



## Obtaining a New Generation Skin Mask for Cosmetic Applications

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As in every field, there is a great variety in the field of cosmetics to increase quality and to meet the demands of customers who prefer personal care products. Among many cosmetic products, the addition of nanomaterials has provided better quality and is widely used in sunscreen creams, anti-aging creams, hair products, face masks and lipsticks. The cosmetic application of electrospun nanofibers is a new area with great emphasis on the development of face masks or membranes capable of revealing skin care products [1-3].

In this study, new generation skin masks that can moisturize the skin and contribute to cell regeneration, electrospinning technique, black cumin (*Nigella sativa* L.) (DO), aloe vera (AV), black rice (*Oryza sativa*) (SP) added polyvinyl alcohol (It was obtained from PVA) composites. In addition to morphological (SEM, Scanning Electron Microscopy), biological (Cell Culture) and mechanical (Attraction) characterization studies of these produced nanocomposites, dermatological testing (in-vivo PATCH test) (for sensitive and normal skin) and toxicology tests were carried out. When the results of the study are evaluated, it will be able to show the feature of a face mask, which is anti-wrinkle, anti-aging, calming, anti-inflammatory cosmetic products that improve the skin, prevent skin aging and help fight free radicals.

**Keywords:** *Black Seed, Aloe vera, Black Rice, Nanotechnological, Composite, Mask.*

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### 1. Introduction

Due to the current problem of today's corona virus (COVID-19), the whole society, especially healthcare professionals, use face masks. However, as a result of long-term use of masks, since the skin tissue cannot breathe, it causes irritation of the skin on the face, rashes up to eczema, acne and wounds. Due to the number of

cases in the pandemic, in a situation where there are closures, the society could not

perform masks and similar personal care activities, since the cosmetic salons were closed, and thus, lubrication on the skin, dead cell formation, wrinkles, etc. problems have also occurred. In addition to the pandemic, in traditional cosmetic applications, creams, skin masks, sprays, laser therapy, injection and feeding from the skin, etc. as

personal care products. products and methods are used today, these create high costs and additional processes depending on the condition of the skin. For this reason, facial paralysis, temporary numbness, permanent deformation and additional diseases are caused by the use of chemicals. After our literature research, studies on composite face masks produced with nanotechnological electrospinning technique have been increasing in recent years. The use of the produced electrospun composite nanofibers in cosmetic applications is in the form of face masks, under-eye patches and acne patch materials that can reveal skin care products [1-7]. Thanks to nanofibers, active substances such as vitamins can be transported to the skin in a controlled manner. Thanks to the small fiber diameters, the bioactive agents that allow deep penetration into the skin are spread to the depths of the pores. Nanofibers with large surface area can show high contact properties. Nanofibers are of great importance in cosmetic applications. It can absorb a large amount of liquid with its improved properties such as small pore size and high porosity. Nanotechnology provides improvements in the qualities of cosmetic products. There is an increasing demand for the use of nanomaterials such as nanoemulsions, nanopigments, nanoliposomes, nanoparticles and nanofibers in cosmetic applications [6-9].

The skin consists of two important layers, the epidermis and the dermis, which are located on the oily layer called the hypodermis (subcutaneous tissue). The epidermis and dermis are also composed of lower layers. The area that connects the epidermis to the dermis is called the dermal-epidermal junction. It is responsible for the exchange of oxygen, nutrients and waste products between the vascular dermis and the avascular epidermis. The largest human organ, the skin, covers an area equivalent to 2 square meters and can weigh up to 10 kg. While maintaining internal homeostasis, it also acts as a protective barrier against the external environment.

Skin care products that give the skin the care it needs and that we can apply to the skin and leave it for 10-15 minutes are called skin masks. Face masks can meet the need for intense moisture, purify the skin, remove dirt and oil, fight blackheads and remove dead skin [2].

From past to present, plants and seeds have been used as a source of healing apart from being food. It is known that plants and their seeds used as a source of healing are used by trial, coincidence and advice [3].

Black cummin (*Nigella sativa L.*) (DO) is a species of the Ranunculaceae family that has been used in the treatment of many diseases since ancient times; It is a small shrub

and a flowering plant belonging to the buttercup family. There are many benefits of consuming DO on a regular basis. These; If DO is consumed regularly today, it is good for skin cracking by moisturizing the dried skin. It is used because it has positive effects against acne and acne formations. It provides healing of problems such as psoriasis and is widely used against eczema and skin disorders. DO consumption brightens the skin and makes the skin look more beautiful and tighter. DO consumption is also widely used against sunburn or sunspots [4].

Aloe Vera (AV) plant is a plant species belonging to the "aloeaceae" family. This plant, which was thought to be in the "liliaceae" family in the old classification, has begun to be used widely due to its medicinal properties such as medicinal value plants such as garlic, onions and asparagus in the liliaceae family. The plant looks like a cactus with its external appearance. In the aloe family, there are about 350 species with medicinal value, four of which are AV. "Aloe arborescens", "Tree Aloe", "Aloe fero", "Prickly aloe", "Aloe saponaria", "Spotted aloe" and "Aloe perryi" and "Curaçao islet aloe" are other medicinal values [5].

Thanks to the high amount of potassium it contains, it has a healing effect on all wounds and irritations. It is also known to be good for sunburns. Due to its high water and mineral content, it destroys the worn and pale appearance of the skin. It also shows excellent results in anti-wrinkle care with its collagen (COL) content. Due to its natural antioxidant and antibacterial properties, it helps to soothe and dry acne. With regular use, it eliminates problems such as skin tone unevenness and redness. It also has skin lightening properties when used with lemon. Thanks to its sebum balancing feature, it is effective against the problem of shine, which is the biggest problem of oily skin. In addition, AV, which is a natural and powerful moisturizer, is an indispensable source of moisture for dry skin [6].

Rice is consumed as a staple food in Asian countries. In addition to the most consumed white rice, there are also red and black varieties. Due to the antioxidant properties of colored rice products, their use in various food production has been supported. Black rice (SP) is obtained from 3-glycoside, which is rich natural anthocyanin compounds. SP has many nutritional advantages over other rice colors. SP is richer in protein, vitamins and minerals compared to white. Because it contains more magnesium than SP white, it also plays a critical role in the energy production of cells, increases the body's energy level, and strengthens the immune and nervous system. Another difference of SP from white rice is its high antioxidant value. Antioxidants strengthen the body's immune system and reduce the effects of aging. The above substances, which have a wide

range of uses in recent years, are also suitable for local use such as moisturizing the skin in the cosmetic sector and cause great developments in the medicine and medical sector [7-10].

Poly(vinyl alcohol) (PVA), a biocompatible and water-soluble hydroxy polymer, has very good chemical resistance, flexibility, mechanical strength and biodegradability. It can form a very good fibrous material with very good physical and mechanical properties, chemical stability at room temperature, alone or mixed with other polymers [11-17].

Electrospinning technique is the process of transforming the fluid into nano-sized fibers by applying a kilovolt tension to the viscous fluids at very small flow rates. In these applications, polymer solution or melt is used as a viscous liquid. The solution coming to the tip of the medical syringe at a uniform flow rate forms a spherical droplet under the influence of surface tensions and is transferred to the grounded collector at a certain distance as nanofibers by conicalizing (Taylor cone) under the influence of electrical forces [12].

Composite materials are polyphase structures formed by two or more materials protecting their own boundaries. Matrix and reinforcement element are essential in composite materials. Matrix and reinforcement material may differ according to the area where it will be used as metal, ceramic or polymer. In nanotechnological electrospinning technique, nanofiber membranes are obtained from polymer solutions by electric field. Composite nanofiber membranes can be produced with polymer matrix and reinforced ceramic, polymer or metal materials [11-20].

Production and distribution of cosmetic products are not subject to strict rules like pharmaceuticals. Nevertheless; New formulations and new active substances in cosmetic products should be dermatologically tested to evaluate their efficacy and safety. Cosmetic manufacturers generally prefer independent laboratories for this type of analysis. Dermatological testing in Turkey is carried out according to the "Guideline on the Evaluation of Human Skin Compatibility of Cosmetic Products or Raw Materials" published by the Turkish Medicines and Medical Devices Agency.

FDA; states that almost all cosmetics can cause allergic reactions in sensitive people. According to the American Academy of Dermatology (AAD), fragrance is the number one cause of cosmetic allergic reactions. In addition, the AAD states that there are over 5000 essential fragrances used in various cosmetic products, including perfume,

cologne, skin care products, soaps, shampoos, lipsticks, sunscreens, lotions, and preservatives can be another trigger for allergic reactions in cosmetic products.

The most common adverse effect seen in cosmetic products is contact dermatitis. The most prominent features of contact dermatitis are symptoms such as redness, scaling, itching, tingling and swelling. Cosmetic products cause 2 types of dermatitis:

**Allergic Contact Dermatitis:** It occurs only in some people and is an immune system reaction.

**Irritant Contact Dermatitis:** It can occur in anyone, depending on exposure, and is more common than allergic contact dermatitis.

In current applications, injection into the skin with a syringe is performed, which sometimes causes side effects. It poses similar problems such as paralysis, numbness on the skin, and permanent scar formations. The chemicals used adversely affect human health, inadequate skin care and the use of carcinogenic substances. Many anti-aging materials and drugs are smoked, both applied and coated. In addition to these, the fact that the existing skin masks are expensive reveals why this project is important.

Due to the pandemic, the whole society, especially health workers, uses mask and protective equipment. The dyes in the fabrics can cause skin irritation. However, the most important side effect is the development of an allergic reaction.

The allergic reaction may become more severe as it manifests itself in the form of tiny blisters and severe itching on a red, edematous surface that starts from the contact area but can spread to the surrounding area.

Among the problems mentioned above, materials and production methods that will not cause allergic reactions and skin irritation should be preferred. The nanotechnological electrospinning system is capable of eliminating these problems [20-25].

In this study, PVA-AV-DO-SP nanocomposite materials were produced by electrospinning method. The nanocomposites produced are thought to be used as an ideal skin mask in cosmetic applications.

## 2. Material and Method

### 2.1. Material

Black seed (*Nigella sativa L.*) (DO), aloe vera (AV), black rice (*Oryza sativa*) (SP) natural skin moisturizers were obtained from herbalists and plant breeders around us. Sigma/Aldrich brand was used as poly (vinyl alcohol) (PVA), molecular weight (Mw): 85.000-124,000 g/mol. In order to produce composite material in the electrospinning stage, oiled paper was used as the substrate.

## 2.2. Method

### 2.2.1. Preparation of composite skin mask solutions

Composite skin mask solutions were prepared by reinforcing the polymer at the values in Table 2.1.

**Table 2.1.** Preparation parameters of composite skin mask solutions

Polymer / Additive	Solvent	Mixture Temperature (°C)	Mixture Time (min.)
10% PVA	Distile water	60	60
10% PVA-2.5% DO	Distile water	60	60
10% PVA-2.5% AV	Distile water	65	60
10% PVA-5% SP	Distile water	65	60
10% PVA-2.5% DO-2.5% AV-%5 SP	Distile water	65	60

### 2.2.2. Production of composite skin mask nanofiber membrane by electrospinning method

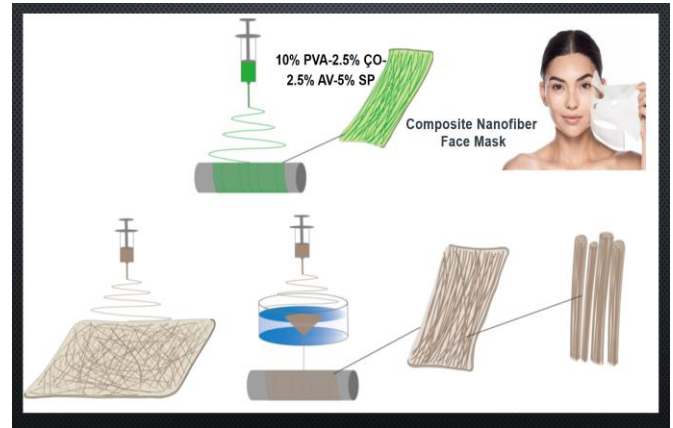
The electrospinning parameters required for the production of composite skin mask nanofiber membrane are shown in Table 2.2.

**Table 2.2.** Electrospinning parameters required for composite skin mask production

Polymer / Additive	Working Distance (cm)	Flow rate (ml/saat)	Voltage (kV)
10% PVA	15	2.5	30
10% PVA-2.5% DO	15	2.5	30
10% PVA-2.5% AV	15	3.0	30

10% PVA-5% SP	15	3.0	30
10% PVA-2.5% DO-2.5% AV-%5 SP	15	3.0	30

Composite skin mask production steps by electrospinning method are given in Figure 2.1.



**Figure 2.1.** Production stages of composite skin mask by electrospinning method

### 2.2.3. Characterization studies

The nanofiber diameters of the produced nanofiber membranes were investigated in the FEI FEGSEM QUANTA 450 brand device at 7 kV potential. The average nanofiber diameter ranges were determined by measuring 40 nanofiber diameters on the images and taking their arithmetic averages. Within the scope of cell culture biological characterization studies, samples were placed on 96 plates and mesenchymal stem cell seeding was performed, and cell viability values were observed at 24, 48 and 72 hour intervals. and Tensile strength values of all samples were prepared in ASTM standard and determined as a result of tensile test at 5 mm<sup>2</sup>/min tensile speed. The samples were repeated in triplicates among themselves and their arithmetic averages were taken as basis [25-30]. Dermatological test was performed in the company. Dermatological test (Patch Test) aims to detect allergic or irritating harmful findings of the skin. It is the only reliable method of detecting the factors that cause contact dermatitis. The test was carried out under the supervision of a dermatologist on 10 volunteers in accordance with the Declaration of Helsinki, the recommendations and guidelines of the European Union of Cosmetics within the framework of Turkish and EU standards. The course and conditions of the test were explained to all participants and a consent form was then filled out. Then, information about

the medical history of the volunteers was obtained. The knowledge that the test can be performed on a group of people with aggravated allergies or dry skin was also explained by expert dermatologists. The test process is respectively; 1- The product is prepared, 2- The test material is placed on the face, 3-After the application, the circumference of the test material is marked and numbered, 4-48 hours later, the test material is removed, the test area is marked with a pencil and evaluated after 30 minutes, and 5-Test area is 72 and/or 96 Reassessed at. It was reported that the evaluation to be made only at the 48th hour was not sufficient. It was said that second and/or third evaluations should be made at 72 and/or 96 hours in order to detect late reactions. The results were calculated by the expert dermatologist with the data of the tested cosmetic product. The classification of the product was made based on the outcome data. These classifications are as follows;

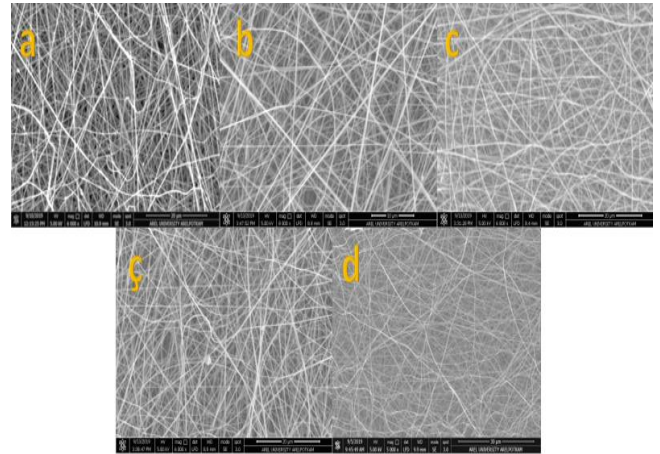
- ▶ No irritant / cause of irritation
- ▶ Very slight irritation/Very mild cause of irritation
- ▶ Slightly mild/minor irritation
- ▶ Moderate to mild irritation/Moderate cause of irritation
- ▶ Cause of irritation/irritation
- ▶ Very irritating / Cause of high irritation

The general toxicity data of 76/768/EEC of our skin mask has been tested. Testing has been reported locally and systemically [21-33].

### 3. Result and Discussion

#### \*SEM analysis

Nanofiber formation was observed in all composites. In addition to the polymer, natural skin moisturizers and electrospinning operating parameters affect the morphology of composite nanofiber membranes. By calculating the diameters of 40 nanofibers, arithmetic averages were taken and the nanofiber diameter distribution range of the samples was calculated. It was observed that the nanofibers of 10% PVA sample were irregularly oriented and thick. When DO, AV, SP additives were added to PVA, the fiber diameters of the skin mask nanofiber membranes became thinner. The thinnest nanofiber average in the study was observed in 10% PVA-2.5% DO-2.5% AV-5% SP composite sample with a value of 50-180 nm [30-35]. Diameter distribution range values of composite nanofiber membranes are shown in Table 3.1. SEM images of skin mask composite nanofiber membranes are shown in Figure 3.1.



**Figure 3.1.** (a) 10% PVA, (b) 10% PVA-2.5% DO, (c) 10% PVA-2.5% AV, (d) 10% PVA-5% SP, (e) 10% PVA-2.5% DO SEM images of -2.5% AV-5% SP skin mask composite nanofiber membranes

**Table 3.1.** Diameter distribution range values of nanofiber membranes

Sample name	Nanofiber diameter distribution range of samples (nm)
10% PVA	200-360
10% PVA-2.5% DO	160-345
10% PVA-2.5% AV	150-300
10% PVA-5% SP	100-250
10% PVA-2.5% DO-2.5% AV-5% SP	50-180

#### \*Cell culture analysis

Skin mask composite nanofiber membranes were placed on 96 plates. Mesenchymal stem cells were seeded into the samples in the plates and cell viability values were examined for 24, 48 and 72 hours. Cell viability values of 10% PVA sample were lower than other samples. However, cell viability values increased as a result of DO, AV, SP contribution. Due to their synergistic effects, cell viability values reached 97% at the end of 72 hours with the combination of natural skin moisturizers [23-37]. Cell live/dead values of skin mask composite nanofiber membranes during 24, 48 and 72 hours are shown in Table 3.2.

**Table 3.2.** Cell live/dead values of nanofiber membranes for 24, 48 and 72 hours

Sample name	24 hour	24 hour	48 hour	48 hour	72 hour	72 hour
	Cell live ratio (%)	Cell dead ratio (%)	Cell live ratio (%)	Cell dead ratio (%)	Cell live ratio (%)	Cell dead ratio (%)
10% PVA	92	8	90	10	88	12
10% PVA-2.5% DO	94	6	93	7	92	8
10% PVA-2.5% AV	97	3	97	3	95	5
10% PVA-5% SP	98	2	97	3	95	5
10% PVA-2.5% DO-2.5% AV-5% SP	99	1	98	2	97	3

**\* Tensile test**

The tensile strength values of the composite specimens were prepared in ASTM standard and carried out in a tensile test device at a tensile speed of 5 mm<sup>2</sup>/min at room conditions. The 1x5 cm sized samples were repeated three times and the arithmetic average strength values were taken as basis. Although the PVA sample has the lowest strength in the study, it has a durable structure in the light of the values in many studies when the literature studies are examined. In our study, the strength values increase as DO, AV, SP and their synergistic effect composites are added to PVA. Additives surround the polymer homogeneously and increase the strength value of the polymer. In this way, the strength has increased compared to PVA [35-38].

**\*Dermatological test results**

The dermatological test (Patch Test) of our composite skin mask was performed on five male and five female volunteers to detect allergic or irritating harmful findings on the skin. It was concluded that there was no evidence of irritation, no dryness and no edema. The results were calculated by the expert dermatologist with the data of the tested cosmetic product. Table 3.3. includes the dermatological test result of the composite skin mask.

**Table 3.3.** Composite skin mask dermatological test result

Irritation	Dryness	Edema
No findings	There was no	There was no formation

were found 0: No finding 0.5: Minimal and suspicious irritation 1: Slightly red, spotty 2: Moderate, uniform-looking redness 3: Strong, uniform-looking redness 4: Inflammatory redness	drought situation. 0: There is no finding 0.5: Dry, tense image 1: Light, thin dry layer formation 2: Medium dry layer formation 3: Serious dry layer with large flakes	of edema. +: Edema formation observed -: No formation of edema
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Ophthalmological test (in-vivo, in-vitro), In-vitro skin irritation test, Hydration test, Skin elasticity test, Transepidermal water loss test (TEWL), Anti-wrinkle product test, Anti-aging product test, Smoothing product test, Whitening Product effect test, Anti-acne test, Mattifying product test, Sebum test, Collagen production stimulating product test, Comedogen test, Cell renewal product test, Anti-eye bag product test, Make-up removal product effect test, Brightening effect test, Sensitization test, Mutagenesis (Ames) test, Healing effect test and Hypo-allergen test will be performed by examining all aspects of the skin, and by producing functional composite skin masks, the diversity of our composite products that will break new ground in the cosmetics industry will increase [27-32].

**\*Toxicology test results**

The local effect and systemic effects have been examined and it has been reported that the local effect of the toxic substance, which occurs at the first contact in the biological system, is at the level of ppm, while the systemic effect, which requires the absorption and distribution of the toxic substance, and the toxic effect seen after reaching the place of action is reported to be at the level of ppm. According to the test results, it was determined that our composite nanofiber skin mask did not sensitize and irritate the skin tissue [17-23]. Table 3.4. shows the local and systemic effect results of the composite skin mask.

**Table 3.4.** Local and systemic effect results of composite skin mask

Sample name	Local effect	Sistemic effect
Composite face mask	0.0001% The local effect of the toxic substance, which occurs at the first contact of the toxic substance in the biological system, is in ppm.	0.0001% The systemic effect requires the absorption and distribution of the toxic substance, and the toxic effect seen after reaching the site of action is again in ppm.

#### 4. Conclusion

Successful nanofiber formation was observed in all composite samples. Compared to PVA, nanofiber diameters were thinned with the addition of natural skin moisturizers. In addition, electrospinning operating parameters can change the morphology of the membranes. Electrospinning technology has resulted in the creation of a paper-like thin hydrogel face mask, a product that can provide anti-aging, whitening and anti-wrinkle benefits for the user. The thinnest nanofibers in the study were in the range of 50-180 nm and were observed in 10% PVA-2.5% DO-2.5% AV-5% SP composite sample. When the tensile test values of the samples were examined, the sample with all the materials together was the most durable nanocomposite in the study with a tensile strength of 45.67 MPa. Within the scope of cell culture studies, cell cultivation was provided to all samples, and cell viability was examined at intervals of 24, 48 and 72 hours. According to cell viability, 10% PVA-2.5% DO-2.5% AV-5% SP nanocomposite has the highest value with 97% viability at the end of 72 hours. As a result of these successful results, it is foreseen that the polymeric matrix nanocomposites produced as an ideal skin mask will be used in cosmetic applications. In addition, considering the features in the literature of the substances in the content of our product, it is also used in sectors such as health, textile, food, agriculture, filtration and defense.

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