Laboratory GmbH, a QIMA Life Science Company, Mendelstrasse 17, 48149, Münster, Germany.
E-mail address: m.bertolini@monasteriumlab.com (M. Bertolini).

https://doi.org/10.1016/j.jff.2024.106124
Received 16 November 2023; Received in revised form 1 March 2024; Accepted 5 March 2024
Available online 16 March 2024
1756-4646/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Hair loss, seen during aging and in HF disorders such as telogen effluvium or male/female pattern hair loss (M/FPHL, also commonly known as androgenetic alopecia, AGA), results from hair cycle disturbances and causes severe psychological distress (Gentile & Garcovich, 2021). These forms of hair loss are characterized by excessive shedding, gradual decrease in anagen growth phase duration, premature catagen entry, and an extended telogen resting phase (Garza et al., 2011; Gentile & Garcovich, 2019). Also, progressive miniaturization of the HF leads to the production of shorter, lighter-colored and thinner hair shafts in pattern hair loss and aging (Pantelireis & Higgins, 2018). The precise underlying mechanisms driving hair thinning and balding are unclear but mouse and human data imply impaired DP fibroblast and/or reduced eHFSC functions (Garza et al., 2011; Mohammadi et al., 2021). Specifically, HFs from aged mice show reduced eHFSC K15+ cell numbers (Matsumura et al., 2016) while those from the bald scalps of MPHL reveal markedly diminished numbers of CD34+ eHFSC progeny (Garza et al., 2011).

There are limited options available for preventing or delaying aging- and M/FPHL-induced hair loss. Dietary supplementation could be a potential solution, since quantity and quality of hair are critically associated to the nutritional state of an individual (Finner, 2013). Recent evidence (Piccini et al., 2022) suggests that HFs from female hair loss patients exhibit nutrient deficiencies and a more quiescent metabolism, making dietary supplementation an appealing intervention to combat hair loss (Avila Rodríguez et al., 2018; Helal et al., 2019).

Collagen peptides (CPs) are gaining attention as bioactive ingredients in nutricosmetic products for improving skin properties (Venkatesan et al., 2017; Salvatore et al., 2020; Proksch et al., 2014). Upon digestion, CPs break down into free amino acids and di- or tripeptides characterized by a high content of the amino acids hydroxyproline, glycine, and proline (Asserin et al., 2015). Following absorption from the gut, they are transported into the skin and support the formation of new collagen and elastin fibers and actively modulate cell functions (León-López et al., 2019; Sibilla et al., 2015). Dietary supplements containing CPs reportedly increased hair thickness (Oesser, 2020) and proved effective for hair loss in patients suffering from patterned hair loss and/or telogen effluvium (Arias et al., 2022; Milani and Colombo, 2023).

Here, the effects of marine- and bovine-derived CPs (m- and b-Collagen, respectively) on human HF functions were investigated using the clinically-relevant human HF organ culture model (Campiche et al., 2022; Chéret et al., 2018; Edelkamp et al., 2020; Jimenez et al., 2021) under physiologically relevant experimental conditions, including the simulation of human digestion of the CPs, to mimic oral ingestion and metabolism, prior to treatment of the HFs (Brodkorb et al., 2019). We set out to detect possible effects of CPs on a range of end points including HF cycle, cell proliferation and apoptosis, the number of eHFSCs and direct progeny, keratin expression, DP cell functions, hair fiber growth rate and melanogenesis.

2. Material & methods

Detailed materials and methods are provided in the "Supplementary file.".

2.1. Donor material and ethics consideration

Human HF specimens were obtained from three male (age 45, 48, 59 years) and female (age 27, 41, 48 years) donors after informed written patient consent and ethics committee approval (Medical Association of Westfalen-Lippe and Westfälischen Wilhelms University, no. 2015-602-f-S). All experiments on human tissue were performed according to the study plan 2020-954-f-S and the Declaration of Helsinki principles.

2.2. Digested collagen peptides and blank preparation

Peptan® Marine 5000 (5 kDa), a hydrolyzed collagen made from fish skins of wild caught fish living in oceans, here called m-Collagen, and Peptan® B 5000 (5 kDa), a mixture of bovine-hide derived collagen peptides, here called b-Collagen, were provided by Rousselot BV (Ghent, Belgium). Both marine and bovine CPs were digested *in vitro* prior to use, according to the Infogest protocol, an international consensus for simulated gastrointestinal digestion in humans (Brodkorb et al., 2019). As a control, the *in vitro* digestion protocol was performed without collagen peptides (blank).

2.3. Hair follicle organ culture and treatment

Human amputated HFs were micro dissected from scalp skin as previously described (Edelkamp et al., 2020) and cultured for 5–6 days *ex vivo* with m-Collagen (0.01 mg/ml), b-Collagen (0.01 mg/ml) or blank (vehicle control; 0.01 mg/ml) dissolved in William's culture medium (WCM). During organ culture, hair follicle length and hair shaft production were measured using a digital light microscope at 50X magnification (VHX900; Keyence Corporation, Osaka, Japan) (Edelkamp et al., 2020).

2.4. Immunofluorescence, histochemistry & (immuno-)histomorphometry

Frozen sections of HFs were collected and used for immunofluorescence or histochemical staining as previously described (Bodó et al., 2007a; Tobin, 2008; Purba et al., 2017; Edelkamp et al., 2020; Campiche et al., 2022). Images were taken with a Keyence fluorescence microscope (BZ9100; Osaka, Japan), maintaining a constant set exposure time throughout imaging for further analysis. Hair cycle staging and scoring was performed at the end of the culture according to established parameters (Kloepper et al., 2010; Bertolini et al., 2021a).

2.5. Statistical analysis

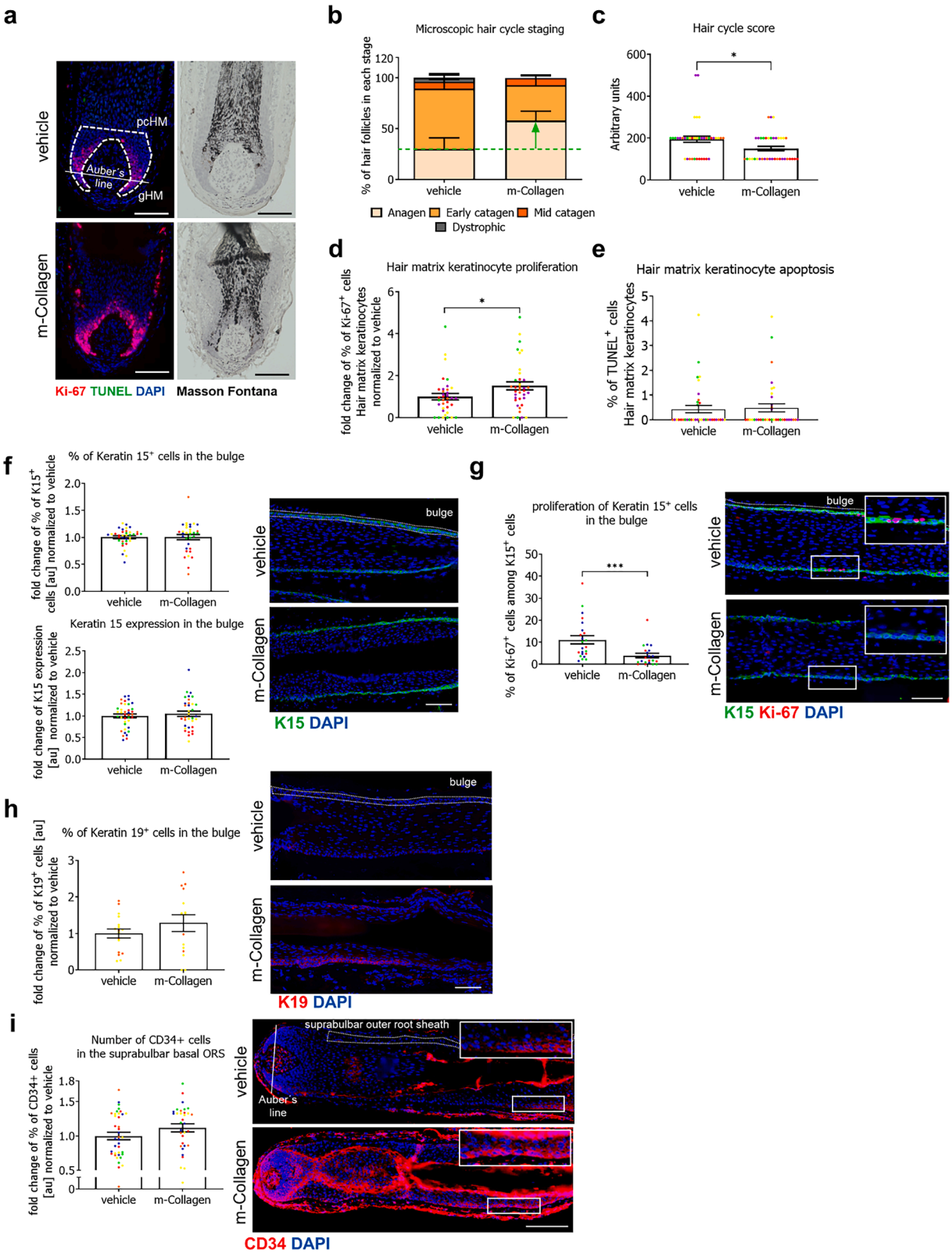
Statistical analyses were performed using Graphpad Prism 9 (GraphPad Software Inc.). Data were tested for normal distribution using the D'Agostino & Pearson omnibus normality test. When data did not follow normal distribution the Mann-Whitney *U* test was used. When data were normally distributed an unpaired student's *t*-test was used. Data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. M-collagen prolongs anagen phase ex vivo

First, it was validated that neither m-Collagen nor b-Collagen application induced cytotoxicity in human HF organ cultures, in that no excessive melanin clumping was observed; **Fig. S1a**. Afterwards, the effect of m- or b-Collagen application on HF *ex vivo* was investigated by examining hair cycle staging and resultant cycle score as previously described (Kloepper et al., 2010; Langan et al., 2015). Application of m-Collagen maintained significantly more HFs in anagen phase when compared to vehicle treated controls (Fig. 1**a-b**). The anagen-prolonging effect of m-Collagen was verified by a significant decrease in hair cycle score (Fig. 1**c**). In line with this, the germinative HM keratinocytes showed significantly higher cell proliferation (Ki-67+) together with an unaffected apoptosis rate (TUNEL) upon treatment with m-Collagen in comparison with control (Fig. 1**a, d-e**). In contrast, treatment with b-Collagen had no effect on the percentage of HFs in anagen, the hair cycle score (**Fig. S1b-d**) or HM keratinocyte proliferation and apoptosis (**Fig. S1e-f**).

Anagen phase is characterized by keratin synthesis, hair shaft production (Schneider et al., 2009), and melanogenesis (Van Neste & Tobin, 2004), all requiring continued production of DP derived hair growth



(caption on next page)

factors (Greco et al., 2009; Kishimoto et al., 2000; Qiao et al., 2009; Schneider et al., 2009). However, m- and b-Collagen did not affect any of these biological processes (Suppl. Results, and Fig. S2a-f). Thus, the catagen preventing effects of m-Collagen in HFs *ex vivo* are linked to other factors important for maintaining hair growth, such as preserving and modulating eHFSCs.

Fig. 1. Marine derived Collagen peptides prolong anagen phase in human hair follicles, increase hair matrix keratinocyte proliferation and reduce K15+ cell proliferation in the bulge. a) Representative images of Ki-67/TUNEL immunostaining and Masson Fontana staining to quantify anagen and catagen HFs, as well as hair matrix keratinocyte proliferation and apoptosis under vehicle or marine derived Collagen (m-Collagen) treatment. Ki-67+ cells (red) in the gHM below the Auber's line were counted for proliferation and in the gHM and pcHM for apoptosis. b) Quantitative analysis of hair cycle staging of anagen and catagen HFs under vehicle or m-Collagen treatment. c) Quantitative analysis of hair cycle score of anagen and catagen HFs under vehicle and m-Collagen treatment. Arbitrary units (au) were assigned to anagen (100), early catagen (200), mid catagen (300), and dystrophic (500) HFs. d) Quantitative analysis of proliferating (Ki-67+) keratinocytes in the gHM of vehicle or m-Collagen treated HFs. e) Quantitative analysis of apoptotic (TUNEL+) keratinocytes in gHM and pcHM of vehicle or m-Collagen treated HFs. f) Quantification of the percentage of Keratin 15 positive (K15+) cells and K15 expression intensity in the bulge in HFs treated with vehicle or m-Collagen (left panel). Representative images of K15 immunostaining in the bulge (right panel). g) Quantification of the percentage of proliferating (Ki-67+) K15+ cells in the bulge in HFs treated with vehicle or m-Collagen (left panel). Representative images of K15/Ki-67 immunostaining in the bulge (right panel). h) Quantification of the percentage of Keratin 19 positive (K19+) cells in the bulge in HFs treated with vehicle or m-Collagen. (left panel). Representative images of K19 immunostaining in the bulge (right panel). i) Quantification of CD34 positive cells in the suprabulbar basal ORS of m-Collagen treated HFs (left panel) and representative images of CD34 immunostaining in the suprabulbar ORS (right panel). All experiments are presented as mean ± SEM of (b) n = 38–39 HFs from five donors, (c) n = 38–39 HFs from five donors, (d) n = 36 HFs from five donors, (e) n = 36 HFs from five donors, (f) n = 33–38 from five donors, (g) n = 19–23 HFs from three donors, (h) n = 13–17 HFs from two donors and (i) n = 33–38 HFs from five donors. The data were tested for normality with the D'Agostino & Pearson omnibus normality test and further compared with an unpaired student's *t*-test when the datasets followed a normal distribution or a Mann-Whitney test if not (c,d,g). *$p < 0.05$, ***$p < 0.001$. Independent experiments are indicated with dots of different colors: green and yellow represent HFs from the female donors, red, blue, and orange represent HFs from the male donors. Scale bars: 100 μm; HF: hair follicle, gHM: germinative hair matrix, pcHM: precortical hair matrix. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. M-collagen reduces K15+ bulge eHFSCs proliferation

The effect of CPs on number and distribution of eHFSCs and their progeny was examined by analyzing the eHFSC markers K15, K19 and CD34 in the bulge and subORS to include pluripotent and progenitor cells (Purba et al., 2017). Treatment with m-Collagen neither affected percentages of eHFSC K15+ cells, nor K15+ expression in the bulge (Fig. 1**f**). However, K15+ cell proliferation was significantly lower in the bulge under treatment with m-Collagen in comparison to vehicle treated controls (Fig. 1**g**), indicating maintenance of quiescence, preservation of the eHFSC niche and a more stem cell-like phenotype (Purba et al., 2017). In the subORS where eHFSC progeny are located, the percentage of K15+ cells and proliferation remained unaltered upon m-Collagen treatment (**Figure S3a-b**). M−Collagen and vehicle treated HFs also showed similar percentages of apoptotic K15+ cells, as well as double positive K15+ K19+ cells in the bulge and subORS (**Figure S3c-f**). The percentage of K19+ cells in the bulge was slightly higher in HF treated with m-Collagen, but not in the subORS SC niche (Fig. 1**h** and **Fig S3g**). Although non-significantly, this trend, together with higher numbers of CD34+ cells in the subORS in HFs treated with m-Collagen (Fig. 1**i**) indicates that m-Collagen treatment promotes differentiation of eHFSCs into K19+ and CD34+ progenitor cells or modulates directly these HFSC subpopulations, while also maintaining a quiescence eHFSC niche *ex vivo*. This suggest that m-Collagen support the maintenance of anagen at least in part by regulating eHFSC activities.

3.3. B-collagen increases the percentage of K15+ bulge eHFSCs, reduces their proliferation and apoptosis and enhances the percentage of K19+ cells

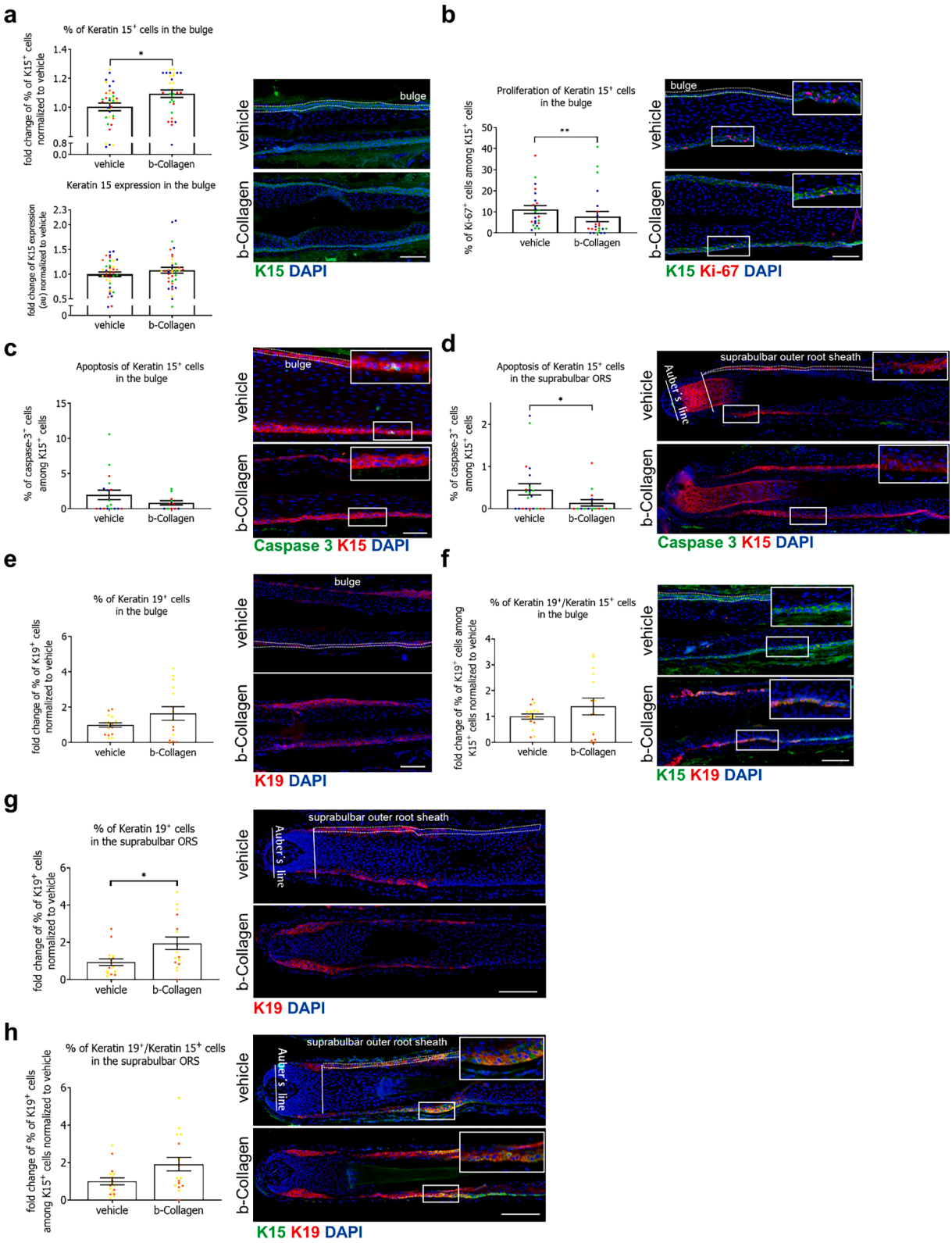
The percentage of K15+ cells was significantly increased in the bulge of b-Collagen treated HFs compared to vehicle treated controls indicating a reinforcement of the stem cell niche (Garza et al., 2011), although no pronounced effect on K15 expression was observed (Fig. 2**a**). Application of b-Collagen had no effect on the percentage of K15+ cells in the subORS (**Figure S4a**) while significantly fewer proliferating K15+ cells were detected in the bulge of HFs exposed to b-Collagen (Fig. 2**b**). This was not seen in the subORS (**Figure S4b**) where cells are typically more proliferative (Chen et al., 2020). B-Collagen treatment resulted in a tendency towards reduced K15+ cell apoptosis in the bulge, while it significantly decreased apoptosis of K15+ cells in the subORS, suggesting protection of pluripotency in this secondary stem cell niche in the HF (Fig. 2**c-d**). Contrary to m-Collagen, b-Collagen treatment had no effect on the percentage of CD34+ cells (**Figure S4c**). However, slightly higher numbers of K19+ single and K15+ K19+ double positive cells were found in the bulge of HFs under b-Collagen treatment (Fig. 2**e-f**). In the subORS the percentage of single positive K19+ cells were significantly higher and that of K15+ K19+ double positive cells marginally higher (Fig. 2**g- h**) versus the control follicles. Thus, b-Collagen treatment reinforces the eHFSC niche, stemness and promotes the generation of K19+ eHFSC progeny.

4. Discussion

Collagen supplementation for skin health is a major area of nutraceuticals development and commercialization, but there is considerably less data on its benefits for hair. This study is the first to demonstrate the beneficial effects of hydrolyzed m-Collagen and b-Collagen peptides on key biological processes of the intact human HF, using the clinically relevant HF organ culture model (Campiche et al., 2022; Chéret et al., 2018; Edelkamp et al., 2020; Jimenez et al., 2021). While only the application of m-Collagen inhibits catagen development *ex vivo*, both collagens demonstrate interesting and divergent effects on distinct eHFSCs populations.

It is widely accepted that amino acid composition, molecular structure and -weight, and hydrophobic properties influence the functional outcomes of CPs and other bioactive peptides (Aguirre-Cruz et al., 2020; González-Serrano et al., 2022; León-López et al., 2019; Nuñez et al., 2020; Venkatesan et al., 2017). Interestingly, teleost fish species express three collagen 1 α-chains, whereas mammals express only two (Kleinnijenhuis et al., 2022), and the hydroxyproline content and/or the degree of hydroxylation of proline amino acids differs between marine and bovine species (Bao et al., 2018; Carvalho et al., 2018). Thus, these differences may explain the divergent functional outcomes described following supplementation with bioactive CPs isolated from fish and mammals (Nuñez et al., 2020), as observed in our study, in the context of HF biology. Yet, in other biological contexts, i.e. modulation of bone cell metabolism, products of bovine, porcine and fish origin all had comparable effects (Wauquier et al., 2019). Although the differences between CPs from different species become less apparent as the mean length of the peptides decreases (Kleinnijenhuis et al., 2020), it is important to note that the potential impact of the raw material source of the product may also depend on the target organ or cell.

Another explanation for the differing effects of m- and b-Collagen on HFSC function could be that CPs regulate the stem cell niche by interacting with extracellular matrix (ECM) components (Ahmed & ffrench-Constant, 2016; Abdal Dayem et al., 2023). CPs can bind to ECM integrin receptors (Leitinger, 2011; Zeltz & Gullberg, 2016), which are also expressed in HFs (Jones & Watt, 1993; Jones et al., 1995; Commo et al., 2000; Kloepper et al., 2008; Ernst et al., 2013). Particularly β1 integrin-mediated signaling is critical for human TAC generation, HM keratinocyte proliferation, anagen prolongation (Ernst et al., 2013; Kloepper et al., 2008), and eHFSC maintenance (Jones et al., 1995; Jones & Watt, 1993). However, human eHFSC subpopulations differ in



(caption on next page)

their responses to β1-integrin activation (Commo et al., 2000; Ernst et al., 2013) suggesting that distinct integrin ligands, such as hydrolyzed m- or b-Collagen, may have different effects within the HFs, particulary within the eHFSC niche, as observed in our study.

Besides direct effects on the HF, the impact of m-Collagen and b-Collagen on HF function could also be indirectly mediated through surrounding cells, as CPs are known to facilitate morphogen and growth factor signaling, and pro-inflammatory cytokine production in different cells and tissues surrounding the HF, all of which are important for anagen maintenance (Ahmed & ffrench-Constant, 2016; Brandao-

Fig. 2. Bovine derived Collagen peptides upregulate K15+ cells in the bulge, diminish K15+ cell proliferation in the bulge and K15+ cell apoptosis in the suprabulbar outer root sheath and increase K19+ cell numbers in the suprabulbar outer root sheath. a) Quantification of the percentage of K15+ cells and K15 expression in the bulge in HFs treated with vehicle or b-Collagen (left panel). Representative images of K15 immunostaining in the bulge (right panel). b) Quantification of the percentage of proliferating (Ki-67) K15+ cells in the bulge in HFs treated with vehicle or b-Collagen (left panel). Representative images of K15/ Ki-67 immunostaining in the bulge. (right panel). c) Quantification of the percentage of apoptotic (caspase 3+) K15+ cells in the bulge of HFs treated with vehicle or b-Collagen (left panel). Representative images of caspase 3/K15 immunostaining in the bulge (right panel). d) Quantification of the percentage of apoptotic (caspase 3+) K15+ cells in the suprabulbar basal ORS in HFs treated with vehicle or b-Collagen (left panel). Representative images of caspase 3/ K15 immunostaining in the suprabulbar ORS (right panel). e) Quantification of the percentage of K19+ cells in the bulge of vehicle or b-Collagen treated HFs. Outliers were removed (left panel). Representative images of K19 immunostaining in the bulge (right panel). f) Quantification of K15+/K19+ double positive cells in the bulge ORS of vehicle or b-Collagen treated HFs (left panel). Representative images of K15/K19 immunostaining in the bulge (right panel). g) Quantification of the percentage of Keratin 19 positive (K19+) cells in the suprabulbar basal ORS of vehicle or b-Collagen treated HFs (left panel). Representative images of K19 immunostaining in the suprabulbar ORS (right panel). h) Quantification of K15+/K19+ double positive cells in the suprabulbar basal ORS and the bulge of vehicle or b-Collagen treated HFs (left panel). Representative images of K15/K19 immunostaining in the suprabulbar ORS (right panel). All experiments are presented as mean ± SEM of (a) n = 31–38 HFs from five donors, (b) n = 21–23 HFs from three donors, (c) n = 11–18 HFs from three donors, (d) n = 15–22 HFs from 3 donors, (e) n = 14–17 HFs from two donors, (f) n = 15–17 HFs from two donors, (g) n = 16–17 HFs from two donors and (h) n = 15–17 HFs from two donors. The data were tested for normality with the D'Agostino & Pearson omnibus normality test and further compared with an unpaired student's *t*-test when the datasets followed a normal distribution (c,e,f,h) or a Mann-Whitney test if not (a,b,d,g). *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$. Independent experiments are indicated with dots of different colors: green and yellow represent HFs from the female donors, red, blue, and orange represents HFs from the male donors. Scale bars: 100 μm; HF: hair follicle, gHM: germinative hair matrix, pcHM: precortical hair matrix, ORS: outer root sheath. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Rangel et al., 2022; Edgar et al., 2018; Nica et al., 2020; Paralkar et al., 1991; Sarrazin et al., 2011). For instance, it has been shown that dietary supplementation with fish derived CPs significantly elevated blood plasma levels of the anagen prolonging growth factor IGF-1 (Ito et al., 2018).

Finally, CPs are potent antioxidants (Li & Yu, 2015; Zou et al., 2016; Wang et al., 2018). Particularly marine-derived CPs efficiently scavenge free radicals and reduce lipid peroxidation (Aguirre-Cruz et al., 2020; González-Serrano et al., 2022). Especially proline and hydroxyproline have been suggested to regulate the cellular redox state and apoptosis (Phang et al., 2008). Thus, the different levels of these two AAs in m- and b-derived CPs (Bao et al., 2018; Carvalho et al., 2018) could influence their antioxidative characteristics, which are of interest in the context of hair growth. For instance, hydrogen peroxide treatment of human HFs *ex vivo* leads to premature catagen induction (Haslam et al., 2017), and treatment with the antioxidants niacinamide or sulforaphane prolong hair growth (Choi et al., 2021; Haslam et al., 2017). Hair aging is affected by oxidative stress that damages the HFs and eHFSCs (Trueb, 2009), while DP spheroids generated from cells of MPHL patients showed increased mitochondrial ROS levels, accompanied by altered activity of electron transport chain complexes and decreased ATP levels (Chew et al., 2022).

Thus, a follow up characterization of the exact amino acid and peptide composition of m- and b-Collagens may provide tangible explanations to the differential response of human HFs to the investigated peptides.

Many studies limit their search for putative effects of hair loss actives using DP cells (Kageyama et al., 2023; J. Lee et al., 2023; Liang et al., 2023; Sung, 2023). However, anagen maintenance and delay or prevention of catagen and subsequent hair shedding also requires maintenance of the eHFSC populations and their progeny in bulge and ORS compartments (J. H. Lee & Choi, 2024). Hence, regardless of the mechanism of action, maintaining eHFSC resilience, i.e. quiescence within their niche, proper functioning and a high potential for self-renewal, is critical for sustained hair cycle induction, anagen maintenance, delayed hair shedding (J. H. Lee & Choi, 2024), and HF regeneration (Purba et al., 2014), as well as overall skin homeostasis (Li & Tumbar, 2021). Additionally, a low proliferation rate of bulge eHFSCs reduces their susceptibility to accumulate mutations. As a result fewer, potentially deleterious, mutations are passed on to daughter cells (Moore & Lemischka, 2006). Thus, our results on HF *ex vivo* suggest that m- and b-Collagens may contribute to the resilience of HFSC populations, in particular, preservation of the quiescent K15+ eHFSCs, in a similar manner as observed in muscle stem cells treated with collagen V *in vitro* (Baghdadi et al., 2018).

Along this line, the enrichment of the K15+ HFSC pool in the bulge observed in HFs after b-Collagen treatment might be particularly interesting in the context of HF aging. In fact, proteolysis of type XVII collagen, a critical molecule for HFSC maintenance, induces loss of K15+ eHFSCs and terminal differentiation into epidermal, not HF, lineages, which results into HF miniaturization and hair loss in aged mice *in vivo* (Matsumura et al., 2016). The HF is regarded as a zoo of SC populations, including K19+ cells (Purba et al., 2014). Although the exact biological role of these progenitor cells remains speculative, the elevated K19+ cell numbers in the presence of b-Collagen suggest an improved regeneration potential of the HF (Purba et al., 2014).

Given the clinical relevance of the HF organ culture model (Chéret et al., 2018; Edelkamp et al., 2020; Jimenez et al., 2021; Campiche et al., 2022), the anagen prolonging effect of m-Collagen seen *ex vivo* suggests that its supplementation may be a promising adjuvant strategy to prevent or reduce hair shedding *in vivo*. While this deserves clinical investigation, m-Collagen may be of particular interest in preventing the sudden decrease in anagen duration, consequent premature catagen induction and prolonged telogen phase seen in telogen effluvium or MPHL patients (Garza et al., 2011; Gentile & Garcovich, 2019). The latter is especially noteworthy given the increased percentage of CD34+ cells detected upon m-Collagen treatment, the progenitor cell type reduced in affected HFs from MPHL patients (Garza et al., 2011).

Thus, the results described herein and those from previous studies suggest that CPs are promising candidates to sustain hair health *in vivo,* and that supplementation may be a promising adjuvant strategy to other therapeutic treatments to delay or reduce hair loss *in vivo*. However, further studies are needed to fully understand the specific mode of action of these peptides, and to confirm their beneficial effects in well-designed clinical trials.

5. Conclusion

Our study is the first of its kind to investigate the mechanisms behind the beneficial effects of CPs on key biological processes of the intact human HF. Our results highlight that CPs, isolated from different sources, exert distinct biological effects, by differentially and selectively targeting diverse HFSC populations. This knowledge could help to predict how individuals suffering from different hair loss conditions may benefit from the dietary supplementation with distinct CPs. Indeed, our data suggests that exploring the effects of a wider set of collagen peptide structures on hair physiology could reveal specific peptide functionalities to target different causes of hair loss, which could lead to a more personalised treatment approach.

Funding

This research was funded by Rousselot BV Ghent, Belgium. Monasterium Laboratory GmbH, Münster, Germany, also financially

contributed to the preparation of this manuscript.

CRediT authorship contribution statement

Karin I. Pappelbaum: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Nicolina Virgilio:** Writing – review & editing, Resources, Conceptualization. **Lisa Epping:** Writing – review & editing, Writing – original draft, Visualization. **Bastiaan van der Steen:** Writing – review & editing, Resources, Methodology. **Francisco Jimenez:** Writing – review & editing, Resources. **Wolfgang Funk:** Writing – review & editing, Resources. **Janne Prawitt:** Writing – review & editing, Resources, Conceptualization. **Marta Bertolini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank Katharzina Figlac, Jasmina Golaboska, Noelia Guedes and Mercedes de Mirecki for the technical support and Gill Westgate for her support in interpreting the data and in proofreading the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106124.

References

Abdal Dayem, A., Lee, S. B., Lim, K. M., Kim, A., Shin, H. J., Vellingiri, B., Kim, Y. B., & Cho, S.-G. (2023). Bioactive peptides for boosting stem cell culture platform: Methods and applications. *Biomedicine & Pharmacotherapy, 160*, Article 114376. https://doi.org/10.1016/j.biopha.2023.114376

Aguirre-Cruz, G., León-López, A., Cruz-Gómez, V., Jiménez-Alvarado, R., & Aguirre-Álvarez, G. (2020). Collagen hydrolysates for skin protection: Oral Administration and topical formulation. *Antioxidants, 9*(2), 181. https://doi.org/10.3390/antiox9020181

Ahmed, M., & ffrench-Constant, C. (2016). Extracellular Matrix Regulation of Stem Cell Behavior. *Current Stem Cell Reports*, *2*(3), 197–206. 10.1007/s40778-016-0056-2.

Arias, E. M., Floriach, N., Moreno-Arias, G., Camps, A., Arias, S., & Trüeb, R. M. (2022). Targeted nutritional supplementation for telogen effluvium: multicenter study on efficacy of a hydrolyzed collagen, vitamin-, and mineral-based induction and maintenance treatment. *International Journal of Trichology, 14*(2), 49–54. https://doi.org/10.4103/ijt.ijt_57_21

Asserin, J., Lati, E., Shioya, T., & Prawitt, J. (2015). The effect of oral collagen peptide supplementation on skin moisture and the dermal collagen network: Evidence from an ex vivo model and randomized, placebo-controlled clinical trials. *Journal of Cosmetic Dermatology, 14*(4), 291–301. https://doi.org/10.1111/jocd.12174

Avila Rodríguez, M. I., Rodríguez Barroso, L. G., & Sánchez, M. L. (2018). Collagen: A review on its sources and potential cosmetic applications. *Journal of Cosmetic Dermatology, 17*(1), 20–26. https://doi.org/10.1111/jocd.12450

Baghdadi, M. B., Castel, D., Machado, L., Fukada, S., Birk, D. E., Relaix, F., Tajbakhsh, S., & Mourikis, P. (2018). Reciprocal signalling by Notch-Collagen V-CALCR retains muscle stem cells in their niche. *Nature, 557*(7707), 714–718. https://doi.org/10.1038/s41586-018-0144-9

Bao, Z., Sun, Y., Rai, K., Peng, X., Wang, S., Nian, R., & Xian, M. (2018). The promising indicators of the thermal and mechanical properties of collagen from bass and tilapia: Synergistic effects of hydroxyproline and cysteine. *Biomaterials Science, 6*(11), 3042–3052. https://doi.org/10.1039/C8BM00675J

Bertolini, M., Botchkareva, N., Westgate, G., Ward, C., Cornwell, P., & Paus, R. (2018). Bridging the "dead hair"-"live follicle" divide in applied hair research. *Journal of Cosmetic Science, 69*(5), 315–322.

Bertolini, M., Chéret, J., Pinto, D., Hawkshaw, N., Ponce, L., Erdmann, H., Jimenez, F., Funk, W., & Paus, R. (2021). A novel nondrug SFRP1 antagonist inhibits catagen development in human hair follicles *ex vivo*. *British Journal of Dermatology, 184*(2), 2. https://doi.org/10.1111/bjd.19552

Bodó, E., Tobin, D. J., Kamenisch, Y., Bíró, T., Berneburg, M., Funk, W., & Paus, R. (2007). Dissecting the Impact of Chemotherapy on the Human Hair Follicle. *The American Journal of Pathology, 171*(4), 4. https://doi.org/10.2353/ajpath.2007.061164

Brandao-Rangel, M. A. R., Oliveira, C. R., da Silva Olímpio, F. R., Aimbire, F., Mateus-Silva, J. R., Chaluppe, F. A., & Vieira, R. P. (2022). Hydrolyzed collagen induces an anti-inflammatory response that induces proliferation of skin fibroblast and keratinocytes. *Nutrients, 14*(23), 4975. https://doi.org/10.3390/nu14234975

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Campiche, R., Le Riche, A., Edelkamp, J., Botello, A. F., Martin, E., Gempeler, M., & Bertolini, M. (2022). An extract of *Leontopodium alpinum* inhibits catagen development ex vivo and increases hair density in vivo. *International Journal of Cosmetic Science, 44*(3), 363–376. https://doi.org/10.1111/ics.12783

Carvalho, A. M., Marques, A. P., Silva, T. H., & Reis, R. L. (2018). Evaluation of the potential of collagen from codfish skin as a biomaterial for biomedical applications. *Marine Drugs, 16*(12), 495. https://doi.org/10.3390/md16120495

Chen, C.-L., Huang, W.-Y., Wang, E. H. C., Tai, K.-Y., & Lin, S.-J. (2020). Functional complexity of hair follicle stem cell niche and therapeutic targeting of niche dysfunction for hair regeneration. *Journal of Biomedical Science, 27*(1), 43. https://doi.org/10.1186/s12929-020-0624-8

Chéret, J., Bertolini, M., Ponce, L., Lehmann, J., Tsai, T., Alam, M., Hatt, H., & Paus, R. (2018). Olfactory receptor OR2AT4 regulates human hair growth. *Nature Communications, 9*(1), 1. https://doi.org/10.1038/s41467-018-05973-0

Chew, E. G. Y., Lim, T. C., Leong, M. F., Liu, X., Sia, Y. Y., Leong, S. T., Yan-Jiang, B. C., Stoecklin, C., Borhan, R., Heilmann-Heimbach, S., Nöthen, M. M., Viasnoff, V., Shyh-Chang, N., Wan, A. C. A., Philpott, M. P., & Hillmer, A. M. (2022). Observations that suggest a contribution of altered dermal papilla mitochondrial function to androgenetic alopecia. *Experimental Dermatology, 31*(6), 906–917. https://doi.org/10.1111/exd.14536

Choi, Y.-H., Shin, J. Y., Kim, J., Kang, N.-G., & Lee, S. (2021). Niacinamide down-regulates the expression of DKK-1 and protects cells from oxidative stress in cultured human dermal papilla cells. *Clinical, Cosmetic and Investigational Dermatology, 14*, 1519–1528. https://doi.org/10.2147/CCID.S334145

Commo, S., Gaillard, O., & Bernard, B. A. (2000). The human hair follicle contains two distinct K19 positive compartments in the outer root sheath: A unifying hypothesis for stem cell reservoir? *Differentiation, 66*(4–5), 157–164. https://doi.org/10.1046/j.1432-0436.2000.660401.x

Edelkamp, J., Gherardini, J., & Bertolini, M. (2020). Methods to Study Human Hair Follicle Growth Ex Vivo: Human Microdissected Hair Follicle and Human Full Thickness Skin Organ Culture. In Natalia V. Botchkareva, & G. E. Westgate (Eds.), *Molecular Dermatology* (Vol. 2154, pp. 105–119). US: Springer. https://doi.org/10.1007/978-1-0716-0648-3_9.

Edgar, S., Hopley, B., Genovese, L., Sibilla, S., Laight, D., & Shute, J. (2018). Effects of collagen-derived bioactive peptides and natural antioxidant compounds on proliferation and matrix protein synthesis by cultured normal human dermal fibroblasts. *Scientific Reports, 8*(1), 1. https://doi.org/10.1038/s41598-018-28492-w

Ernst, N., Yay, A., Bíró, T., Tiede, S., Humphries, M., Paus, R., & Kloepper, J. E. (2013). B1 Integrin signaling maintains human epithelial progenitor cell survival in situ and controls proliferation, apoptosis and migration of their progeny. *PLoS ONE, 8*(12), e84356. https://doi.org/10.1371/journal.pone.0084356

Finner, A. M. (2013). Nutrition and Hair. *Dermatologic Clinics, 31*(1), 167–172. https://doi.org/10.1016/j.det.2012.08.015

Garza, L. A., Yang, C.-C., Zhao, T., Blatt, H. B., Lee, M., He, H., Stanton, D. C., Carrasco, L., Spiegel, J. H., Tobias, J. W., & Cotsarelis, G. (2011). Bald scalp in men with androgenetic alopecia retains hair follicle stem cells but lacks CD200-rich and CD34-positive hair follicle progenitor cells. *Journal of Clinical Investigation, 121*(2), 613–622. https://doi.org/10.1172/JCI44478

Gentile, P., & Garcovich, S. (2019). Advances in regenerative stem cell therapy in androgenic alopecia and hair loss: wnt pathway, growth-factor, and mesenchymal stem cell signaling impact analysis on cell growth and hair follicle development. *Cells, 8*(5), 466. https://doi.org/10.3390/cells8050466

Gentile, P., & Garcovich, S. (2021). Systematic review: The platelet-rich plasma use in female androgenetic alopecia as effective autologous treatment of regenerative plastic surgery. *Journal of Plastic, Reconstructive & Aesthetic Surgery: JPRAS.*. https://doi.org/10.1016/j.bjps.2021.11.004

González-Serrano, D. J., Hadidi, M., Varcheh, M., Jelyani, A. Z., Moreno, A., & Lorenzo, J. M. (2022). Bioactive peptide fractions from collagen hydrolysate of common carp fish byproduct: antioxidant and functional properties. *Antioxidants, 11*(3), 509. https://doi.org/10.3390/antiox11030509

Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., dela Cruz-Racelis, J., & Fuchs, E. (2009). A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. *Cell Stem Cell*, *4*(2), 155–169. 10.1016/j.stem.2008.12.009.

Haslam, I. S., Jadkauskaite, L., Szabó, I. L., Staege, S., Hesebeck-Brinckmann, J., Jenkins, G., Bhogal, R. K., Lim, F.-L., Farjo, N., Farjo, B., Bíró, T., Schäfer, M., & Paus, R. (2017). Oxidative Damage control in a human (Mini-) organ: Nrf2 activation protects against oxidative stress-induced hair growth inhibition. *Journal of*

Investigative Dermatology, 137(2), 295–304. https://doi.org/10.1016/j.jid.2016.08.035
Helal, N. A., Eassa, H. A., Amer, A. M., Eltokhy, M. A., Edafiogho, I., & Nounou, M. I. (2019). Nutraceuticals' novel formulations: the good, the bad, the unknown and patents involved. *Recent Patents on Drug Delivery & Formulation, 13*(2), 105–156. https://doi.org/10.2174/1872211313666190503112040
Hsu, Y.-C., Li, L., & Fuchs, E. (2014). Emerging interactions between skin stem cells and their niches. *Nature Medicine, 20*(8), 847–856. https://doi.org/10.1038/nm.3643
Hsu, Y.-C., Pasolli, H. A., & Fuchs, E. (2011). Dynamics between stem cells, niche, and progeny in the hair follicle. *Cell, 144*(1), 1. https://doi.org/10.1016/j.cell.2010.11.049
Inoue, K., Aoi, N., Sato, T., Yamauchi, Y., Suga, H., Eto, H., Kato, H., Araki, J., & Yoshimura, K. (2009). Differential expression of stem-cell-associated markers in human hair follicle epithelial cells. *Laboratory Investigation, 89*(8), 8. https://doi.org/10.1038/labinvest.2009.48
Ito, N., Seki, S., & Ueda, F. (2018). Effects of composite supplement containing collagen peptide and ornithine on skin conditions and plasma igf-1 levels—a randomized, double-blind, placebo-controlled trial. *Marine Drugs, 16*(12), 482. https://doi.org/10.3390/md16120482
Jimenez, F., López, E., Bertolini, M., Alam, M., Chéret, J., Westgate, G., Rinaldi, F., Marzani, B., & Paus, R. (2021). Topical odorant application of the specific olfactory receptor OR2AT4 agonist, Sandalore ®, improves telogen effluvium-associated parameters. *Journal of Cosmetic Dermatology, 20*(3), 784–791. https://doi.org/10.1111/jocd.13608
Jones, P. H., Harper, S., & Watt, F. M. (1995). Stem cell patterning and fate in human epidermis. *Cell, 80*(1), 83–93. https://doi.org/10.1016/0092-8674(95)90453-0
Jones, P. H., & Watt, F. M. (1993). Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell, 73*(4), 713–724. https://doi.org/10.1016/0092-8674(93)90251-K
Kageyama, T., Seo, J., Yan, L., & Fukuda, J. (2023). Effects of oxytocin on the hair growth ability of dermal papilla cells. *Scientific Reports, 13*(1), 15587. https://doi.org/10.1038/s41598-023-40521-x
Kishimoto, J., Burgeson, R. E., & Morgan, B. A. (2000). Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes & Development, 14*(10), 1181–1185. https://doi.org/10.1101/gad.14.10.1181
Kleinnijenhuis, A. J., van Holthoon, F. L., Maathuis, A. J. H., Vanhoecke, B., Prawitt, J., Wauquier, F., & Wittrant, Y. (2020). Non-targeted and targeted analysis of collagen hydrolysates during the course of digestion and absorption. *Analytical and Bioanalytical Chemistry, 412*(4), 973–982. https://doi.org/10.1007/s00216-019-02323-x
Kleinnijenhuis, A. J., van Holthoon, F. L., & van der Steen, B. (2022). Identification of collagen 1α3 in teleost fish species and typical collision induced internal fragmentations. *Food Chemistry: X, 14*, Article 100333. https://doi.org/10.1016/j.fochx.2022.100333
Kloepper, J. E., Hendrix, S., Bodó, E., Tiede, S., Humphries, M. J., Philpott, M. P., Fässler, R., & Paus, R. (2008). Functional role of β1 integrin-mediated signalling in the human hair follicle. *Experimental Cell Research, 314*(3), 498–508. https://doi.org/10.1016/j.yexcr.2007.10.030
Kloepper, J. E., Sugawara, K., Al-Nuaimi, Y., Gáspár, E., van Beek, N., & Paus, R. (2010). Methods in hair research: How to objectively distinguish between anagen and catagen in human hair follicle organ culture. *Experimental Dermatology, 19*(3), 305–312. https://doi.org/10.1111/j.1600-0625.2009.00939.x
Langan, E. A., Philpott, M. P., Kloepper, J. E., & Paus, R. (2015). Human hair follicle organ culture: Theory, application and perspectives. *Experimental Dermatology, 24*(12), 903–911. https://doi.org/10.1111/exd.12836
Lee, J. H., & Choi, S. (2024). Deciphering the molecular mechanisms of stem cell dynamics in hair follicle regeneration. *Experimental & Molecular Medicine*. https://doi.org/10.1038/s12276-023-01151-5
Lee, J., Kim, N., Jung, A., Jang, J., Lee, J., & Bae, J. (2023). Effect of *Plantago asiatica L.* Extract on the anagen phase in human hair follicle dermal papilla cells. *Journal of Cosmetic Dermatology, 22*(8), 2324–2332. https://doi.org/10.1111/jocd.15720
Leitinger, B. (2011). Transmembrane collagen receptors. *Annual Review of Cell and Developmental Biology, 27*(1), 265–290. https://doi.org/10.1146/annurev-cellbio-092910-154013
León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., & Aguirre-Álvarez, G. (2019). Hydrolyzed Collagen—Sources and Applications. *Molecules, 24*(22), 4031. https://doi.org/10.3390/molecules24224031
Li, K. N., & Tumbar, T. (2021). Hair follicle stem cells as a skin-organizing signaling center during adult homeostasis. *The EMBO Journal, 40*(11). https://doi.org/10.15252/embj.2020107135
Li, Y., & Yu, J. (2015). Research Progress in structure-activity relationship of bioactive peptides. *Journal of Medicinal Food, 18*(2), 147–156. https://doi.org/10.1089/jmf.2014.0028
Liang, Y., Tang, X., Zhang, X., Cao, C., Yu, M., & Wan, M. (2023). Adipose mesenchymal stromal cell-derived exosomes carrying MiR-122-5p antagonize the inhibitory effect of dihydrotestosterone on hair follicles by targeting the TGF-β1/SMAD3 signaling pathway. *International Journal of Molecular Sciences, 24*(6), 5703. https://doi.org/10.3390/ijms24065703
Lim, Y. S., Harland, D. P., & Dawson, T. L. (2019). Wanted, dead and alive: Why a multidisciplinary approach is needed to unlock hair treatment potential. *Experimental Dermatology, 28*(5), 517–527. https://doi.org/10.1111/exd.13898
Matsumura, H., Mohri, Y., Binh, N. T., Morinaga, H., Fukuda, M., Ito, M., Kurata, S., Hoeijmakers, J., & Nishimura, E. K. (2016). Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science, 351*(6273), aad4395. https://doi.org/10.1126/science.aad4395
Milani, M., Colombo, F., & GFM-O-Trial Investigators Group: Chiara Baraldo (Padova), Mauro Barbareschi (Milano), Paolo Chieco (Ruvo di Puglia), Laura Colonna (Roma), Mandel Victor Desmond (Modena), Maria Cristina Fiorucci (Genova). (2023). Efficacy and tolerability of an oral supplement containing amino acids, iron, selenium, and marine hydrolyzed collagen in subjects with hair loss (androgenetic alopecia, AGA or FAGA or telogen effluvium). A prospective, randomized, 3-month, controlled, assessor-blinded study. *Skin Research and Technology*, *29*(6), e13381. 10.1111/srt.13381.
Mohammadi, P., Nilforoushzadeh, M. A., Youssef, K. K., Sharifi-Zarchi, A., Moradi, S., Khosravani, P., Aghdami, R., Taheri, P., Hosseini Salekdeh, G., Baharvand, H., & Aghdami, N. (2021). Defining microRNA signatures of hair follicular stem and progenitor cells in healthy and androgenic alopecia patients. *Journal of Dermatological Science, 101*(1), 49–57. https://doi.org/10.1016/j.jdermsci.2020.11.002
Moore, K. A., & Lemischka, I. R. (2006). Stem cells and their niches. *Science, 311*(5769), 1880–1885. https://doi.org/10.1126/science.1110542
Nica, C., Lin, Z., Sculean, A., & Asparuhova, M. B. (2020). Adsorption and release of growth factors from four different porcine-derived collagen matrices. *Materials, 13*(11), 2635. https://doi.org/10.3390/ma13112635
Nuñez, S. M., Guzmán, F., Valencia, P., Almonacid, S., & Cárdenas, C. (2020). Collagen as a source of bioactive peptides: A bioinformatics approach. *Electronic Journal of Biotechnology, 48*, 101–108. https://doi.org/10.1016/j.ejbt.2020.09.009
Oesser, S. (2020). The oral intake of specific bioactive collagen peptides has a positive effect on hair thickness. *Nutrafoods, 1*, 134–138. https://doi.org/10.17470/NF-020-0019
Pantelireis, N., & Higgins, C. A. (2018). A bald statement—Current approaches to manipulate miniaturisation focus only on promoting hair growth. *Experimental Dermatology, 27*(9), 959–965. https://doi.org/10.1111/exd.13690
Paralkar, V. M., Vukicevic, S., & Reddi, A. H. (1991). Transforming growth factor β type 1 binds to collagen IV of basement membrane matrix: Implications for development. *Developmental Biology, 143*(2), 303–308. https://doi.org/10.1016/0012-1606(91)90081-D
Phang, J. M., Donald, S. P., Pandhare, J., & Liu, Y. (2008). The metabolism of proline, a stress substrate, modulates carcinogenic pathways. *Amino Acids, 35*(4), 681–690. https://doi.org/10.1007/s00726-008-0063-4
Piccini, I., Sousa, M., Altendorf, S., Jimenez, F., Rossi, A., Funk, W., Bíró, T., Paus, R., Seibel, J., Jakobs, M., Yesilkaya, T., Edelkamp, J., & Bertolini, M. (2022). Intermediate hair follicles from patients with female pattern hair loss are associated with nutrient insufficiency and a quiescent metabolic phenotype. *Nutrients, 14*(16), 3357. https://doi.org/10.3390/nu14163357
Proksch, E., Segger, D., Degwert, J., Schunck, M., Zague, V., & Oesser, S. (2014). Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: A double-blind, placebo-controlled study. *Skin Pharmacology and Physiology, 27*(1), 1. https://doi.org/10.1159/000351376
Purba, T. S., Haslam, I. S., Poblet, E., Jiménez, F., Gandarillas, A., Izeta, A., & Paus, R. (2014). Human epithelial hair follicle stem cells and their progeny: Current state of knowledge, the widening gap in translational research and future challenges. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology, 36*(5), 513–525. https://doi.org/10.1002/bies.201300166
Purba, T. S., Peake, M., Farjo, B., Farjo, N., Bhogal, R. K., Jenkins, G., & Paus, R. (2017). Divergent proliferation patterns of distinct human hair follicle epithelial progenitor niches in situ and their differential responsiveness to prostaglandin D2. *Scientific Reports, 7*(1). https://doi.org/10.1038/s41598-017-15038-9
Qiao, J., Zawadzka, A., Philips, E., Turetsky, A., Batchelor, S., Peacock, J., Durrant, S., Garlick, D., Kemp, P., & Teumer, J. (2009). Hair follicle neogenesis induced by cultured human scalp dermal papilla cells. *Regenerative Medicine, 4*(5), 667–676. https://doi.org/10.2217/rme.09.50
Rahmani, W., Abbasi, S., Hagner, A., Raharjo, E., Kumar, R., Hotta, A., Magness, S., Metzger, D., & Biernaskie, J. (2014). Hair follicle dermal stem cells regenerate the dermal sheath, repopulate the dermal papilla, and modulate hair type. *Developmental Cell, 31*(5), 543–558. https://doi.org/10.1016/j.devcel.2014.10.022
Salvatore, L., Gallo, N., Natali, M. L., Campa, L., Lunetti, P., Madaghiele, M., Blasi, F. S., Corallo, A., Capobianco, L., & Sannino, A. (2020). Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare. *Materials Science and Engineering: C, 113*, Article 110963. https://doi.org/10.1016/j.msec.2020.110963
Sarrazin, S., Lamanna, W. C., & Esko, J. D. (2011). Heparan Sulfate Proteoglycans. *Cold Spring Harbor Perspectives in Biology, 3*(7), a004952–a. https://doi.org/10.1101/cshperspect.a004952
Schneider, M. R., Schmidt-Ullrich, R., & Paus, R. (2009). The Hair Follicle as a Dynamic Miniorgan. *Current Biology*, *19*(3), Article 3. 10.1016/j.cub.2008.12.005.
Sibilla, S., Godfrey, M., Brewer, S., Budh-Raja, A., & Genovese, L. (2015). An overview of the beneficial effects of hydrolysed collagen as a nutraceutical on skin properties: scientific background and clinical studies. *The Open Nutraceuticals Journal, 8*(1), 29–42. https://doi.org/10.2174/1876396001508010029
Sung, J.-H. (2023). Effective and economical cell therapy for hair regeneration. *Biomedicine & Pharmacotherapy, 157*, Article 113988. https://doi.org/10.1016/j.biopha.2022.113988
Tobin, D. J. (2008). Human hair pigmentation biological aspects. *International Journal of Cosmetic Science, 30*(4), 233–257. https://doi.org/10.1111/j.1468-2494.2008.00456.x
Trueb, R. (2009). Oxidative stress in ageing of hair. *International Journal of Trichology, 1*(1), 6. https://doi.org/10.4103/0974-7753.51923
Van Neste, D., & Tobin, D. J. (2004). Hair cycle and hair pigmentation: Dynamic interactions and changes associated with aging. *Micron, 35*(3), 193–200. https://doi.org/10.1016/j.micron.2003.11.006

Venkatesan, J., Anil, S., Kim, S.-K., & Shim, M. (2017). Marine fish proteins and peptides for cosmeceuticals: A review. *Marine Drugs, 15*(5), 143. https://doi.org/10.3390/md15050143

Wang, J., Luo, D., Liang, M., Zhang, T., Yin, X., Zhang, Y., Yang, X., & Liu, W. (2018). Spectrum-effect relationships between high-performance liquid chromatography (HPLC) fingerprints and the antioxidant and anti-inflammatory activities of collagen peptides. *Molecules, 23*(12), 3257. https://doi.org/10.3390/molecules23123257

Wauquier, F., Daneault, A., Granel, H., Prawitt, J., Fabien Soulé, V., Berger, J., Pereira, B., Guicheux, J., Rochefort, G. Y., Meunier, N., Blot, A., & Wittrant, Y. (2019). Human enriched serum following hydrolysed collagen absorption modulates bone cell activity: from bedside to bench and vice versa. *Nutrients, 11*(6), 1249. https://doi.org/10.3390/nu11061249

Zeltz, C., & Gullberg, D. (2016). The integrin–collagen connection – a glue for tissue repair? *Journal of Cell Science, 129*. https://doi.org/10.1242/jcs.180992

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules, 21*(1), 72. https://doi.org/10.3390/molecules21010072