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Regeneration of the Skin of Oncology Patients after Radiation Therapy Using Upcycled Fish Collagen

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Abstract

Oncological therapy is a very complex process. An accurate diagnosis is the first and most important stage of therapy for the patient, as it allows the medical staff to determine what treatment is necessary and how to plan it. Cancer treatment is now widely recognized as holistic, in which the body is viewed as one interconnected system. Cancer therapy should not focus on just one area of care, but should also include treatment support such as proper nutrition or pain management. The aim of the article was to confirm the level of hydration, limit excessive water loss and regulate the pH value of the skin of patients after cancer treatment who were administered fish collagen. The cosmetic preparation collagen laminate obtained by ultrafiltration was tested on patients who underwent therapy at the University Hospital in Zielona Góra and who underwent radiotherapy. Patients are currently no longer undergoing treatment. The research group of 50 women suffered from breast cancer (n=50). Before the experiment, the patients' skin was examined to diagnose its condition (zero condition). The study was conducted with the consent of the Bioethics Committee (consent number 692/23). It was confirmed that the product tested for an oncological patient has an appropriate level of skin hydration, supports the reconstruction of the skin and its energy barrier by reducing trans epidermal water loss and restoring appropriate pH values. Thanks to this, the effects of pathological changes can be removed and the effects of pathological changes can be accelerated.

Keywords: Collagen; Upcycling; Radiology; Oncological skin

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Introduction

Radiotherapy is one of the most popular cancer treatment methods, using ionizing X-rays with high ionization energy.

Ionization energy causes electrons to be ejected. As the energy increases, the ability of the rays to penetrate the skin increases, this property helps to reduce the dose. Cells undergoing division are most susceptible to radiation. Cancer cells have a higher division frequency than healthy cells, making them more susceptible to damage caused by radiotherapy [1].

Therapies using ionizing radiation are characterized by local effects and are limited to places where cancer lesions occur. The aim of treatment is to induce specific but desired physicochemical phenomena [2]. They cause biological effects such as: damage to cell membranes and DNA, as a result of which their ability to divide and further metabolic processes is impaired, or immediate cell apoptosis. Radiotherapy can be implemented as an independent form of treatment or as an accompanying therapy to chemotherapy [3].

Unfortunately, the use of radiotherapy also causes side effects, both in the epidermis, dermis and subcutaneous tissue. Skin reactions after radiotherapy can be divided into early and late reactions. Early reactions usually appear within a few weeks of starting treatment. It is characterized by excessive skin dryness, pigmentation disorders, erythema and hair loss. As a result of exposure, sebaceous and sweat glands, hair follicles and pigment cells are damaged. The body produces more pro-inflammatory cytokines, including: interleukins 1 and 6, TGF-β and TNF-α [4]. Erythema on the skin is a photochemical reaction, as a result of which the cells of the spinous layer of the epidermis are damaged and the proteins of this layer are denatured, and then histamine is released, blood vessels dilate, resulting in skin hyperemia. The duration of erythema depends on the dose and frequency of radiation to which the skin was exposed. The consequence of erythema is exfoliation of the epidermis and its thickening.

In the early stage of post-radiation reaction, we observe exfoliation of the epidermis, which occurs as a result of damage to keratinocytes in the basal layer, accompanied by itching. Dry exfoliation turns into wet exfoliation, accompanied by the exudation of serous fluid. An early reaction is also permanent dilation of blood vessels, i.e., the development of telangiectasia.

Late reaction occurs several months after the end of therapy. It is caused by the reaction of fibroblasts to radiation. Fibroblasts are cells characterized by low proliferation potential. With the degradation of collagen fibers, atrophic changes appear in the skin. Increased synthesis of collagen with an irregular arrangement of fibers contributes to the appearance of thickening and fibrosis in the skin tissue, and the skin loses its elasticity [5]. Ionizing radiation, which is used in cancer radiotherapy, is responsible for the formation of free oxygen radicals (Reactive Oxygen Species - ROS), which interact with biomolecules. They contribute to the development of the above-mentioned radiation reactions in the skin and subcutaneous tissue [6-7]. More and more women, after undergoing anti-cancer treatment, are looking for solutions to specific skin problems in dermatological and cosmetic clinics. The appropriate care treatment should be selected very individually, taking into account the type of cancer, the time since the end of treatment, the general condition of the patient, as well as factors such as smoking, previous care and skin condition. All treatments that regenerate, moisturize and soothe itchy skin are applicable. Proper skin moisturizing is important primarily due to the damage to the function of the sebaceous and sweat glands during anti-cancer therapy. You should not decide on treatments that are invasive, irritating and damaging the outer protective layer of the skin [8-10]. The aim of the study was to check skin tolerance and the effectiveness of a cosmetic preparation whose main active substance was collagen of fish origin, and to select other ingredients in such a way as to minimize the occurrence of side effects of oncological therapy after radiotherapy. Most skin reactions associated with anticancer treatment should subside within a few weeks of its completion. However, there are so-called late skin complications that can persist for months or years after the end of therapy. They usually take the form of pigmentation disorders (darker skin, areas of lighter spots), fibrosis, skin hardening, and the appearance of telangiectasia, i.e. visible dilation of small blood vessels. Much less common are complications in the form of ulcers and increased susceptibility to skin infections. The formation of late skin reactions is associated with a decrease in the population of fibroblasts in the skin and their production of collagen molecules with an irregular fiber arrangement, which clinically causes hardening, fibrosis, and scarring. The formation of keloids promotes lymph circulation disorders and the formation of edema, and consequently trophic changes and ulcers. All of the above symptoms may take the form of recurrent symptoms, but sometimes they take the form of chronic changes. It should also be remembered that secondary cancers may develop in the area of irradiated skin years after the end of treatment [11-14]. The most important building blocks of the skin are collagen type I and type III. Collagen I is a fibrillar collagen that has long fibers and serves as a support in human skin. However, its proper functioning is closely linked to collagen type III, which in turn determines the appropriate spatial arrangement of collagen type I fibers. The state of oxidative stress occurring in the skin of a patient undergoing radiotherapy causes significant dysfunctions in the functioning and structure of collagen. One of the few forms of collagen used in cosmetology and care that is not subject to chemical processing is collagen type III - interchangeably called native collagen or tropocollagen. Type III collagen, which is derived from the skins of mostly young animals. Nowadays, marine collagen, which is obtained from fish processing waste, is the most popular. The waste materials usually include skin, bones, fins, and scales. Using upcycled substances instead of disposing of them allows for the maximum utilization of resources and is an environmentally friendly solution. Sources of marine collagen include fish, invertebrates, marine mammals, and algae. This type of collagen is characterized by high biocompatibility, biodegradability, lack of allergic reactions and anesthesia, ease of acquisition, and high solubility [14-16]. The aforementioned properties of fish collagen, obtained as waste from skins, have become the basis for using it as the most important bioactive substance. The substance derived from waste, firstly, reduces the amount of waste that needs to be processed for disposal, and secondly, it has properties identical to the most active form, which is tropocollagen, and due to its small molecular weight, it effectively works on the regeneration of the skin of subjects after treatment [17].

Experimental

The cosmetic preparation collagen laminate obtained by ultrafiltration was tested on patients who underwent therapy at the University Hospital and who underwent radiotherapy. Collagen produced from fish skin, waste from the food industry, was used as an active substance in the study. The fish - silver carp - were bred in a local pond. Patients are currently no longer undergoing treatment. The research group of 50 women suffered from breast cancer (n=50). Before the start of the study, patients were interviewed to diagnose their skin condition and well-being (baseline). The experiment was conducted with the approval of the Bioethics Committee (approval number 692/23). The subjects received a commercial cosmetic product that they agreed to apply. All participants had undergone radiotherapy; therefore, they applied the given product to the area exposed to radiation, i.e., the breasts. The participants applied the product twice a day, in the morning and evening. Patients were informed about the purpose of applying the cosmetic. The study aimed to reduce the side effects on the skin of patients who had undergone radiotherapy. The entire study lasted 6 months. The patients came mainly from small towns and villages in the Lubusz Voivodeship. The average age in the study group was 54 years (age range: 35-74 years).

The study also included a control group, another 50 people were the control group. Patients used a product that contained only a cosmetic base without an active substance. Probands and researchers did not know what the cosmetics contained. They also had their skin biophysical parameters tested. Then, digital measurements were performed monthly using a Courage + Khazaka Electronic GmbH device to measure hydration, TEWL and pH value. Patients were asked to wash their skin with water. Then wait 10 minutes before applying the preparation. The above-mentioned skin parameters (stratum corneum) were measured using probes. The experiment was conducted with the patients' informed consent. They were also interviewed to collect information about their health and skin condition. Patients used a commercial collagen laminate cream that contained the following INCI ingredients: Aqua, Pectin, Collagen (Soluble Collagen), Saccharomyces Cerevisiae (Yeast Hulls) /Hexapeptide-11 Ferment Lysate, Pyrus Malus Fruit Extract/ Pectin, Lactobionic Acid, Linum Usitatissimum Seed Extract, Gluconolactone, Panthenol, Polysorbate 20, Citric Acid, Trisodium Ethylenediamine Disuccinate, Calcium Gluconate, Sodium Benzoate,

Potassium Sorbate.

Statistical analysis

Changes over time of the scored data and measured data were determined by the *post-hoc* test.

Results

The figures below 1-3 show the average variation in the levels of hydration, transepidermal water loss and skin pH value, before the application of the tested formula and throughout the entire study. Measurement checks were performed once a month. All parameters were measured in a group of 50 people in the place that was exposed to radiation, i.e., the pedestrian area. A placebo consisting of the base alone was also applied to 50 probands.

Hydration Level

The average degree of hydration of the epidermis before laminate application and after 1-6 months is shown in Figure 1. Based on the above graph, it can be seen that all the people who took part in the study had better results of the hydration parameter as the experiment progressed. This parameter was very important throughout the experiment. The skin is a complex mechanism that tries to restore the proper biophysical parameters. In the natural process of exfoliation of epidermal cells, the proteolytic degradation of desmosomes plays a key role, conditioned by the activity of proteases and the deactivation of inhibitors. This process is also very dependent on the lipids of the

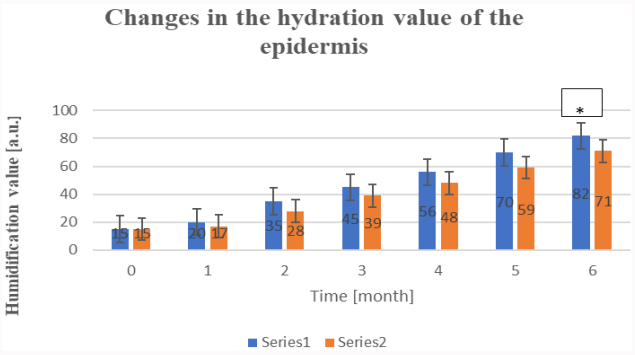

Figure 1: A Change in the skin hydration level of patients after the first application of the preparation series 1 and during its use after radiotherapy **p<0.01 - significant increase from month 1 to 6 (T6) based on the post hoc test. Group size test (n=50). and series 2 application of the placebo preparation.

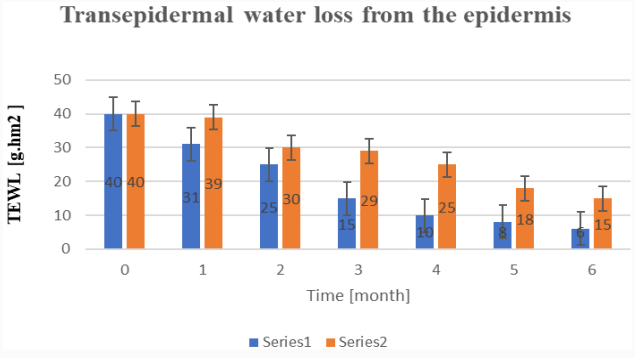

Figure 2: Change in the TEWL value of the patients' skin after the first application of the preparation series 1 and during its use after radiotherapy **p<0.01 - significant increase from month 1 to 6 (T6) based on the post hoc test. Group size test (n=50). Series 2 application of the placebo preparation.

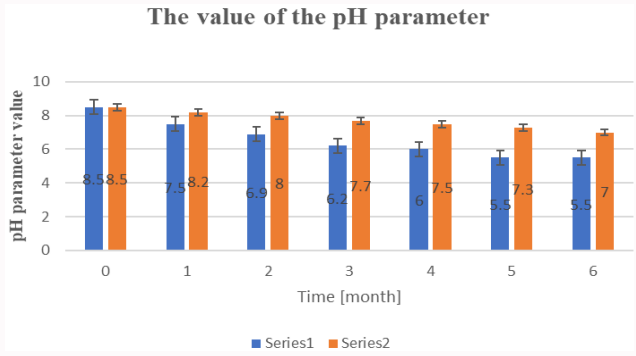

Figure 3: Change in the pH value of the skin of patients after the first application of the preparation and during its use after radiotherapy **p<0.01 - significant increase from month 1 to 6 (T6) based on the post hoc test. Group size test (n = 50). Series 2 shows the probands using only cosmetic bases.

intercellular matrix. "Turn over" exfoliation affects the maintenance of proper moisture, with proper NMF synthesis and thus the pH value of the lipid coat. Epidermal turnover is a natural process that cleanses the skin of toxins, bacteria, mite excrements, the rate of which decreases with age. Impairment of the barrier function is manifested by a significant decrease in the level of hydration, which is why it requires in particular the application of a cream containing a ready-made moisturizing complex, which can supplement the necessary concentration of NMF. Thanks to the use of fish collagen, lunasin, placenta extract and speedwell in the formula, these ingredients have an active effect that improves the epidermal barrier. The time needed to restore the appropriate moisture parameter would be much longer if not for the use of the discussed care. Changes in the moisture parameter ranged on average from 15-82 [j.u.]. Table 1 presents the interpretation of the results. The longer the time of using the cosmetic, the greater the improvement of the parameter.Already after the first use, the device's skin hydration level increases by 5 [a.u.]. In the next month it is increased by 15 [a.u.] in the fourth month it increases by 30 [IU]. Staff skin hydration levels increased linearly over time. After 5 parameters enabling 55 [a.u.]. And after 6 a.m., another one until 6 a.m. [a.u.]. During and after radiotherapy, the subjects' skin was hypersensitive, itchy and prone to cracking. The hydrolipid barrier weakened and the body was unable to protect itself against the consequences [9]. In order to achieve a disease state as a result of treatment, moisturizing preparations should be used. It has been shown that collagen, which is a component of the product but is also detached from our skin, is very harmful to it due to its structure. A long-term state of oxidative stress that is affected by radiation therapy, dysfunction in the role and development of collagen. The form that does not undergo chemical degradation is native collagen. It is obtained from fish skins, isolated from fish skins obtained from fish processing. their existence allows for resource management and is excluded from environmental friendliness. Collagen obtained from marine resources that do not have an allergenic potential, due to its molecular content and biocompatibility with human collagen. The effect of skin regeneration after radiotherapy. Moreover, it's good water-soluble and biodegradable. Increases the resistance of skin cells to environmental factors, including radiation. Hexapeptide-11 Peptide Encapsulated in Yeast (INCI: Saccharomyces Cerevisiae (Yeast Hulls) / Hexapeptide-11 Ferment Lysate) is a substance that is especially beneficial for the condition of the skin. Research results suggest that this compound improves skin hydration by stimulating the production of glycosaminoglycans and collagen, which are

Table 1: Interpretation of epidermal hydration measurement results.

The degree of hydration of the epidermis	Measurement result
Very dry skin	<30
Dry skin	30–45
The skin is sufficiently moisturized	>45

Table 2: Interpretation of epidermal TEWL measurement results.

Interpretation of the results	Value TEWL [g/hm^2]
Very healthy skin	0–10
Healthy skin	10–15
Normal skin	15–25
Leather in poor condition	25–30
The skin is in critical condition	>30

responsible for the proper hydration of the deeper layers of the skin. On the surface of the epidermis, they create an impenetrable filter for evaporating water. The hydrophobic active substance penetrates deep into the epidermis, thus replenishing the lipids in the protective layer. Consequently, the emulsion increases the skin's moisturizing parameter [8-10]. Pectins from UPCYCLING apple pomace (INCI: Pyrus Malus Fruit Extract/Pectin), which were obtained after juice production. They contain antioxidants that help protect the skin. Pectins support the skin through their moisturizing properties. They act as a moisture-binding agent, which helps maintain the appropriate level of hydration.

Transepidermal Water Loss Level from the Epidermis (TEWL)

Figure 2 shows the average level of variation in the transepidermal water loss parameter.

Based on the above graph, it can be seen that all the people who took part in the study had better TEWL parameter results as the experiment progressed. Changes in the transepidermal water loss parameter ranged on average between 40-6 [g/hm2]. The longer the time of using the product, the better the parameter. The natural lipid barrier of the skin protects not only against excessive transepidermal water loss (TEWL), but also against excessive penetration of toxic substances. The efficiency of the epidermal barrier is determined by the value of the transepidermal water loss index - TEWL (transepidermal water loss). Based on the graph, it can be seen that the ingredients and physicochemical form of the product with collagen contributed to the improvement of the efficiency of the epidermal barrier of the test subjects.Properly moisturized skin binds about 20% of the water contained in the body, with most of the water being located in the dermis. This determines, among other things, its proper tension and balance in maintaining the function of metabolic processes. Water in the skin always migrates from its interior towards the surface and evaporates (Transepidermal Water Loss - TEWL). Drying out of the skin is therefore a natural phenomenon. In addition, water introduced into the skin from the outside is not retained in it and evaporates quickly. The state of skin hydration does not depend only on the amount of water supplied from the outside, but primarily on the skin's ability to retain it. There should be a balance between the speed of water migration to the outside and its evaporation, and the speed of its penetration. Inhibition of natural water loss from the skin can be achieved by limiting its evaporation (including by reducing the concentration gradient), by increasing the skin's ability to bind water (the presence of hygroscopic substances) and by creating barrier systems (including a lipid barrier). Inadequate skin hydration, dryness and loss of elasticity disrupt the integrative processes of this organ in terms of protective and defensive functions, which is manifested by its hypersensitivity (i.e. lack of tolerance to commonly occurring environmental factors). Dehydrated skin becomes more susceptible to the adverse effects of physicochemical factors (including temperature, wind, UV radiation, micro-injuries and water, detergents). Insufficiently moisturized skin is the result of an increase in the pH value of the epidermis towards alkaline and the TEWL index [1].

Ph Parameter Value

The normal reaction of human skin is acidic, ranging between 4 and 6 in the outermost layers of the skin. The dermis has a pH value of approximately 7. The slightly acidic pH on the skin has a bacteriostatic effect, prevents excessive colonization of pathogenic bacteria and facilitates the proper course of the skin cell exfoliation process. The hydrolipid coat, sebaceous gland secretions and NMF are responsible for maintaining the appropriate pH level of the skin. Many external factors can affect the pH level, such factors include: too aggressive detergents, UV radiation, atmospheric factors, inadequate skin care, lack of a balanced diet. Each of these factors affects the deficiency of the ingredients that make up the hydrolipid coat and the natural moisturizing factor.

The average change in pH value before laminate application and after 1-6 months is presented in Figure 3.

A very important factor influencing the enzymatic processes involved in the proliferation of epidermal cells is pH. In healthy children, after birth, its value is close to neutral (pH=6.5). It changes after a few weeks and, similarly to adults, reaches pH 5.3-5.5. The pH value of the epidermis is influenced by endogenous and exogenous factors, such as: phospholipase A2, NMF components, sweat and sebum components, bacterial metabolites, organic and inorganic chemical compounds applied to the skin. Symptoms similar to the frequently reported problem of cosmetic allergy may also be caused by increased sensitivity of the TRPV1 receptor (transient receptor potential vanilloid subtype). This receptor is sensitive to many chemical stimuli, including temperatures above 42°C and high pH values. The skin becomes more alkaline immediately after washing with preparations characterized by high pH values. In pathological conditions, the use of e.g. soap can lead to a much greater difference in the pH of the lipid coat, perceived by the receptor as a chemical burn. These symptoms can be intensified by increased contact of cosmetic ingredients with infected skin. In the tested product, lactic acid is responsible for regulating the pH value of the skin. It was found that acquired hypersensitivity to cosmetics is caused, among other things, by a weakening of the lipid-epidermal barrier and an increase in the pH value of the epidermis.

Discussion

Collagen is the main protein of connective tissue, made exclusively of amino acids (including glycine, proline and hydroxyproline). It has the form of a triple helix (it can be compared to a strong rope woven from three strands). An important ingredient used in the formula is lunasin, an extract from placenta and speedwell.

Based on the above Graph 1, it can be seen that all people who participated in the study had better results in the hydration parameter

as the experiment progressed. Table 1 presents the interpretation of the results of the discussed parameter. Changes in the hydration parameter ranged on average from 15-82 [a.u.]. Using the base itself to a lesser extent but also contributed to improving the level of hydration. Already after the first use, the device's skin hydration level increases by 5 [a.u.]. In the next month it is increased by 15 [a.u.] in the fourth month it increases by 30 [IU]. Staff skin hydration levels increased linearly over time. After 5 parameters enabling 55 [a.u.]. And after 6 a.m., another one until 6 a.m. [a.u.]. During and after radiotherapy, the subjects' skin was hypersensitive, itchy and prone to cracking. The hydrolipid barrier weakened and the body was unable to protect itself against the consequences [9]. In order to achieve a disease state as a result of treatment, moisturizing preparations should be used. It has been shown that collagen, which is a component of the product but is also detached from our skin, is very harmful to it due to its structure. A long-term state of oxidative stress that is affected by radiation therapy, dysfunction in the role and development of collagen. The form that does not undergo chemical degradation is native collagen. It is obtained from fish skins, isolated from fish skins obtained from fish processing. their existence allows for resource management and is excluded from environmental friendliness. Collagen obtained from marine resources that do not have an allergenic potential, due to its molecular content and biocompatibility with human collagen. The effect of skin regeneration after radiotherapy. Moreover, it's good water-soluble and biodegradable. Increases the resistance of skin cells to environmental factors, including radiation. Hexapeptide-11 Peptide Encapsulated in Yeast (INCI: Saccharomyces Cerevisiae (Yeast Hulls)/Hexapeptide-11 Ferment Lysate) is a substance that is especially beneficial for the condition of the skin Based on the results obtained, it can be concluded that collagen derived from fish skins contributed to the improvement of the subjects' skin hydration. It is suggested that the improvement in this biophysical skin parameter is caused by the stimulation of the production of our own glycosaminoglycans and collagen. These compounds are responsible for the proper hydration of the deeper layers of the skin. An impermeable film is formed on the outermost layer of the epidermis, which prevents water evaporation, thereby indirectly stabilizing the hydration of the subjects' skin. The active substance used has the ability to penetrate deeply into the epidermis, thus replenishing the amount of lipids in the protective layer. Consequently, the emulsion increases the skin's hydration parameter [8-10]. Pectin from upcycling apple pomace (INCI: Pyrus Malus Fruit Extract/Pectin), which were obtained after juice production. They contain antioxidants that help protect the skin. pectin supports the skin through their moisturizing properties. They act as a moisture-binding agent, which helps maintain the appropriate level of hydration. The ability of the stratum corneum to retain water is an important parameter that indirectly regulates skin hydration and indicates whether the hydro-lipid barrier is functioning properly. The dermis contains 80% water, while the stratum corneum contains only 13%. The average TEWL value measured in probands after radiotherapy was 40 [g/hm2]. With the duration of the study, the TEWL parameter gradually improved, oscillating after 6 months of study at an average level of 6 [g/hm2] (Figure 2). As a result of regular use of the preparation, which contained flax extract (INCI: Linum Usitatissimum Seed Extract) rich in fatty acids, vitamins and minerals, as well as alpha-linolenic acid, which has a moisturizing effect and supports the condition of the skin. This ingredient supports the occlusive effect of fatty raw materials and the physicochemical effect on intercellular cement. Lipids are incorporated into cement structures, which allows the properties of the epidermal barrier to change. Free radicals oxidize fatty acids that make up cell membranes and the hydrolipid coat of the skin (this is the process of lipid peroxidation) and damage structural proteins, among others. collagen and enzymatic proteins (aggregation and denaturation process) [18]. Lipid peroxidation involves the oxidation of unsaturated fatty acids, resulting in the formation of fatty acid peroxides, which, like reactive oxygen species, are characterized by very high chemical reactivity. As a result of this process, excessive water loss may be observed, which is inextricably linked to the dry skin that occurs during and after radiotherapy. In summary, the TEWL value improved after application of the product, also thanks to the phenomenon of merging/gluing irregular epidermal cells [19,20]. Based on the graph, it can be concluded that the TEWL parameter improved significantly for people using the cream with the tested active substance, whereas the base itself did not result in a satisfactory improvement. X-ray therapy also has an adverse effect on the condition of the skin, increasing the normal slightly acidic reaction on the skin surface. Based on the research, it was found that the longer the time passed since the end of the therapy, the less alkaline the pH became Figure 3. Patients who used only bases did not have satisfactory changes in the pH parameter value. Patients who used only bases did not have satisfactory changes in the pH parameter value. In order to accelerate the return of the appropriate pH reaction and thus the ability to perform protective functions, during the study the subjects were advised not to wash their skin with aggressive detergents, which also contribute to many irritations, excessive evaporation of water from the skin layers and increased sensitivity to external factors. To speed up the process of restoring the appropriate pH value after therapy, the preparation contains glucanolactone (INCI: Gluconolactone), panthenol and citric acid (INCI: Citric Acid). In addition to their pH-restoring properties, these compounds also have soothing and moisturizing properties. They have a soothing effect on irritations. Panthenol (INCI: Panthenol), which is another ingredient of the cosmetic formula tested in our study, accelerates healing, soothes irritations, burns and allergies [21-23].

Conclusions

It was confirmed that the product tested for an oncological patient has an appropriate level of skin hydration, supports the reconstruction of the skin and its energy barrier by reducing transepidermal water loss and restoring appropriate pH values. Thanks to this, the effects of pathological changes can be removed and the effects of pathological changes can be accelerated. For the emulsion to be more effective in patients exposed to ionized effects, it should be used long-term. After interviewing the test subjects about their sensory and application experiences, moisturization occurred and the wound healing process was accelerated. The respondents observed that regular use influences better results. The entire group of test subjects agreed that the effect of the product was consistent with the manufacturer's claims. Maintaining balance on the surface of the skin should be a priority in the equipment, and then it can be used to prepare lubricating and moisturizing preparations. The new modern methodology, based on obtaining fish skins from the fishing industry, is an innovative method due to the use of the upcycling trend. The formula of the test cosmetic is used to remove CO_2 emissions. They give them a second life. The upcycling trend to a future solution in terms of providing utility functions and additionally gaining access to active ingredients [24-37]. Based on studies conducted only on the control group. It can be seen that the examined biophysical parameters of patients using collagen improved significantly more than those who used only the

cosmetic base.

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Conflicts of Interest

The authors declare no conflict of interest.

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