bend
BEAUTY

MARINE COLLAGEN + CO-FACTORS

Scientific Research & Evidence Summary

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1.0 SYNOPSIS

Bend Beauty Marine Collagen + Co-Factors:

- Helps build, support, protect and repair healthy skin
- Contains hydrolyzed fish collagen rich in lysine, glycine, proline, and hydroxyproline; amino acids that are major components of collagen and are known to stimulate skins cells to produce collagen
- Includes the added benefit of vitamin C and silicon from highly bioavailable sources, and that are both necessary to build collagen
- Contains natural product formulation enhancers including Beetroot Red, Maltodextrin, Stevia leaf and Strawberry flavour
- Hydrolysed collagen is clinically proven to enhance skin hydration, improve elasticity that decreases wrinkles (Sibilla 2015), thicken hair (Scala 1976), improve brittle nails (Tyson 1950) and enhance wound healing (Brett 2008).
- Silicon is clinically proven to improve skin smoothness, brittle nails, and hair strength and thickness (Barel 2005, Wickett 2007).
- Vitamin C has been clinically shown to improve skin anti-oxidant capacity (Bertuccelli 2016, Placzek 2005, Barbosa 2009) and in combination with vitamin E reduce sunburn sensitivity (Placzek 2005). With additional nutrients, it improves elasticity, moisture (Bertuccelli 2016), redness, overall appearance (Costa 2015), and wound healing (Barbosa 2009).

Bend Beauty Marine Collagen + Co-Factors is a mixture of hydrolyzed fish collagen, vitamin C and silicon designed to help build, support, protect and repair healthy skin. The natural strawberry flavoured product is available in amber glass bottles containing 146 g of powder. A daily dose of 1 tablespoon (5.2 g), provides 4500 mg of hydrolyzed collagen from fish skin (cod, haddock and/or pollock) and that contains 140 mg of lysine, 50 mg of vitamin C from acerola fruit, and 10 mg of silicon from bamboo.

Bend Beauty Marine Collagen + Co-Factors is authorized for sale by Health Canada and has four skin related health claims. Benefits reported in clinical studies for individual ingredients within the formulation include:

Hydrolyzed Marine Collagen

- Many human clinical studies, involving a variety of types of hydrolysed collagen using a dosage range that includes the amount included in *Bend Beauty Marine Collagen + Co-Factors*, have reported benefits on skin properties (Figueres 2015), including enhanced hydration and improved elasticity that decreases visible fine lines and wrinkling (Sibilla 2015) and thicken hair (Scala 1976), improvements in brittle nails (Tyson 1950) and enhanced wound healing (Brett 2008).

Silicon

- Randomized, double blind, placebo-controlled studies using the same dose of silicon as provided in *Bend Beauty Marine Collagen + Co-Factors* have measured improvements in skin smoothness as well as less brittle nails and hair within 20 weeks (Barel 2005), and enhanced hair morphology and mechanical properties including tensile strength, break load and cross sectional diameter, resulting in thicker hair within 9 months (Wickett 2007).

Vitamin C

- Preclinical studies provide strong evidence for use of vitamin C to enhance collagen production, provide photo-protection against sunburn, reduce photo-damage including wrinkles and pigmentation, reduce skin thinning, and enhance wound healing, skin moisture and hair growth. Few human studies have tested the effects of vitamin C alone on skin health. Those that have, reported increased blood and/or skin vitamin C content (McArdle 2002, Lauer 2013) accompanied by enhanced anti-oxidant capacity in one study (Lauer 2013). When combined with vitamin E, vitamin C reduced sunburn sensitivity (Placzek 2005) and with additional nutrients, it improved elasticity, moisture (Bertuccelli 2016), redness, overall appearance (Costa 2015), and wound healing (Barbosa 2009). Unfortunately, it is difficult to attribute all these benefits to vitamin C since other nutrients were simultaneously provided within these studies.

2.0 Recommended use

Bend Beauty Marine Collagen + Co-Factors is licenced by the NNHPD of Health Canada. The licence number is NPN 80076621 and is associated with the following approved health claims.

Claim 1: Helps in collagen formation

Claim 2: Helps to maintain healthy skin, hair and nails

Claim 3: Source of an antioxidant that helps protect against free radicals

Claim 4: Helps in wound healing

3.0 Formulation details

Bend Beauty Marine Collagen + Co-Factors is available in amber glass bottles containing 146 g of powder. The recommended daily dose of 2.5 teaspoons (5.2 g), provides the medicinal ingredients listed in Table 1.

Table 1: Medicinal ingredients per serving (2.5 tsp. (5.2 g)

PROPER NAME	SOURCE MATERIAL(S)	DOSAGE
Hydrolyzed collagen	Fish skin (cod, haddock and/or pollock)	4500 mg
Lysine	Hydrolyzed collagen [Fish skin (cod, haddock and/or pollock)]	140 mg
Vitamin C (L-ascorbic acid)	*Malpighia glabra* (acerola) – fruit	50 mg
Silicon (as silicon dioxide)	*Bambusa breviflora* (bamboo) - whole plant	10 mg

Non-medicinal ingredients: Strawberry flavour (natural source), maltodextrin, red beetroot, stevia leaf.

3.1 Dosage Rationale

3.1.1 Marine Collagen

Bend Beauty Marine Collagen + Co-Factors provided 4500 mg = 4.5 g of hydrolysed collagen daily. The following doses within clinical trials have resulted in the following improvements:

1 g/day for 6 weeks
increased skin haemoglobin and collagen content (Schwartz 2012)]

1 g/day for 12 weeks
significantly reduced skin dryness, scaling, lines and wrinkles (Schwartz 2012).

2.5 g/day for 4 weeks
reduced eye wrinkles (Proksch 2014a)

2.5 g/day for 8 weeks
increased Type I procollagen by 65%, elastin by 18% and fibrillin by 6% (Proksch 2014a)

2.5 and 5 g/day for 8 weeks
improved skin elasticity (Proksch 2014b)

3 g/day for 12 weeks
improved skin hydration and elasticity (Choi 2014)

5 g and 10 g/day for 4 weeks
improved skin hydration in subjects older than 30 years of age (Matsumoto 2006).

10 g/day for 8 weeks
increased skin hydration by 28% (Sibilla 2015)

10 g/day for 12 weeks
decreased deep wrinkles by 30% (Sibilla 2015)

Since *Bend Beauty Marine Collagen + Co-Factors* provides more hydrolysed collagen per day than was provided in most of the studies listed above, one would expect it to also provide benefits similar to those reported.

3.1.2 Silicon

Randomized, double blind, placebo-controlled studies using the same dose of silicon as provided in *Bend Beauty Marine Collagen + Co-Factors* (i.e. 10 mg/day) have measured improvements in skin smoothness as well as less brittle nails and hair within 20 weeks (Barel 2005), and enhanced hair morphology and mechanical properties including tensile strength, break load and cross sectional diameter, resulting in thicker hair within 9 months (Wickett 2007). Therefore, *Bend Beauty Marine Collagen + Co-Factors* would be expected to provide similar benefits attributable to its silicon content.

3.1.3 Vitamin C

Few studies have tested the effects of vitamin C alone on skin health, making it difficult to determine an effective dose of this ingredient for inclusion within *Bend Beauty Marine Collagen + Co-Factors*. One study reported rapidly improved oxygen radical scavenging in a dose dependent manner following treatment with 100 mg vitamin C/day for 4 weeks, with an increased radical-scavenging activity of 22% (Lauer 2013). This study included twice the dose of vitamin C provided in *Bend Beauty Marine Collagen + Co-Factors*.

The current recommended dietary allowance (RDA) of vitamin C is 90 mg/day and 75 mg/day, for men and women aged 19 years, respectively. The RDA is defined as the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life-stage and gender group (Health Canada: DRI Tables). A 2004 nutrition survey of Canadians found that among Canadians 19 and older, 10-35% had vitamin C intakes below the Estimated Average Requirement (i.e. the amount of a nutrient that is estimated to meet the requirement of half of all healthy people in a particular life-stage and gender group) (Government of Canada). Therefore, any amount of supplemental vitamin C would be expected to provide some health benefits that may or may not become apparent in skin. In addition, since vitamin C is known to be a co-factor in collagen synthesis, it is a necessary addition to the *Bend Beauty Marine Collagen + Co-Factors* formulation at any dose.

4.0 Scientific Background

During the aging process, hyaluronic acid, collagen, and elastin fibers undergo structural and functional changes (Genovese 2017) that contribute to fine lines, furrows, roughness, wrinkles, brown spots, and thickened and sagging skin attributed to skin aging. Such damage is accelerated by smoking, excessive alcohol consumption, physical and psychological stress, poor nutrition, over eating, lack of sleep, environmental pollution, autoimmune diseases and chronic ultraviolet (UV) radiation from the sun (Sibilla 2015). In addition, the ability to replenish lost or damaged collagen decreases by about 1.5% annually, partly because the fibroblasts make smaller amounts (Sibilla 2015).

During youth, collagen accounts for up to 75% of the dermis and is responsible for skin structure, firmness, and elasticity. However, the amount of collagen in the skin is reduced by about 1% per year after 21 years of age, resulting in thickness reduction and elasticity loss, which is directly related to the wrinkles depth. Changes occurring after menopause are even more striking, including loss of about 30% of skin collagen in the first 5 years and annual loss of 0.55% of elastin. In addition, collagen biosynthesis after the third or fourth decade of life remains low and is insufficient to enable mature skin to repair or even replace collagen lost as part of the degradation processes associated with age (Araújo 2016).

Skin aging occurs by intrinsic and extrinsic mechanisms. Intrinsic aging is unavoidable and involves irreversible degenerative changes including atrophy, fibroblasts reduction and thinning of blood vessels that impacts collagen fibers. Extrinsic aging primarily results from damage caused by UV radiation and other environmental factors including smoking, pollution and inadequate nutrition. These injuries lead to increased collagen and elastin degradation, reduced number of extracellular matrix proteins and fibroblasts, and reduced silicon and hyaluronic acid levels in the connective tissues (Araújo 2016). However, it is possible to slow the extrinsic aging process by avoiding factors that contribute to the process and ensuring adequate nutrition for optimal collagen production. *Bend Beauty Marine Collagen + Co-Factors* is designed to provide nutrients including hydrolysed collagen, silicon and vitamin C that are known to slow the extrinsic aging process and enhance skin health.

4.1 Hydrolyzed Marine Collagen

Collagen is the main structural protein within connective tissue of all animals, including humans. Type I collagen is the most abundant type of collagen in the human body (Huey-Jine 2010) and accounts for 80% of the collagen in skin, with the remaining being Type III. These collagen fibers form a dense network throughout the dermis (deep layer) and provide structural support for the epidermis (surface layer) of skin. In addition, collagen is the main insoluble fibrous protein found in the extracellular matrix (the mixture of substances that are secreted by cells and fill the spaces between cells). It, combined with hyaluronic acid and elastin within the extracellular matrix, give the skin its structure, elasticity and firmness, and overall health and longevity. Therefore collagen is a critical building block of the skin. But it is also the main structural protein in all fibrous tissues within the body including tendons and ligaments, and is also abundant in cornea, cartilage, bone (Sibilla 2015), gums, muscles, hair and nails.

Collagen is produced within the body by fibroblasts and epithelial cells (Sibilla 2015). However, diet derived collagen, found in animal connective tissue (Figueres 2015), is also a significant source for physiological function. Today, powdered hydrolyzed collagen derived from cattle, pigs and fish, bones, skin and connective tissue is commercially available and can be mixed with beverages or added to certain foods. Hydrolyzed collagen consists of small peptides with low molecular weight (0.3 - 8 kDa) (Sibilla 2015). Many of these collagen peptide products are derived from pig and cow hides (Huey-Jine 2010), primarily because they are abundant by-products and cheap to produce. However, fish skin derived collagen peptides, although more costly, are significantly better absorbed and utilized within the body. In addition, the risk of exposure to Creutzfeld-Jacob disease (CJD) and the bovine spongiform encephalopathy (BSE), whose occurrence is associated with prions carried within bovine collagen, has led to an increased preference for fish derived hydrolyzed collagen (Huey-Jine 2010, Sibilla 2015, Figueres 2015, Gauza-Włodarczyk 2017). It is made from skin and bones of fresh or salt water fish and since these parts are typically discarded during fish processing, using them to make hydrolyzed collagen is environmentally friendly. Fish hydrolyzed collagen consists of smaller peptides (pieces of protein) than other sources and as a consequence is 1.5 times (Sripriya 2015) more easily digested, absorbed and distributed throughout the body (Sibilla 2015).

4.1.1 Marine Collagen Pharmacokinetics

In order to be active in the deeper layer of the skin, hydrolyzed collagen must cross the intestinal barrier and reach the blood stream. The first step of digestion involves degradation of hydrolyzed collagen to form dipeptides and tripeptides or free amino acids by several proteases including pancreatic proteases, small intestinal brush-border proteases and peptidase. Both free amino acids and peptides are absorbed into the circulation. For example, both hydroxyproline and the peptide, proline-hydroxyproline (Pro-Hyp) are absorbed, with the latter being the major peptide found in human plasma after oral ingestion of any hydrolyzed collagen. The mechanism whereby peptides are absorbed through the intestine includes PEPT1 mediated transcellular transport for di- and tripeptides, a transcytotic route known to be used for the transport of macromolecules (such as proteins), and intracellular passive transport. The transport of these peptides across intestinal epithelial cells occurs in two-steps across two different membranes (i.e. from the lumen across the brush-border membrane and into the blood stream across the basolateral membrane). The first step is mediated by H+-coupled peptide transporters (PEPT1 and PEPT2). PEPT1 operates an enantioselective transport of neutral and mono or polyvalently charged peptides including Pro-Hyp and glycine-proline-hydroxiproline (Sibilla 2015).

Distribution studies have shown that when consumed, hydrolyzed collagen is carried as peptides and free amino acids, in particular, to the dermis, where it can remain for up to 14 days (Watanabe-Kamiyama 2010). In rats given an oral dose of hydrolyzed collagen (gelatin hydrolysate) at 10g/kg bodyweight, the overall bioavailability of the protein supplement over the course of the next 12 hours was 95% reaching peak plasma concentrations after six hours, when testing the serum, peptides ranging from 500 daltons to 15 kilodaltons in weight from collagen appear to be absorbed intact. Despite being 85% eliminated from plasma within 24 hours, hydrolyzed collagen appeared to accumulate in the skin. Peak skin levels were seen within 12 hours, but there was still 58% of the dose detectable in the skin after 192 hours. Other organs including liver, kidney, spleen, and skeletal muscle did not appear to accumulate hydrolyzed collagen relative to amino acid control (Oesser 1999).

4.1.2 Marine Collagen Pharmacodynamics/Mechanism of Action

Within the dermis, collagen peptides stimulate the multiplication and motility of fibroblasts, increase collagen production including fiber density and diameter, increase hyaluronic acid production and activate protection against UVA radiation (Sibilla 2015).

Hydrolyzed collagen, including that contained within the *Bend Beauty Marine Collagen + Co-Factors* formulation, has a high content of the amino acids: lysine, glycine, proline, and hydroxyproline (Sibilla 2015). Combined, these nutrients stimulate cells in the skin, joints and bones to synthesize proteins including collagen and help build body tissues including muscle, bone and skin. Lysine not only plays an essential role in collagen production, but also promotes healthy immune function. Lysine plus arginine, has been shown to prevent infection, which could also help prevent acne (Azzarà 1995). A double-blind, study found that 12 months supplementation with lysine significantly decreased the recurrence of cold sores (Thein 1984).

Historically, it was assumed that all non-essential amino acids (NEAA) were synthesized sufficiently in the body to meet the needs for maximal growth and health. However, there has been no compelling experimental evidence to support this assumption over the past century and NEAAs including proline and glycine have been shown to play important roles in regulating gene expression, cell signalling, antioxidative responses, neurotransmission, and immunity (Wu 2013). Proline plays important roles in protein synthesis and structure, metabolism (particularly the synthesis of arginine, polyamines, and glutamate via pyrroline-5-carboxylate), as well as wound healing, antioxidative reactions, and immune responses. The requirement for proline for whole-body protein synthesis is the greatest among all the amino acids. Proline and hydroxyproline are major amino acids in the collagen proteins which contain three chains of polypeptides (two α1 chains and one α2 chain) and are major extracellular components in connective tissues within skin. Although hydroxyproline has been traditionally considered to have little nutritional significance, it is now recognized as a substrate for the synthesis of glycine and it may scavenge oxidants and regulate the redox state of cells (Wu 2011). Glycine, proline, and hydroxyproline typically account for up to 57% of total amino acids in collagen (Li 2017). Pro-Hyp, one of the major food-derived collagen peptides, enhances the growth of fibroblasts and synthesis of hyaluronic acid, which partially explains the beneficial effects of collagen hydrolysate ingestion on the enhancement of wound healing and improvement in skin condition (Sato 2017).

4.1.3 Marine Collagen Preclinical Studies

Collagen polypeptides from cod skin have been shown to protect against UV-induced damage to mouse skin. The mechanisms of action mainly involved enhanced immunity, reduced moisture and lipid loss, enhanced anti-oxidative properties, inhibition of increased glycosaminoglycans, repair of endogenous collagen and elastin protein fibers, and maintenance of the ratio of type III to type I collagen (Hou 2012).

Collagen peptides from tilapia skin enhanced embryonic skin fibroblast cell proliferation and procollagen synthesis in human and mouse embryonic skin fibroblast cell lines. In addition, peptides up to molecular weights of 3500-4500 Da were able to effectively penetrate stratum corneum to be delivered to the epidermis and dermis when topically applied to mouse skin (Chai 2010).

Some in vitro studies have demonstrated that Pro-Hyp and Hydroxyproline-Glycine (Hyp-Gly) exert chemotaxis effects on dermal fibroblasts and enhance cell proliferation. Additionally, Pro-Hyp enhances the production of hyaluronic acid by dermal fibroblasts. Combining these results with results from human observation studies showing the occurrence of two major collagen peptides, Pro-Hyp and Hyp-Gly within blood, suggests that the amounts of Pro-Hyp and Hyp-Gly in blood are important factors to show the efficacy of collagen hydrolysates on skin health (Inoue 2016).

4.1.4 Marine Collagen Human Intervention Trials

Many human clinical studies, involving a variety of types of hydrolyzed collagen, have reported the benefits of hydrolyzed collagen on skin properties (Figueres 2015) including enhanced hydration and improved elasticity that decreases visible fine lines and wrinkling (Sibilla 2015). Ten grams of hydrolyzed collagen daily can increase skin hydration by 28% in 8 weeks and decrease deep wrinkles by 30% in 12 weeks (Sibilla 2015), while as little as 2.5 g daily for 4 weeks can reduce eye wrinkles, and by 8 weeks can increase Type I procollagen by 65%, elastin by 18% and fibrillin by 6% (Proksch 2014a). Other studies have reported increases up to 78% in dermis density (Beguin 2005), improved elasticity (Choi 2014), and in combination with other nutrients including anti-oxidants such as Vitamin C, which is needed for collagen production, improved elasticity (De Luca 2016), reduced skin dryness (Borumand 2014) and improved skin texture (Genovese 2017).

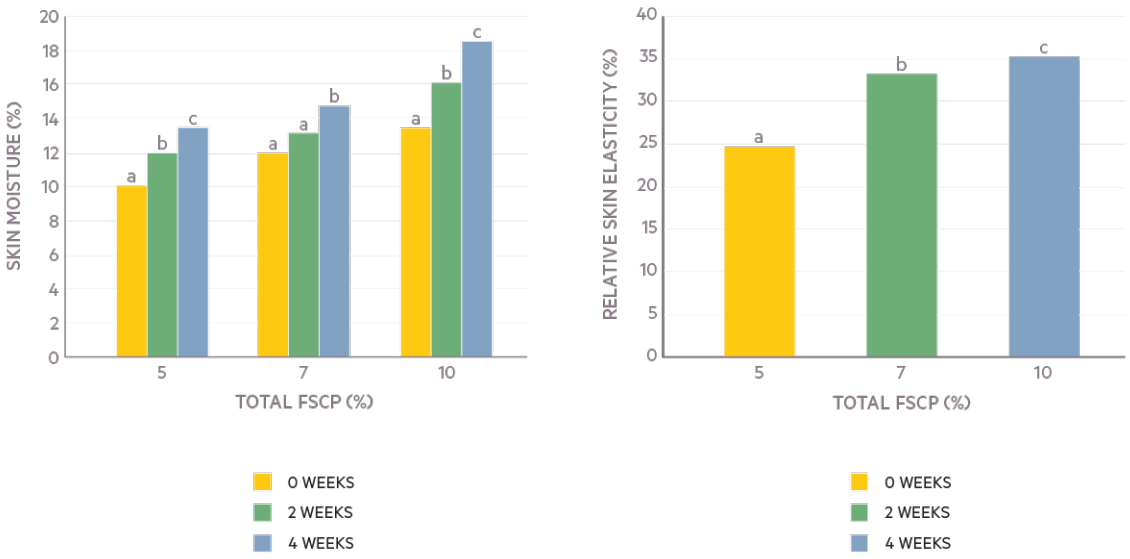
Taking hydrolyzed collagen can also thicken hair (Scala 1976), improve nail disorders such as brittle nails (Tyson 1950), may smooth cellulite appearance (Proksch 2014b), prevent Staphylococcus aureus infections in the skin (Ennaas 2016), help with weight loss (Sibilla 2015) partly because it is more filling that other types of collagen (Hays 2009), reduce muscle loss, enhance wound healing (Brett 2008), help balance blood sugar levels (Gannon 2002), lower LDL (bad) and increase HDL (good) cholesterol (Zhu 2010), increase bone mineral density (Yamada 2013) thereby reducing the risk of osteoporosis, and provide anti-inflammatory (Alemán 2011) effects that can reduce osteoarthritis.

A randomised double-blind placebo-controlled trial studied the effects of two types of collagen hydrolysates, containing either high or low amounts of Pro-Hyp and Hyp-Gly, on skin condition following 8 weeks supplementation. Use of the collagen hydrolysate with a higher content of Pro-Hyp and Hyp-Gly led to significantly greater improvements in facial skin condition, including facial skin moisture, elasticity, wrinkles and roughness (Inoue 2016).

A 4-month randomized, double-blind, placebo-controlled study included 40 subjects supplemented with 220 mg marine protein, 308 mg marine lipids, of which 180 mg were omega-3, 8 mg natural tocopherols, 18 mg plant flavonoids, and 6 mg natural carotenes daily for 3 months followed by a 1-month supplement-free period for both groups to assess lasting effects. Efficacy measurements included skin surface evaluation, ultrasound measurement of sun-exposed and protected areas of the skin (back of the hand and ventral forearms, respectively), and photographic assessment. All parameters showed a continuous and significant improvement in the active group during the 3 months of supplementation as compared to placebo. Photographs showed visible improvement of the overall skin appearance and reduction of fine lines. Ultrasound measurements showed an increase in dermis density of up to 78% in the active group ($P < 0.0001$). The final assessment after 1 month without supplementation showed no further improvements, but a slight decrease was observed in most improved parameters. No treatment-related side effects were reported. This study showed that the product was effective and safe as an oral supplement to protect the skin and support its repair process (Beguin 2005).

At least one human intervention trial has included hydrolyzed fish collagen alone as follows:

- Sixty-two Taiwanese women (within 23 to 60 years of age) were topically treated on their face with hydrolyzed collagen from tilapia skin twice daily for 30 days. Skin moisture contents improved significantly in a time- and dose-dependent manner during the trial. In addition, relative elasticity of facial skin also increased during treatment (Chai 2010).



4.2 Silicon

Silicon is the second most abundant element on Earth, and the third most abundant trace element in the human body (Araújo 2016). Silicon dioxide or silica (SiO_2), the form included in Bend Beauty Marine Collagen + Co-Factors, is the most studied chemical compound following water, and the most important Si-containing substance. Silica is a silicic acid anhydride of monomeric ortho-silicic acid (H_4SiO_4), which is water soluble and stable in highly diluted aqueous solutions. Ortho-silicic acid plays a crucial role in delivering silicon to cells within living organisms and thus is a major sources of silicon for both humans and animals (Jurkić 2013).

Within the body, the highest silicon concentration has been measured in connective tissues, especially in the aorta, trachea, bone, and skin. Silicon is present in 1–10 parts-per-million (ppm) in hair, nails, epidermis, and the hair epicuticle (Jurkić 2013). Higher silicon content in hair results in a lower rates of hair loss and increased brightness. Nails are also affected by the presence of silicon, since this is the predominant mineral within nails. The presence of soft and brittle nails can indicate systemic silicon deficiency (Araújo 2016).

Silicon is naturally present in food as silicon dioxide, free ortho-silicic acid, silicic acids bound to certain nutrients, and in its silicate form. It is found in foods derived from plants such as: cereals, oats, barley, white wheat flour, polished rice and beer, but is less abundant in animal products (Jurkić 2013).

4.2.1 Silicon Pharmacokinetics

There is little information pertaining to the intake of dietary silicon by humans. In Finland, a mean daily intake of 29 mg has been reported and a typical British diet provides roughly 20–50 mg silicon/day. This corresponds to 0.3-0.8 mg silicon/kg bw/day for a 60 kg person. This agrees with estimated mean intakes of silicon in the USA of 30 and 33 mg silicon/day in men, and 24 and 25 mg silicon/day in women, respectively. However, silicon intake decreases with age to less than 20 mg silicon/day (Jurkić 2013).

Only small amounts of silicon (as H_4SiO_4) are actually released in the gastrointestinal tract from ingested amorphous silicon dioxide and subsequently absorbed in the systemic circulation. Absorption studies indicate that the ortho-silicic acid is the main readily bioavailable source of silicon for humans, whereas its higher polymers are poorly absorbed (Jurkić 2013).

Most of the silicon present in serum is filtered by the kidneys, suggesting the kidneys as its major excretion route. Silicon levels in serum correlate with those in urine. Serum silicon levels are similar to other trace elements including iron, copper, and zinc, and silicon is excreted through the urine in similar orders of magnitude as calcium. However, it is still not clear how and if the body can efficiently retain adequate doses of silicon (Jurkić 2013). As a consequence, a daily intake of silica may be necessary to ensure a supply needed for normal physiological function.

4.2.2 Silicon Pharmacodynamics/Mechanism of Action

Silicon is important for activation of hydroxylating enzymes necessary for optimal collagen synthesis, which improves skin strength and elasticity. Silicon is also associated with the synthesis of glycosaminoglycans, which are complex polysaccharides containing amino groups, that are found in connective tissue along with collagen (Araújo 2016).

4.2.3 Silicon Preclinical Studies

Ortho-silicic acid stimulates collagen type 1 synthesis in human osteoblast-like cells and skin fibroblasts and enhances osteoblastic differentiation in the MG-63 cells in vitro. Ortho-silicic acid does not alter collagen type 1 gene expression, but it modulated the activity of prolyl hydroxylase, an enzyme involved in the production of collagen (Keeting 1992).

In controlled animal studies, silicon deficiency is connected with bone defects and impaired synthesis of connective tissue compounds, such as collagen and glycosaminoglycans. It is therefore reasonable to assume that silicon deficiency or lower bioavailability may be linked to problems with collagen production (Jurkić 2013).

4.2.4 Silicon Human Intervention Trials

To date, only a few studies have tested the effects of silicon on skin, hair and nails as follows:

- The effect of choline-stabilized orthosilicic acid (ch-OSA) on skin, nails and hair was investigated in a randomized, double blind, placebo-controlled study including 50 women between 40–65 years with photodamaged facial skin. They were treated with either 10 mg Si/day in the form of ch-OSA pellets or placebo for 20 weeks. Noninvasive methods were used to evaluate skin variations in surface elevation (microrelief) and hydration on the forearm and mechanical properties of the skin on the forehead. Volunteers were also evaluated on a virtual analog scale (VAS) from 0 = none to 3 = severe brittleness of hair and nails. The serum silicon concentration was significantly higher after a 20-week supplementation in the active group compared to placebo. Skin roughness increased in the placebo group but decreased in the ch-OSA group. The difference in longitudinal and lateral shear propagation time increased after 20 weeks in the placebo group but decreased in the ch-OSA group suggesting improvement in skin smoothness. VAS scores for nail and hair brittleness were significantly lower after 20 weeks in the ch-OSA group compared to baseline scores (Barel 2005).

- The effect of ch-OSA on hair was investigated in a randomized, double blind, placebo-controlled study including 48 women with fine hair who were given 10 mg Si/day in the form of ch-OSA beadlets or placebo for 9 months. Hair morphology and mechanical (tensile) properties were evaluated before and after treatment. Urinary silicon concentration increased significantly in the ch-OSA supplemented group but not in the placebo group. The elastic gradient decreased in both groups but the change was significantly smaller in the ch-OSA group (-4.52%) compared to placebo group (-11.9%). Break load changed significantly in the placebo group (-10.8%) but not in the ch-OSA supplemented group (-2.20%). Break stress and elastic modulus decreased in both groups but the change was smaller in the ch-OSA group. The cross sectional area increased significantly after 9 months compared to baseline in ch-OSA supplemented subjects but not in the placebo group. The change in urinary silicon excretion was significantly correlated with the change in hair cross sectional area. This study showed that oral intake of silicon had a positive effect on hair tensile strength including elasticity and break load and resulted in thicker hair (Wickett 2007).

4.3 Vitamin C

Vitamin C (L-ascorbic acid) is a water-soluble vitamin, probably best known for its ability to prevent scurvy with symptoms that include fatigue, widespread connective tissue weakness, skin and capillary fragility (Li 2007), bleeding gums, corkscrew hairs and impaired wound healing (Pullar 2017). It is a powerful antioxidant that helps protect cells from damage caused by free radicals, but it also helps the body form and maintain connective tissue, including bones, blood vessels, and skin.

Daily intake of vitamin C is essential for humans, because unlike most animals, humans are unable to synthesize vitamin C endogenously (Li 2007). It is naturally present in some foods, is added to others, and is available as a dietary supplement. The best food sources are fruits and vegetables including citrus fruits, tomatoes, potatoes, red and green peppers, kiwifruit, broccoli, strawberries, Brussels sprouts, and cantaloupe (Institute of Medicine 2000). However, the vitamin C content of food may be reduced by prolonged storage and by cooking because ascorbic acid is water soluble and is destroyed by heat (Weinstein 2000, Institute of Medicine 2000).

The Recommended Dietary Allowance (RDA), which is the average daily level of intake sufficient to meet the nutrient requirements of 97%–98% of healthy individuals, for vitamin C is 90 mg/day for males and 75 mg/day for females 19 years of age and older (Institute of Medicine 2000). A 2004 nutrition survey of Canadians found that among Canadians 19 and older, 10-35% had vitamin C intakes below the Estimated Average Requirement (i.e. the amount of a nutrient that is estimated to meet the requirement of half of all healthy people in a particular life stage and gender group) (Government of Canada).

Although today vitamin C deficiency is rare in developed countries, vitamin C inadequacy can occur when intakes fall below the RDA but are above the amount required to prevent overt deficiency (approximately 10 mg/day). This can occur in groups at risk including:

- Smokers where studies consistently show lower plasma and leukocyte vitamin C levels than in nonsmokers, due in part to increased oxidative stress. For this reason, smokers are recommended to eat 35 mg more vitamin C per day than nonsmokers. In addition, exposure to second hand smoke also decreases vitamin C levels (Institute of Medicine 2000).
- People who have limited food variety. This may include some elderly, people who abuse alcohol or drugs, food faddists, people with mental illness and, occasionally, children (Jacob 2002,Wang 2007, Weinstein 2000, Institute of Medicine 2000, Stephen 2001, Francescone 2005).

One of the best sources of vitamin C is reported to be Acerola (Malpighia sp. fruit that is found throughout Central America and northern parts of South America. Acerola and extracts made from it, also contain numerous functional phytochemicals, including carotenoids and polyphenols including cyanidin-3-α-O-rhamnoside, pelargonidin-3-α-O-rhamnoside, quercetin-3-α-O-rhamnoside, kaempferol glycosides, astilbin, and proanthocyanidin (Sato 2017). A human study has shown increased absorption of vitamin C into plasma and reduced urinary excretion of vitamin C when vitamin C is consumed from acerola juice, which suggests improved bioavailability of vitamin C from this source (Uchida 2011).

4.3.1 Vitamin C Pharmacokinetics

Intestinal absorption of vitamin C is regulated by at least one specific dose-dependent, active transporter. Cells accumulate vitamin C via a second specific transport protein. In vitro studies have found that oxidized vitamin C, or dehydroascorbic acid, enters cells via a facilitated glucose transporters and is then reduced internally to ascorbic acid (Jacob 2002).

Tissue and plasma concentrations of vitamin C are tightly controlled. Approximately 70%–90% of vitamin C is absorbed at moderate intakes of 30–180 mg/day. However, at doses above 1 g/day, absorption falls to less than 50% and absorbed, unmetabolized ascorbic acid is excreted in the urine (Jacob 2002). Pharmacokinetic studies indicate that oral doses of 1.25 g/day ascorbic acid produce mean peak plasma vitamin C concentrations of 135 micromol/L, which are about two times higher than those produced by consuming 200–300 mg/day ascorbic acid from vitamin C-rich foods. Pharmacokinetic modelling predicts that even doses as high as 3 g ascorbic acid taken every 4 hours produce peak plasma concentrations of only 220 micromol/L (Padayatty 2004).

The total body content of vitamin C ranges from 300 mg (near scurvy concentration) to about 2 g. Uptake kinetics vary between tissues, with vitamin C levels in some organs such as the brain reaching a plateau at fairly low plasma vitamin C status, whereas other tissues including skeletal muscle, continue to increase in close association with increasing plasma supply (Pullar 2017). High levels of vitamin C (millimolar concentrations) are maintained in cells and tissues, and are highest in leukocytes (white blood cells), eyes, adrenal glands, pituitary gland, and brain. Relatively low levels of vitamin C (micromolar concentrations) are found in extracellular fluids, such as plasma, red blood cells, and saliva (Jacob 2002).

Vitamin C is also a normal component of skin. It is found at high levels in both the dermis and epidermis (Shindo 1994, Rhie 2001, Pullar 2017), although its concentration is higher in the epidermis than the dermis, with differences of 2–5-fold between the two layers being consistently reported (Pullar 2017). Aging, however, causes a decline in vitamin C content in both of these skin layers (Rhie 2001). Excessive exposures to UV light or pollutants (e.g., cigarette smoke and ozone) may also lower vitamin C content, primarily in the epidermis (Shindo 1993, Thilel 1997, Podda 1998, Pullar 2017).

Vitamin C is normally transported to the skin from the bloodstream and is present at concentrations well above those in plasma, which suggests active accumulation from the circulation. Transport proteins specific for ascorbic acid are found on cells in all layers of the skin (Steiling 2007). In fact, cells in the epidermis express both types of vitamin C transporter proteins (i.e. SVCT1 and SVCT2), which contrasts with most other tissues, which express SVCT2 only. SVCT1 is associated with active inter-cellular transport of Vitamin C (Pullar 2017), and keratinocytes have a high capacity for vitamin C transport, possibly to compensate for limited vascularization of the epidermis (Steiling 2007, Kang 2007). The specific localisation of SVCT1 in the epidermis is of interest due to the lack of vasculature in this tissue, and suggests that the combined expression of both transporters 1 and 2 ensures effective uptake and intracellular accumulation of the vitamin. Together with the high levels of vitamin C measured in the epidermal layer, the dual expression of the SVCTs suggests a high dependency on vitamin C in this tissue (Pullar 2017).

Oral supplementation with vitamin C has been shown to effectively increase vitamin C levels in the skin (McArdle 2002, Fuchs 1998). However, when plasma vitamin C levels are saturated, skin vitamin C concentrations no longer increase. Therefore, dietary supplementation is only expected to effectively elevate skin vitamin C in individuals who have below-saturation plasma levels prior to intervention. Optimum skin concentrations of the vitamin are not yet known (Pullar 2017).

4.3.2 Vitamin C Pharmacodynamics/Mechanism of Action

Vitamin C is an essential part of skin health both as a small molecular weight antioxidant and as a critical factor for collagen synthesis, which is needed for adequate wound healing. Within skin, its powerful antioxidant capacity helps contribute to photoprotection and decreases photodamage. Vitamin C also plays a role in the body's immune innate and adaptive immune system, both of which potentially involve the skin.

4.3.2.1 Interaction with Cell Signalling Pathways

Vitamin C can play a role in the differentiation of keratinocytes. For example, it enhanced the differentiation of rat epidermal keratinocytes cells in an organotypic culture model, with markedly improved ultrastructural organisation of the stratum corneum, accompanied by enhanced barrier function. It also increased numbers of keratohyalin granules and levels of the late differentiation marker filaggrin, possibly through altered gene expression, and promoted synthesis and organization of barrier lipids and increased cornified envelope formation during differentiation. This vitamin C modulated keratinocyte differentiation may be under the control of protein kinase C and AP-1 (Pullar 2017).

Vitamin C increases proliferation and migration of dermal fibroblasts, and regulates the stabilization and activation of the hypoxia-inducible factor (HIF)-1, a metabolic sensor that controls the expression of hundreds of genes involved with cell survival and tissue remodelling, including production of collagenases. Vitamin C both stimulates and inhibits elastin synthesis, activates glycosaminoglycan synthesis, and may also influence gene expression of antioxidant enzymes, including those involved in DNA repair including oxidatively damaged bases. The modulation of gene expression may be important for its ability to protect during UV exposure via its inhibition of pro-inflammatory cytokine secretion and apoptosis (Pullar 2017).

4.3.2.2 Modulation of Epigenetic Pathways

Vitamin C has a role in epigenetic regulation of gene expression by functioning as a co-factor for the ten-eleven translocation (TET) family of enzymes, which catalyse the removal of methylated cytosine through its hydroxylation to 5-hydroxymethylcytosine (5 hmC). Aberrant epigenetic alterations are thought to have a role in cancer progression, and there is data to suggest that vitamin C can cause alterations in the transcriptome and a decrease in malignant phenotype (Pullar 2017). For example, vitamin C has been shown to protect against UV-induced apoptosis of an epidermal cell line via a TET-dependent mechanism, which involved increases in p21 and p16 gene expression (Lin 2014).

4.3.2.3 Collagen Biosynthesis

Vitamin C is required for the biosynthesis of collagen and is involved in protein metabolism, so it is not surprising that vitamin C is needed for healthy skin (Li 2007, Carr 1999). Since collagen is an essential component of connective tissue, it is also necessary to grow and repair tissue, and so plays a vital role in wound healing. The role of vitamin C in the hydroxylation of collagen molecules is well characterized (Peterkofsky 1991). It is a co-factor for the proline and lysine hydroxylases that stabilise the collagen molecule tertiary structure, and it also promotes collagen gene expression by fibroblasts. In the skin, collagen formation is carried out mostly by the fibroblasts in the dermis, resulting in the generation of the basement membrane and dermal collagen matrix (Pullar 2017). Hydroxylation of collagen is necessary for extracellular stability and support of the epidermis.

4.3.2.4 Antioxidant Capacity

Vitamin C is also an important physiological antioxidant (Frei 1989). It has been shown to regenerate other antioxidants within the body, including vitamin E (alpha-tocopherol) (Jacob 2002), thereby effectively recycling this important lipid-soluble radical scavenger and limiting oxidative damage to cell membrane structures (Pullar 2017). It plays an active role in photo-protection because it limits damage induced by UV light exposure. Vitamin C is not a "sunscreen" because it does not absorb light in the UVA or UVB spectrum. Rather, the antioxidant activity of vitamin C protects against UV-induced damage caused by free radicals (Darr 1992). Vitamin C transport proteins are increased in keratinocytes in response to UV light, which suggests an increased need for vitamin C uptake for adequate protection (Steiling 2007, Kang 2007).

4.3.2.5 Immune Function

Vitamin C contributes to immune defence by supporting various cellular functions. It supports epithelial barrier function against pathogens and promotes the oxidant scavenging activity of the skin, thereby potentially protecting against environmental oxidative stress. It accumulates in phagocytic cells, such as neutrophils, and can enhance chemotaxis, phagocytosis, generation of reactive oxygen species, and ultimately microbial killing, an important defence against invasion through damaged skin. It is also needed for apoptosis and clearance of the spent neutrophils from sites of infection by macrophages, thereby decreasing necrosis/NETosis and potential tissue damage. Vitamin C has been shown to enhance differentiation and proliferation of B- and T-cells, likely due to its gene regulating effects. Vitamin C deficiency results in impaired immunity and higher susceptibility to infections. In turn, infections significantly impact on vitamin C levels due to enhanced inflammation and metabolic requirements. Prophylactic prevention of infection requires dietary vitamin C intakes that provide at least adequate, if not saturating plasma levels (i.e., 100-200 mg/day), which optimize cell and tissue levels. In contrast, treatment of established infections requires significantly higher (gram) doses of the vitamin to compensate for the increased inflammatory response and metabolic demand (Carr 2017).

4.3.2.6 Wound Healing

Vitamin C levels decrease rapidly at wound sites (Kim 1994, Shukla 1997), possibly because of the increased demand for dermal collagen synthesis which increases vitamin C utilization. Additional roles of vitamin C in wound healing include promoting keratinocyte differentiation (Duarte 2009, Savini 2002), stimulating the formation of the epidermal barrier (Boyce 2002), and re-establishing the stratum corneum (Ponec 1997).

4.3.3 Vitamin C Preclinical Studies

4.3.3.1 Collagen Production

The dependence of the collagen hydroxylase enzymes on vitamin C has been demonstrated in a number of studies with fibroblast cells in vitro, showing both decreased total synthesis and decreased crosslinking when vitamin C is absent. Animal studies with vitamin-C-deficient mice indicate that synthesized collagen stability varies with vitamin C availability, reflecting the stabilising function of the collagen crosslinks formed by hydroxylation (Pullar 2017). In addition, dietary vitamin C supplementation increased collagen production and decreased elastin loss in pregnant females rats compared to those not supplemented (Findik 2016).

4.3.3.2 Photo-protection

In cultured keratinocytes, the addition of vitamin C reduces UV-related DNA damage and lipid peroxidation, limits the release of pro-inflammatory cytokines, and protects against apoptosis (Tebbe 1997, Stewart 1996). It also modulates redox-sensitive cell signalling in cultured skin cells and consequently increases cell survival following UV exposure (Savini 1999, Nakamura 1997). In animals, addition of vitamin C to the diet reduced the size and number of dermal neoplasms and skin tumors induced by chronic UV exposure (Dunham 1982, Pauling 1982).

4.3.3.3 Photo-damage/Wrinkles

Vitamin C supplementation has been shown to provide many beneficial effects in combating photodamage in cell culture models. It stabilizes collagen mRNA, thus increasing collagen protein synthesis needed to repair damaged skin (Geesin 1988). This occurs concurrently with a decrease in elastin production, which is beneficial since elastin protein is often overproduced in response to photodamage (Davidson 1997). Vitamin C also increases the proliferation rate of fibroblasts, a capacity that is decreased with age (Phillips 1994), and it stimulates DNA repair in cultured fibroblasts (Duarte 2009).

4.3.3.4 Photo-damage/Pigmentation

Vitamin C and its derivatives, have been shown to decrease melanin synthesis both in cultured melanocytes and in vivo (Kameyama 1996, Matsuda 2008). Vitamin C deficiency leads to UVB-induced skin pigmentation in mice that are genetically unable to synthesize vitamin C. These animals typically have suppressed epidermal hyperplasia and excessive skin pigmentation when chronically exposed to UVB radiation. A study to investigate the effects of acerola juice intake compared to vitamin C supplementation, or a vitamin C deficient diet, on the skin of UVB-irradiated mice, found that vitamin C and water content of the skin was the same in the acerola juice and vitamin C treated groups, and was significantly higher than in the vitamin C deficient group following treatment. In addition, massive skin pigmentation occurred in the vitamin C deficient group, but not in the acerola juice and vitamin C groups. However, this protective effect was more pronounced in the acerola juice group, which had significantly lighter skin than either group throughout the study. This suppression of skin pigmentation corresponded with a significant decrease in the expression level of dopachrome tautomerase, an enzyme that is involved in melanin biosynthesis, within the acerola juice treated group. These results suggest that the polyphenol content of acerola juice contributes to its UVB protective effects, in addition to the vitamin C content of the product (Sato 2017). The results of this study are directly relevant to Bend Beauty Marine Collagen +Co-Factors since the vitamin C content of the product is derived from acerola juice concentrate. That concentrate likely contains the same polyphenol components that magnified the UVB protective effects of vitamin C in this mouse study.

4.3.3.5 Skin Thinning

Age-related skin thinning is correlated with a decrease in the content of collagen in the skin. In vivo studies have shown that collagen peptides and vitamin C transcriptionally upregulate type I collagen in vivo. In addition, combined supplementation with these two substances also corrected age-related skin thinning via reduced oxidative damage in superoxide dismutase 1 (Sod1)-deficient mice. Co-treatment significantly normalized the altered gene expression of Col1a1, Has2, and Ci1, and a proton-coupled oligopeptide transporter, in their skin. Further analyses revealed that collagen oligopeptide, a digestive product of ingested collagen peptides, significantly promoted vitamin C bioactivity with respect to the migration and proliferation of fibroblasts. These findings suggest that combined treatment with collagen peptides and vitamin C effectively reduces skin thinning (Shibuya 2014).

4.3.3.6 Wound healing

Vitamin C deficiency results in poor wound healing in animals (Ross 1962, Kramer 1979). Moderate and high doses of vitamin C increase the healing rate and strength of skin integrity in dorsal wound healing (Silverstein 1999). In addition, studies have shown that vitamin C strengthens the healing effects of honey in burn wounds including strengthened contraction effects and increased dermal proliferation (Schencke 2016).

4.3.3.7 Skin Moisture

Dry skin is very common in older adults and is largely due to a loss of glycosaminoglycans accompanied by a reduction in the ability to maintain moisture levels because of alterations in the keratinisation process and lipid content of the stratum corneum (Pullar 2017). In cell culture models, addition of vitamin C promotes the synthesis of barrier lipids needed to produce a stratum corneum with low water permeability (Ponec 1997, Pasonen-Seppanen 2001). It also induces the differentiation of keratinocytes and therefore may be instrumental in the formation of the stratum corneum (Pullar 2017).

4.3.3.8 Hair Growth

A mouse study investigating the effects of vitamin C deficiency on the expression of genes involved in cell growth and the hair cycle found that changes in the expression of the genes are involved in delaying hair growth in vitamin C deficient animals. Mice given water with or without the addition of vitamin C for 8 weeks, were tested for hair growth, gene expression, cDNA synthesis and RNA-seq. In addition, hair growth was compared between groups after shaving. There were 1,736 differentially expressed genes between the two groups. The functional analysis of the differentially expressed genes between the two groups predicted cell death and cytotoxicity increases within the vitamin C group. In addition, hair growth was significantly promoted in the vitamin C treated group. This difference in hair growth between the vitamin C supplemented and deficient group was caused by the expression of keratin-related genes and the Sonic hedgehog gene (Wakame 2017).

4.3.4 Vitamin C and Human Epidemiology Studies

Two observational studies have reported that higher intakes of dietary vitamin C are associated with better skin appearance, in particular decreased skin wrinkling (Cosgrove 2007, Purba 2001). In addition, higher intakes of dietary vitamin C have been correlated with a decreased risk of dry skin (Cosgrove 2007), suggesting that ascorbic acid may have effects on trans-epidermal water loss (TEWL).

People with skin inflammation have lower Vitamin C status than unaffected individuals which may reflect increased turnover of the redox-labile vitamin C (Pullar 2017). For example, significantly compromised vitamin C status was found in atopic dermatitis patients. Their plasma levels ranged between 6 and 31 μmol/L (optimal healthy levels > 60 μM), and there was an inverse relationship between plasma vitamin C and total ceramide levels in their epidermis (Shin 2016). Ceramide is the main lipid of the stratum corneum and its synthesis involves an essential hydroxylation step catalysed by ceramide synthase, an enzyme with a co-factor requirement for vitamin C.

4.3.5 Vitamin C Human Intervention Trials

Few human intervention trials have measured the effects of solely vitamin E supplementation on skin health. The only two to date have evaluated its anti-oxidant/free radical scavenging capacity.

4.3.5.1 Anti-oxidant/free radical scavenging capacity

- Supplementation of 33 healthy men and women (aged 22–50), 100 mg vitamin C or 180 mg vitamin C daily for four weeks rapidly improved oxygen radical scavenging in a dose dependent manner. After 4 weeks, 100 mg vitamin C/day increased the radical-scavenging activity by 22% and the 180 mg/day dose increased it by 37% (Lauer 2013).
- Eight weeks oral vitamin C supplementation of 500 mg/day in 12 volunteers resulted in significant rises in plasma and skin vitamin C content. However, supplementation had no effect on the UVR-induced erythemal response. In addition, the skin malonaldehyde, total glutathione and protein thiols content was surprisingly reduced by vitamin C supplementation. The authors speculated that this apparently paradoxical effect could be due to regulation of total reductant capacity by skin cells, such that vitamin C may have been replacing other reductants in these cells (McArdle 2002).

4.3.5.2 Photo-protection

- Supplementation of 12 males and 6 females (21–77 y) with 2 g of vitamin C and 1000 IU of D-alpha-tocopherol for 3 months, doubled serum vitamins C and E concentration. In addition, minimal erythema dose increased and DNA damage halved within the skin (Placzek 2005).

4.3.3.3 Skin-aging

- Oral supplementation with a fermented papaya preparation or an antioxidant cocktail containing 10 mg trans-resveratrol, 60 μg selenium, 10 mg vitamin E and 50 mg vitamin C, for 90 days in 60 healthy non-smoker males and females aged 40–65 years, all with clinical signs of skin aging, improved skin elasticity, moisture and antioxidant capacity in both groups. The antioxidant components of the fermented papaya were unknown and direct link of either treatment with vitamin C effects is difficult to confirm in this study (Bertuccelli 2016).
- Treatment of 47 men aged 30–45 with 54 mg or 22 mg of vitamin C, 28 mg tomato extract, 27 mg grape seed extract, 210 mg of marine complex and 4 mg zinc gluconate for 180 days, improved erythema, hydration, radiance, and overall skin appearance. In addition, intensity of general skin spots, UV spots, and brown spots decreased and skin texture and appearance of pores improved. Collagen and elastin increased by 43%–57% and 20%–31%, respectively (Costa 2015). Again, it is difficult to attribute these improvements solely to vitamin C.

4.3.3.4 Wound healing

- Vitamin C turnover is high at wound sites due to both local inflammation and the demands of increased collagen production (Pullar 2017). Vitamin C deficiency results in poor wound healing and vitamin C supplementation in deficient individuals shows significant benefits (Young 1988, Dunphy 1956). Although vitamin C levels appear to increase collagen synthesis and decrease inflammatory responses at the wound site, neither vitamin C supplementation (Thompson 2005, Vaxman 1995) nor increased plasma vitamin C status (Sorensen 2010) increases wound closure time in otherwise healthy individuals in some studies. This suggests that vitamin C may only affect specific facets of the wound healing response, or that there are other rate limiting factors involved in wound healing.

- Supplementation with vitamin C combined with vitamin E and zinc improved the rate of wound healing in children with extensive burns. This double-blind placebo controlled pilot study included 32 patients taking either no supplementation or an antioxidant supplement consisting of 1.5 times the upper intake level of vitamin C, 1.35 times the upper intake level of vitamin E and 2.0 times the recommended dietary allowance of zinc during 7 days starting on the second day of admittance into the hospital. Increased serum vitamin E and C, but not zinc was confirmed following treatment. There was a decrease in lipid peroxidation measured by malondialdehyde level and the time of wound healing was lower in the supplemented group ($P < .001$) (Barbosa 2009).

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Lauer 2013	Randomized, double-blind, placebo-controlled (R, DB, PC)	33 healthy men and women (aged 22 – 50)	100 mg vitamin C, 180 mg vitamin C or placebo daily for 4 weeks	• Rapidly improved oxygen radical scavenging in a dose dependent manner. • 100 mg vitamin C/day increased the radical-scavenging activity by 22% and the 180 mg/day dose increased it by 37%
McArdle 2002	Open label	12 volunteers	500 mg/day of vitamin C for 8 weeks	• Significant rises in plasma and skin vitamin C content. • No effect on the UVR-induced erythemal response. • Skin malonaldehyde, total glutathione and protein thiols content was reduced. • Authors speculated that this was due to regulation of total reductant capacity by skin cells, such that vitamin C may have been replaced other reductants within cells
Placzek 2005	Open label	12 males and six females (21–77 y)	2 g of vitamin C and 1000 IU of D-alpha-tocopherol for 3 months	• Doubled serum vitamins C and E concentration. • Minimal erythema dose increased • DNA damage halved, based on quantification of thymine dimers
Bertuccelli 2016	R, DB, PC	60 healthy non-smoker males and females aged 40–65 years, all with clinical signs of skin aging	Fermented papaya preparation or an antioxidant cocktail containing 10 mg trans-resveratrol, 60 µg selenium, 10 mg vitamin E and 50 mg vitamin C, for 90 days	• Improved skin elasticity, moisture and antioxidant capacity in both groups.

				• The antioxidant components of the fermented papaya were unknown and direct link of either treatment with vitamin C effects is difficult to confirm in this study
Costa 2015	Open label	47 men aged 30 – 45 with phototypes I-IV on the Fitzpatrick scale.	54 mg or 22 mg of vitamin C, 28 mg tomato extract, 27 mg grape seed extract, 210 mg of marine complex and 4 mg zinc gluconate for 180 days	• Improved erythema, hydration, radiance, and overall skin appearance. • Intensity of general skin spots, UV spots, and brown spots decreased and skin texture and appearance of pores improved. • Collagen and elastin increased by 43%–57% and 20%–31%, respectively. • It is difficult to attribute these improvements solely to vitamin C.
Barbosa 2009	R, DB, PC	32 children with severe burns	No supplement or an antioxidant supplement consisting of 1.5 times the upper intake level of vitamin C, 1.35 times the upper intake level of vitamin E and 2.0 times the recommended dietary allowance of zinc during 7 days starting on the second day of admittance into the hospital.	• Increased serum vitamin E and C, but not zinc was confirmed following treatment. • Decreased lipid peroxidation measured by malondialdehyde level • Decreased time of wound healing in the supplemented group

4.4 Human Intervention Trials with Combination Products

A randomized, double-blind, placebo-controlled trial including 80 healthy female volunteers aged 35 – 55 years with phototype II–IV skin, evaluated the efficacy of an oral supplement in preventing the negative effects of winter weather on skin quality over 4 months. A daily dose of the supplement contained 200 mg of marine collagen, 90 mg vitamin C and 50 mg of silicon, along with various other vitamins, minerals and botanical extracts. The volunteers were examined at baseline, 4 months, and 6 weeks after treatment termination for skin microrelief, macrophotography, skin tension, skin high-frequency ultrasound, and self-assessment. The results showed that skin roughness increased in the placebo group, but did not change in the active group. High-frequency ultrasound determined that skin thickness was significantly decreased in the placebo group during winter but was stable in the treated group even though the photography scaling and self-assessment questionnaires revealed no significant changes in either group. These results showed that skin is prone to seasonal changes during winter, particularly in exposed areas and that oral supplementation with nutrients included within *Bend Beauty Marine Collagen + Co-Factors* can be a safe treatment, with no serious side effects, and may prevent or even eliminate the negative effects of winter on the skin (Fanian 2013).

5.0 Relevant Clinical Trials in Progress

Multiple searches of the clinical trial registration database at https://clinicaltrials.gov, on December 16, 2017, pertaining to hydrolyzed collagen and skin, collagen and skin, marine collagen and skin, silicon and skin, and vitamin C and skin, did not result in retrieval of any information pertaining to relevant ongoing studies.

6.0 Safety

6.1 Marine Collagen

Hydrolyzed Collagen is nontoxic when administered orally or dermally in acute animal toxicity studies. In clinical studies, hydrolyzed collagen produces no skin irritation, sensitization, or indication of phototoxicity (No Author 1985). The European Commission for Health and Consumer Protection have deemed hydrolysed collagen to be safe (EFSA 2005). Rarely minor side effects, such as nausea, flatulence or dyspepsia, may occur in some people following ingestion of collagen peptides (Sibilla 2015). The NNHPD of Health Canada has approved safe use of hydrolyzed collagen as described in their Hydrolyzed Collagen Product Monograph at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/atReq.do?atid=hydrolized.collagen&lang=eng , provided that the risk information described in Section 6.4 is recorded on the product label.

6.2 Silicon

There is no recommended dietary allowance (RDA) for silicon, since an essential biological role for it has not been identified. However, the safe upper intake level (UIL) for amorphous silicon dioxide is 700 mg/day for adults, the equivalent of 12 mg silicon/kg bw/day or **720 mg daily** for a 60 kg adult (Jurkić 2013). That is more than ten times higher than the maximum of 50 mg per day that would be consumed in an average adult diet (Jurkić 2013) plus 10 mg per day that could also be supplied within *Bend Beauty Marine Collagen + Co-Factors*. Therefore, the quantity of silicon provided in *Bend Beauty Marine Collagen + Co-Factors* is well within the safe intake range for silicon.

6.3 Vitamin C

Vitamin C has low toxicity and is not believed to cause serious adverse effects at high intakes. The most common complaints are diarrhea, nausea, abdominal cramps, and other gastrointestinal disturbances due to the osmotic effect of unabsorbed vitamin C in the gastrointestinal tract (Jacob 2002, Institute of Medicine 2000).

There is a theoretical concern that high vitamin C intakes might cause excess iron absorption due to its enhancement of nonheme iron absorption. In healthy individuals, this does not appear to be a concern (Institute of Medicine 2000). However, in individuals with hereditary hemochromatosis, chronic consumption of high doses of vitamin C could exacerbate iron overload and result in tissue damage (Jacob 2002, Institute of Medicine 2000).

The Tolerable Upper Intake Levels (ULs) for Vitamin C in adults aged 19 years of age and older is 2000 mg per day (Institute of Medicine 2000). This is also the maximum intake recommendation provided in the vitamin C monograph issued by the NNHPD of Health Canada (Vitamin C Monograph). That amount is about 16 times more than the combined RDA of 75 mg daily for females plus 50 mg that could be provided through *Bend Beauty Marine Collagen + Co-Factors* supplementation. Therefore, the quantity of vitamin C included within *Bend Beauty Marine Collagen + Co-Factors* would pose no health risk.

6.4 Risk Information

The following risk information is based on requirements by the NNHPD of Health Canada for products containing hydrolysed collagen (Hydrolysed Collagen Monograph).

6.4.1 Caution(s) and Warning(s)

- If you are pregnant or breastfeeding, consult a health care practitioner prior to use.
- If you have liver or kidney disease or if you have been instructed to follow a low protein diet, consult a health care practitioner prior to use.

6.4.2 Contraindication(s) – None

6.4.3 Known adverse reaction(s)

- May cause mild gastrointestinal disturbances.

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