



Research Article

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The Synergistic Effects of Fish Collagen Peptide, Bonito Elastin Peptide, Fish Maw Powder and Rose Water on The Improvement of Skin Conditions: A Mechanistic Study

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Abstract

The consumption of beauty from within has been growing rapidly in recent years due to the increase number of female individuals who care about facial appearance. The formulation originally designed by Bio-E composed of fish collagen peptide, bonito elastin peptide, fish maw powder and rose water was demonstrated to stimulate the fibroblast to produce type I collagen. Compared to individual ingredients alone, the scientific Bio-E's formulation of the four ingredients at specific ratio had synergistic benefits on improvement of skin conditions potentially due to the multi-target effects including skin fibroblast regeneration, bone recovery, hydration and free radicals' eradication. Beyond traditional care of skin rejuvenation, rejuvenating changes in the facial skeleton was also highly appreciated against facial ageing. All ingredients were produced from high value-added natural resources by environmental, sustainable and patented processes. Apart from functionality, scientific literature demonstrated the Bio-E's formulation consisting of the four ingredients had no adverse events during oral supplementation. Overall, the synergistic effects of the f Bio-E's formulation on rejuvenating the structures of both bone and skin should provide a valuable and novel insight for facial anti-ageing.

Introduction

Female individuals do not hesitate to consume expensive oral and topical skin care products to resist facial ageing and retain their youthful appearance, leading to a potential market with a rapid annual growth rate. The skin, which widely spreads above the subcutaneous fat, muscle and bone, is smooth and tight for young individuals. Wrinkles might appear especially visible to the naked eyes during ageing [1]. From microscopic angle, loss of tissue cells is one of the reasons that lead to facial ageing. Individuals have varied cell loss rate while the average rate amounts to 7% every decade [2]. There are internal and external causes for skin ageing [3]. Internal causes refer to the ageing of skin resulted from metabolic and physiological alterations while exogenous factors include life patterns such as unhealthy western diet and smoking

and environmental exposures such as ultraviolet radiation, wind blowing and exposure to chemicals [3] (Figure 1).

On the other hand, skin ageing is not the sole reason for facial ageing. The common absorption of facial bones especially around the eye sockets lead to reduced density and support of skeletal tissues, causing the lateral upper eyelid to be pulled by the lower eyelid and aggravating the performance of "lateral ptosis" [4]. Moreover, bone absorption of maxilla and jawbone results in the hollow facial appearance, manifesting nose lengthening and nose tip drooping and even the protrusive "witch's chin" [4]. Thus, failure to consider ameliorating changes in the skeletal foundation of the face may restrict the potential benefit of any rejuvenation procedure (Figure 2). Various functional food from natural resources have



been used as anti-aging agents due to their evidence-based support regarding functionality and safety. As an essential component for the extracellular matrix of skin, orally administered collagen

peptide has been found effective in improving skin elasticity and hydration and ameliorating skin itchiness and sensitivity in dermis and epidermis [5-8].

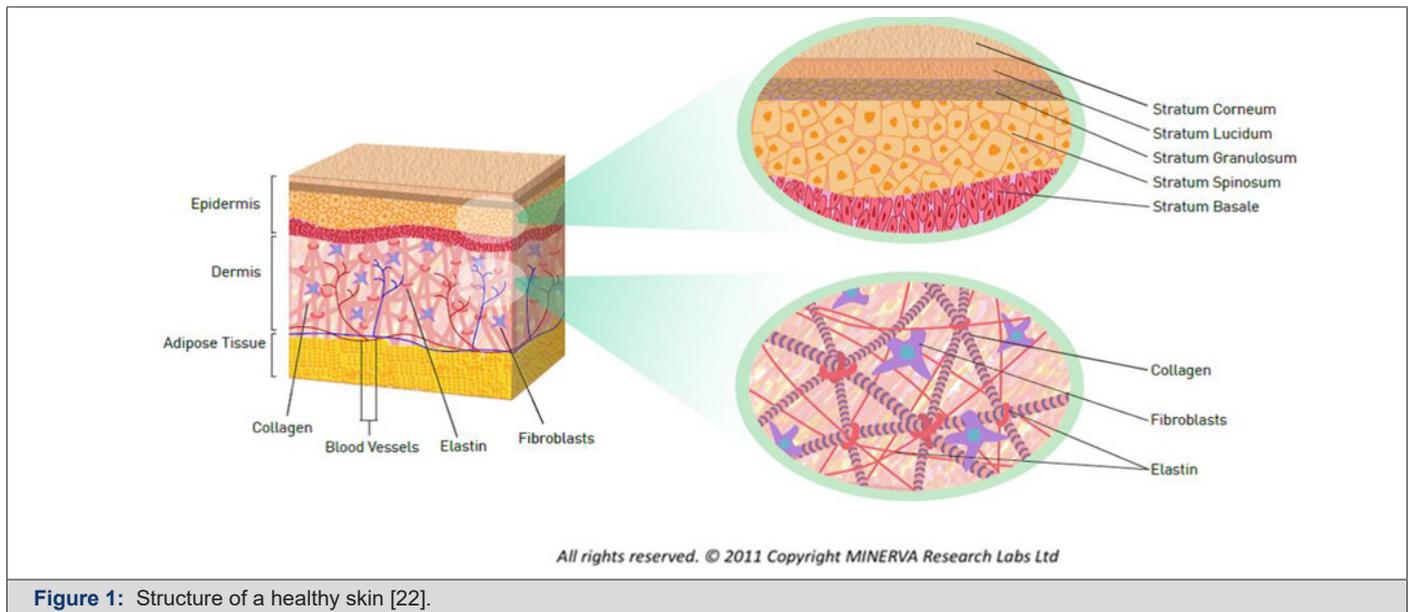


Figure 1: Structure of a healthy skin [22].

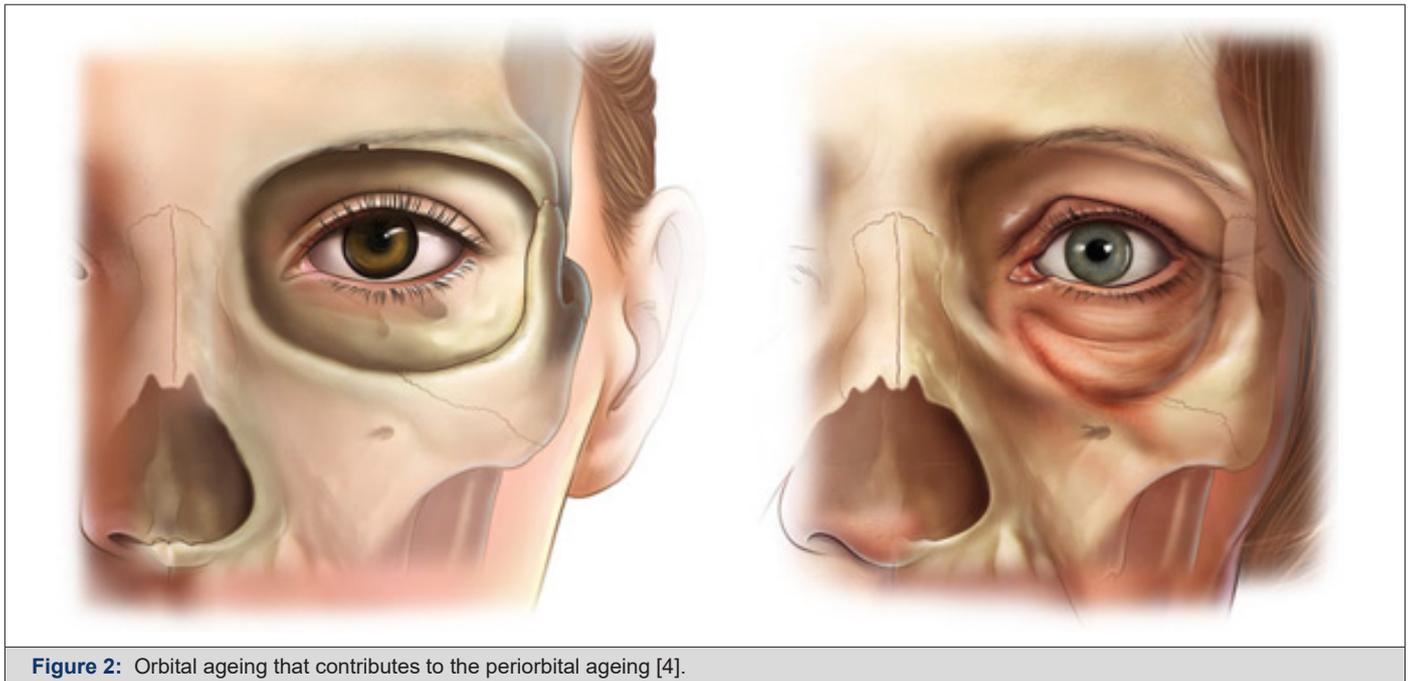


Figure 2: Orbital ageing that contributes to the periorbital ageing [4].

Elastin peptides including proline-glycine (PG) also critically impose beneficial effects on skin conditions [9]. Fish maws, commonly known as the dried swim bladders, have been consumed over many centuries as traditional medicines, tonics and a luxurious gourmet food in Southeast Asia and China [10]. The health benefits of fish maw include improving amnesia, insomnia, dizziness, and weakness [11]. The Glycosaminoglycans in fish maw can mediate skin repair, regeneration and wound healing through FGF-signal pathway [11]. In addition, plant-based extracts

rich in various phytochemicals such as anthocyanins, vitamin C and proanthocyanins can scavenge free radicals generated by UV exposure, which is crucial for skin whitening and hydration [12]. As skin ageing is a complex process involving different targets, it is assumed that multiple components should have synergistic effects on skin conditions compared to each single component. In our study, we aim to verify the Bio-E's formulation of collagen peptide, elastin peptide, rose water on expression of type I collagen in vitro compared to each single component.

Materials and Methods

Chemicals

Primary cultured normal human dermal fibroblasts (NHDF) were obtained from DS Pharma Biomedical (Osaka, Japan). Dulbecco's modified eagle's medium (DMEM) and phosphate buffered saline (PBS) were obtained from Invitrogen (Carlsbad, CA). Preparation: Fish collagen peptide, bonito elastin peptide, fish maw powder and rose water were formulated by Bio-E at specific proportion considering scientific matter, flavour and cost. The Bio-E's formulation and individual ingredients are diluted by PBS to 5mg/ml and 1mg/ml for stimulating fibroblasts in type I collagen production.

Cell Culture

when the plating rate of fibroblasts reached 75% ~ 85%, the culture solution was discarded and was washed twice with PBS. 1ml

of 0.25% trypsin was added to digest the cells at 37 °C for 3-5min. Then the digestion was terminated when about 80% of the cells are suspended. The cells are centrifuged at 1000 rpm/min for 1min. After centrifugation, the supernatant was discarded and counted with a cell counter. Then the cell suspension was inoculated into a 96-well plate with a volume of 2ml per well. After inoculation, it was placed in an incubator (37°C, 5% CO₂, 95% RH) for 24h±2h. Then it was collected in a 1.5ml centrifuge tube and placed in an ultra-low temperature refrigerator at - 80 °C. The supernatant of cells after incubation was collected and analysed for the content of type I collagen by Elisa kit [13].

Results

The type I collagen production by fibroblasts in blank and stimulated by the Bio-E's formulation and other four ingredients are shown in Table 1. The improvement of type I collagen stimulated by the Bio-E's formulation were much better than those from individual ingredients (Table 1 & Figure 3).

Table 1: Type I collagen improvement stimulated by Bio-E's formulation and other four ingredients.

Sample	Concentration	Type I Collagen (ng/ml)	Type I Collagen Improvement
Blank	/	5.73±0.33	
Bio-E's Formulation	5 mg/ml	7.03±0.41	22.69%
	1 mg/ml	6.70±0.31	16.93%
Fish collagen peptide	5 mg/ml	6.86±0.32	19.72%
	1 mg/ml	6.47±0.35	12.91%
Bonito elastin peptide	5 mg/ml	6.77±0.51	18.15%
	1 mg/ml	6.24±0.49	8.90%
Rose water	5 mg/ml	6.45±0.23	12.56%
	1 mg/ml	6.12±0.29	6.80%
Fish maw powder	5 mg/ml	6.39±0.24	11.52%
	1 mg/ml	6.25±0.22	9.08%

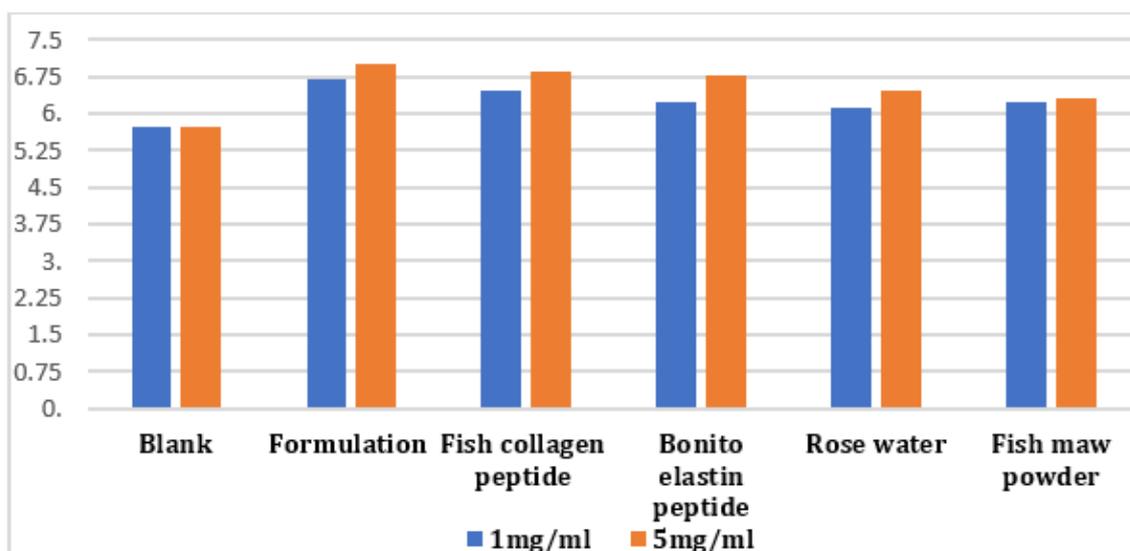


Figure 3: Type I collagen improvement stimulated by Bio-E's formulation and the four ingredients alone.

Discussion

Human skin contains fibroblasts that produce extracellular matrix consisting of collagen, proteoglycans, elastin fibres and so on. Collagen fibres constructs the key structure of skin and are responsible for skin strength while proteoglycans with great amount of water lubricate and cushion the skin. Healthy skin depends on a sustainable and balanced process that breaks down collagen and proteoglycans and recovery with newly formed matrix. However, due to internal and external causes, the disturbed process with faster breakdown of extracellular matrix led to skin ageing. Also, certain areas of the facial skeleton particularly the maxilla including the pyriform region of the nose, the superomedial and inferolateral aspects of the orbital rim, and the pre-jowl area of the mandible undergo resorption that contributes the stigmata of facial ageing. Addressing the changes of such skeleton foundation should not be under-appreciated in any facial rejuvenation procedures [4].

Fish collagen peptides have a unique sequence of amino acid containing high levels of glycine, hydroxyproline, proline, alanine – and thus providing specific nutritional benefits not found in other protein sources. Collagen peptides are key components of extracellular matrix of skin and bone, which are fundamental against facial ageing when collagen is degraded and absorbed in skin and bone respectively [5,8]. Proline–Glycine (PG peptide), a marker peptide contained in elastin fibres, could significantly enhanced elastin synthesis of normal human dermal fibroblast without impacting the rate of cell proliferation [14]. Besides, the low yield from 10000 precious skipjack tuna's hearts to 1 kg elastin peptide make it a very high value ingredient. Elastin could also induce mineral formation during bone regeneration by initiating mineral deposition in the aorta and promoting osteogenic differentiation of bone marrow mesenchymal stem cells [15].

Fish maws are commonly recommended in Asia over many centuries as tonics for people recovering, warding off illness especially for women after child delivery [16]. Fish maws are excellent protein sources with low fat content. They are rich in glycine, proline, glutamic acid, alanine and arginine with a well-balanced composition of FAAs [16]. Glycosaminoglycans (GAGs) contained in fish maws form proteoglycan aggregates and provide essential structural components of the extracellular matrix in addition to collagen fibres, which results in the formation of supermolecular structures and increase the capacity of water retention in the dermis and epidermis [17]. Facial ageing also results from a great number of free radicals generated from UV exposure like sun and cell phone radiation. They are responsible for degradation of extracellular matrix, melanin production and deposition [18-19].

Rose contains different varieties of phytochemicals and antioxidants as flavonoids, anthocyanins and proanthocyanins [20]. Rose water can be easily absorbed into the blood circulation

through oral administration and can quickly and directly exert the beauty and soothing effects on the human body. In particular, French thousand leaf rose in this formulation is organically grown in France and known as the "Queen of flowers". Clinical studies show orally administered rose water can improve the dullness of the skin and deeply purify the underlying layer of the skin [21]. Safety is another significant concern from consumers for daily consumed food products. In the Bio-E's formulation, collagen peptide and elastin peptide were produced from fish and bonito heart respectively by edible enzymatic hydrolysis, which produces small molecule peptide for easy bio-access. The rose water was produced by pure water extraction process in which no organic solvent was used. Essentially, this Bio-E's formulation comprised four natural, clean label food ingredients, free of additives and preservatives. Also, research have shown that no adverse events occurred during orally supplementation of these ingredients [9,16,21].

In our study, we demonstrated the synergistic efficacy of Bio-E's formulated compositions consisting of fish collagen peptide, bonito elastin peptide, rose water and fish maw powder on improving collagen synthesis by fibroblast stimulation. Compared to individual ingredients, Bio-E's combined components had better performance. The Bio-E's scientifically composed formulation provide a novel insight for facial anti-aging based on the consideration and appreciation of the rejuvenation of both skin structure and bone structure.

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