

Universal Molecular Constructor of Protein Nature for Preserving the Functional Health of Connective and Epithelial Tissues

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Abstract

This article presents the development and scientific substantiation of a new biologically active supplement (BAS) "Collagen 5000 Multivitamins," designed as a vitamin-collagen biocomplex for the nutritional support of connective and epithelial tissues. The authors detail a unique formulation centered around "Metacollagen Silicon+," a short-chain animal-derived collagen with low molecular weight, which significantly enhances absorption and bioavailability compared to standard hydrolyzed collagen. A key innovation is the integration of natural silicon sourced from the "Chazhemto" mineral waters of Western Siberia, which acts as a collagen promoter to improve tissue structural integrity. The study describes the synergistic roles of 13 essential vitamins and minerals, such as Ascorbic Acid, Biotin, and a full B-vitamin complex, which facilitate endogenous collagen biosynthesis and provide antioxidant protection. The technological process is highlighted by an enzymatic hydrolysis method (confirmed by RF Patent No. 2827228) conducted under mild physiological conditions without pH correction, ensuring the preservation of bioactive properties. Quality control parameters, including amino acid profiles (L-glycine, L-proline, L-oxypyrrolidine) and micronutrient stability, were verified over a 27-month storage period. The resulting product is positioned as a functional tool for preventing premature aging of the skin, vascular systems, and osteoarticular apparatus, produced in compliance with ISO 9001, 22000, and GMP standards.

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Introduction

Modern nutrition science and preventive medicine regard the state of connective tissue as an integral indicator of a person's biological age. Connective tissue, which accounts for more than 50% of total body mass, forms the structural framework for the skin, blood vessels, ligaments, joints, and internal organs. The key protein within this matrix is collagen; its degradation and diminished biosynthesis with age lead to systemic degenerative processes, impaired regenerative potential of epithelial tissues, and the progression of age-associated pathologies (Kiselova-Kaneva & Ivanova, 2019; Lupu & Popustoi, 2020; Zhang & Chen, 2020; Zhu & Cheng, 2020; Boehm & Waldvogel, 2021; Sadeghian & Jafari, 2021; Al-Atif, 2022; Belousov & Ahmed, 2022; Ryabinin & Poznyakovsky, 2022; Tusevski *et al.*, 2022; Karayathil *et al.*, 2023; Krivoshchekov *et al.*, 2023; Malthesh *et al.*, 2023; Padma *et al.*, 2023; Vasiljeva, 2023; Karthikeyan *et al.*, 2024; Najjar, 2024; Obisesan & Egbetokun, 2024; Temirbekova *et al.*, 2024).

Traditional methods of dietary correction for collagen deficiency often face the challenge of low bioavailability of high-molecular-weight proteins. Consequently, the utilization of enzymatic hydrolysis products has become an increasingly relevant area of study. Low-molecular-weight collagen peptides (metacollagen) possess the capacity for efficient absorption within the gastrointestinal tract, serving not only as raw building materials for endogenous synthesis but also as signaling molecules that activate fibroblasts (Asserin & Lati, 2015; Bilek & Bayram, 2021; Oesser, 2022; Mislislayeva *et al.*, 2023; Nurcahyo *et al.*, 2023; Sato, 2023; Yagubova *et al.*, 2023; Aslanov *et al.*, 2024; Bulovina *et al.*, 2024; Mohammadi *et al.*, 2024; Ramadaniati *et al.*, 2024).

However, the efficacy of connective tissue restoration depends not only on the availability of an amino acid substrate but also on the presence of specific cofactors and promoters. Among these, silicon occupies a unique role as an essential trace element involved in the formation of collagen and elastin cross-links, which ensures the structural integrity and elasticity of biological tissues (Jurkić & Cepanec, 2013; Martin, 2013; Farooq & Dietz, 2020; Price *et al.*, 2021; Febriandika *et al.*, 2023; Zoubi *et al.*, 2023; Cachón-Rodríguez *et al.*, 2024; Joungtrakul & Smith, 2024; Khanh, 2024;



Nguyen Ha *et al.*, 2024). The use of natural silicon sources, such as mineral waters from the Siberian region, represents an innovative technological approach that enhances the affinity of exogenous collagen for human tissues (Jurkić & Cepanec, 2013; AlShammasi *et al.*, 2023; Govindaraj *et al.*, 2023; Mickevičius *et al.*, 2023; Chakraborty & Rajasekar, 2024; Kokoev *et al.*, 2024).

Synergy between collagen peptides and a complex of vitamins and minerals is a prerequisite for a complete metabolic cycle. Vitamin C, acting as a classical antioxidant and a participant in proline and lysine hydroxylation reactions, serves as the primary catalyst for biosynthesis (Pullar & Carr, 2017; DePhillipo & Aman, 2018; Jannath *et al.*, 2024; Kumar *et al.*, 2024; Lee *et al.*, 2024; Nakamura *et al.*, 2024; Santos *et al.*, 2024). In turn, B-vitamins, fat-soluble vitamins (A, E, D3, K2), and magnesium provide cellular energy metabolism, protect membranes from oxidative stress, and facilitate the mineralization of the bone matrix (Gröber & Schmidt, 2021; Zidroun, 2022; Schmid & Walther, 2023; Yurievna *et al.*, 2023; Weber *et al.*, 2024).

The development of the specialized biocomplex "Collagen 5000 Multivitamins" aims to create a universal "molecular construct" capable of providing comprehensive support for the functional health of epithelial and connective tissues. This study is dedicated to the scientific substantiation of the formulation, the technology for obtaining metacollagen, and the evaluation of the quality indicators of this new product in accordance with international standards for safety and efficacy (Tokhiriyon & Poznyakovsky, 2019; Kalimullin *et al.*, 2020; Tokhiriyon *et al.*, 2021; Dorn *et al.*, 2022; Sergun *et al.*, 2022; Vekovtsev *et al.*, 2022; Alzahrani, 2023; Belfiore *et al.*, 2023; European Food Safety, 2023; Ivanov & Poznyakovsky, 2023; Lascu *et al.*, 2023; Lobach *et al.*, 2023; Ranganadhareddy, 2023; Rechmann & Buu, 2023; Boulaares *et al.*, 2024; Delgado-Montemayor *et al.*, 2024; Dorn *et al.*, 2024; Kwak & Jukes, 2024; Lobach *et al.*, 2025; Sergun *et al.*, 2025).

Materials and Methods

A new form of biologically active supplement (BAS) "Collagen 5000 Multivitamins" has been developed, representing a vitamin-collagen biocomplex for the nutritional support of connective and epithelial tissues and the prevention of their premature aging.

To substantiate the qualitative and quantitative composition of the biocomplex, a description of the initial ingredients determining the functional orientation is provided.

"Metacollagen Silicon+."

Short-chain collagen of animal origin, consisting of two or three amino acids, which, compared to hydrolyzed collagen, has a lower molecular weight. This ensures efficient absorption and digestion in the gastrointestinal tract, increases the supply of necessary amino acids to cartilage tissue, and facilitates their use in the biosynthesis of the body's own collagen. "Metacollagen Silicon+" is obtained using a natural source of silicon-mineral water extracted in Western Siberia at the "Chazhemto" sanatorium in the Tomsk region. The ortho-silicic acid content per liter is 75.7 ± 11.3 mg. Silicon is a collagen promoter; it increases its effectiveness and the body's affinity for metacollagen, providing strength and elasticity to connective and epithelial tissues, which is especially important for the skin, blood vessels, eyes, hair, nails, and teeth. Along with

silicon, the mineral water contains potassium, magnesium, calcium, iron, fluorine, bromine, iodine, and other vital macro- and microelements, allowing it to be positioned as therapeutic water.

Ascorbic Acid

Used in the form of sodium ascorbate, it represents a classic antioxidant, inhibiting destructive free radicals by donating electrons. It acts as a collagen promoter, serving as a cofactor in enzymatic reactions for the formation of collagen bonds, and contributes to the absorption and biosynthesis of collagen. Vitamin C regulates the levels of the stress hormone cortisol, improves immunity, and accelerates antibody production during viral and infectious attacks. On the other hand, it increases the production of the "happiness hormone" serotonin, influencing the emotional state of the body. Folic Acid. Performs a coenzyme function for numerous enzymes of protein metabolism; it is necessary for energy production, DNA and RNA synthesis, and the formation of white and red blood cells. It is positioned as "brain food," given its role in the functioning of nervous tissue. Biotin (Vitamin H). Participates in carbohydrate, protein, and fat metabolism, as well as the absorption and functional realization of other B-group vitamins. It is necessary for the normal functioning of bone marrow and the nervous system and maintains the healthy state of hair and skin.

Thiamine (B1)

Has a positive effect on blood circulation processes, red blood cell formation, carbohydrate metabolism, and the muscle tone of organs and systems. It activates cognitive and mental brain activity, preserving memory and learning ability. As an antioxidant, it performs protective functions against nicotine and alcohol.

Riboflavin (B2)

Promotes the absorption of pyridoxine and iron, maintaining the state of the mucous membranes of the gastrointestinal tract. As prosthetic groups-flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)—it is part of flavin enzymes that play diverse and exceptionally important roles in metabolism.

Pyridoxine (B6)

Realizes its numerous physiological functions through participation in the formation of coenzyme forms-pyridoxal-5-phosphate (PLP) and pyridoxamine phosphate, influencing physical and mental health. It regulates potassium and sodium levels, nucleic acid synthesis, and the absorption of cyanocobalamin.

Cyanocobalamin (B12)

Along with folic acid, it participates in hematopoiesis—the formation of red blood cells and the utilization of iron. It is a biologically active component of protein, lipid, and carbohydrate metabolism. It helps preserve the protective sheath of nerve cells, preventing damage, and controls the synthesis of acetylcholine—a neurotransmitter responsible for learning and memory processes. As a coenzyme, it participates in the biosynthesis of creatine and DNA. Nicotinamide (PP). The biological role of nicotinic acid (nicotinamide) is determined by its role in building nicotinamide coenzymes—nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which

ensure the activity of more than 100 dehydrogenases catalyzing the metabolism of carbohydrates, proteins, and fats. It contributes to the normal functioning of the nervous system, maintenance of healthy skin, and hormone biosynthesis. Pantothenic Acid (B5). Realizes its numerous functions through the coenzyme form (Coenzyme A), into which it is converted enzymatically. It enters the metabolic "furnace"-the Krebs cycle-in the form of acetyl-CoA to produce energy from carbohydrates, fats, and proteins. It is positioned as an "anti-stress vitamin," being involved in the biosynthesis of neurotransmitters, adrenal hormones, and the formation of antibodies. Vitamin K (Phylloquinone). Necessary for the biosynthesis of the bone tissue protein osteocalcin, its formation, and restoration. Based on these properties, it helps prevent osteoporosis. It is a neuroprotector, preventing nerve cell death. The classic function of Vitamin K is due to its importance in the blood clotting mechanism.

Vitamin A (Retinol, Retinal, Retinoic Acid)

Among the numerous functions of Vitamin A, the most important are related to vision, the processes of differentiation of connective and epithelial tissues, reproduction and growth, and immune status. In the form of retinal, it maintains the health of the retina and forms the compound necessary for night vision. Retinoic acid is a hormone-like growth factor for epithelial cells and cellular structures.

Vitamin E (α -tocopherol)

Realized in metabolism as an active peroxy antioxidant, preventing the destructive action of free radicals. Being a fat-soluble vitamin, it protects cellular and subcellular membranes from oxidative damage. Other important functions relate to gene expression and enzymatic activity; it interacts with other antioxidants-ascorbic acid, selenium, α -lipoic acid, and glutathione. Vitamin D – Cholecalciferol (D3) and Ergocalciferol (D2). The most important function of Vitamin D3 is the regulation of calcium and phosphorus metabolism, which underlies the mineralization and growth of the skeleton. It has an oncoprotective effect, supporting anti-tumor immunity by influencing cell proliferation, differentiation, and apoptosis. It participates in the biosynthesis of insulin receptor substrate 1 (IRS1), the regulation of peroxisome proliferator-activated receptors, and overall fat and carbohydrate metabolism. It holds key positions in cytokine modulation, T-helper lymphocyte division, and B-lymphocyte differentiation, ensuring the functioning of the immune system and non-specific protection against viral and bacterial infections, including tuberculosis. It has been established that Vitamin D intake prevents autoimmune diseases and reduces respiratory tract infections. Each of the vitamins described above possesses individual properties and specific functions, making them indispensable in cellular life. At the same time, they all act as collagen promoters, ensuring its accessible biotransformation, effectiveness, and overall functional orientation of the biocomplex.

Results and Discussion

Table 1 provides the formulation of the developed biocomplex.

Table 1. Formulation of BAS "Collagen 5000 Multivitamins

Name of components	Content, mg / 1 dose	% of RDI ¹ / AI ² for adults
Metacollagen Silicon+	5000	-
Magnesium dicitrate (as Magnesium)	500 (80)	20 ¹
Sodium ascorbate (as Ascorbic acid - Vit C)	67.44 (60)	100 ¹
MenaQ7 Natural 2000 ppm WD Powder (as Menaxinon - Vit K2)	30 (0.06)	50 ²
Tocopherol acetate 50% (as Vit E / TE)	29.8 (10)	100 ¹
Beta-carotene 20% (as Beta-carotene)	25 (5)	100 ²
Nicotinamide (as Niacin - Vit B3)	18.14 (18)	100 ¹
Retinol acetate 325 IU/mg (as Vit A / RE)	8.22 (0.8)	100 ¹
Calcium pantothenate (as Pantothenic acid - Vit B5)	6.54 (6)	100 ¹
Pyridoxine hydrochloride (Vit B6)	2.44 (2)	100 ¹
Cholecalciferol 100 IU/mg (Vit D3)	2 (0.005)	100 ¹
Thiamine mononitrate (Vit B1)	1.74 (1.4)	100 ¹
Riboflavin (Vit B2)	1.6	100 ¹
Folic acid (Vit B9)	0.2	100 ¹
Biotin	0.05	100 ¹
Cyanocobalamin (Vit B12)	0.001	100 ¹

¹RDI – Recommended Daily Intake according to Annex 2 to TR CU 022/2011.

²AI – Adequate Intake level according to Annex 5 of the Uniform Sanitary-Epidemiological and Hygienic Requirements of the EAEU.

All raw materials comply with the requirements of technical regulations: TR CU 021/2011 "On Food Safety" and TR CU 029/2012 "Safety Requirements for Food Additives, Flavorings, and Technological Aids."

Along with the main ingredients, auxiliary substances are used in the formulation:

- Natural flavor additives (apple, cherry, cranberry, orange concentrates), natural flavorings (cherry, blueberry, grapefruit);
- Preservatives (potassium sorbate, sodium benzoate);
- Acidity regulator (citric acid, E330);
- Thickener (gum arabic, E414).

Technology for obtaining the composition based on hydrolyzed collagen (Alexander *et al.*, 2024; Delgado-Montemayor *et al.*, 2024). Mineral water with a silicic acid content of 27.9 ± 5.6 mg/1000 cm³ is heated to 75°C; gelatin is added in a ratio of 1:99 to 30:70; the solution is cooled to 60°C, and the enzyme preparation—protozyme in liquid form (5.0 g per 1.0 kg of gelatin)—is added. Hydrolysis is carried out for 4 hours at 55–60°C. The resulting hydrolysate is filtered twice followed by pasteurization for 30 minutes at $90 \pm 2^\circ\text{C}$. The enzymatic process of collagen breakdown is carried out under mild conditions, at temperatures and pH values close to physiological ones. Silicon in the form of silicic acids possesses high bioavailability for the human body compared to known analogs. pH correction during enzymatic hydrolysis is not performed, which significantly simplifies the technology.

Biocomplex production technology. Consists of a series of sequential stages:

Solution Preparation: Performed at the liquid form filling level. All components are dosed separately in the order and quantity according to the formulation and loading card; **Mixing:** Components are loaded into a homogenizer in parts, one by one, at a temperature not exceeding 40°C, each after the previous one has dissolved.

The solution is drained through a nylon filter with a hole diameter of 0.315 mm. The resulting semi-finished product must be homogeneous without lumps or foreign inclusions. Filling and packaging are carried out in 50 cm³ sachet, zip, or stick bags on an automatic line; 3 samples of finished products are sent to the collection of arbitration samples.

Regulated indicators characterizing the quality of the developed products have been established (**Table 2**).

Table 2. Regulated Quality Indicators of BAS "Collagen 5000 Multivitamins"

Indicator Name	Characteristic Content
Color	From orange to brown
Appearance	Liquid, slight sediment allowed
Taste and Aroma	Specific, corresponds to flavor additive
Micronutrient content in 50 g:	
L-glycine*, mg	Not less than 100
L-proline*, mg	Not less than 820
L-oxypoline*, mg	Not less than 500
Magnesium, mg	80 (64 – 96)
Vitamin A (RE), mg	0.8 (0.56 – 1.04)
Vitamin E (TE), mg	10 (7 – 13)
Vitamin D3, mcg	5.0 (3.5 – 6.6) / 200 (140-260) IU
Vitamin C, mg	60 (48 – 72)
Vitamin K2, mcg	60 (48 – 72)
Vitamin B1, mg	1.4 (1.12 – 1.68)
Vitamin B2, mg	1.6 (1.28 – 1.92)

Vitamin B3, mg	18 (14.4 – 21.6)
Vitamin B5*, mg	6.0 (4.8 – 7.2)
Vitamin B6, mg	2.0 (1.6 – 2.4)
Vitamin B9*, mg	0.2 (0.14 – 0.26)
Vitamin B12*, mcg	1.0 (0.7 – 1.3)
Vitamin H, mcg	50 (35 – 65)
Beta-carotene, mg	5.0 (4.0 – 6.0)

*Indicator regulated by input; quantitative content controlled in the substance during incoming raw material inspection.

The nutritional value of the specialized product is determined by the regulated indicators of micronutrient content. Studies of organoleptic, physicochemical, sanitary-hygienic, and sanitary-toxicological indicators of the quality and safety of the BAS were conducted after 27 months of storage at a temperature of 0°C to 25°C and relative humidity of no more than 60%.

According to sanitary-hygienic and sanitary-toxicological indicators, the product meets sanitary-hygienic requirements, which allowed for the establishment of a shelf life of 2 years under the specified conditions.

The nutritional value reflects the functional properties of the specialized product aimed at supporting connective tissue, where the main role is played by silicon metacollagen and, as promoters, the other components of the formulation, the most important of which are glucosamine and chondroitin sulfate.

Conclusion

Taking into account available literature data and results of our own research, the developed product can be used as a source of fermented collagen enriched with silicon and its synergists for the prevention and complex treatment of connective and bone tissues.

Recommendations for use: Adults take 50 cm³ (content of one sachet, zip, or stick pack) once a day during meals. Duration – 1 month.

The product has passed the state registration procedure, has been tested, and is produced at the enterprises of the biotechnological cluster of the "ArtLife" company in accordance with the requirements of international standards ISO 9001, 22000, and GMP.

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