In Vitro Characterization of the Mineral-Rich Magnetic Mud Mask

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Abstract

Modern research findings have connected skin exposure to air pollution with premature skin aging. The $SEACRET^{TM}$ Mineral-Rich Magnetic Mud Mask, is a cosmetic product currently focused on cleansing and improving the quality of the skin by the use of a colloid of oils, iron powder, and Dead Sea mud components. The mud mask should be applied to the surface of skin and magnetically removed after five minutes, resulting in facial skin rejuvenation. Product exploration began with characterization of the mask content including oil, iron powder, and minerals by X-ray Fluorescence, Fourier-Transform Infrared Spectroscopy, and Ultraviolet Spectroscopy. Further, to study the ability of the mud mask to remove ambient particulate matter of air pollution from skin, we collected particles from a car exhaust pipe and applied them on a lamb collagenous membrane as a skin model. To evaluate the efficiency of iron-based mask pollution removal after the completed procedure, particle counting and size identification on the skin model were quantified using ImageJ. The components of the mask remaining on the membranes led to an in vitro study of the effect on skin cells. Considering the possibility of components penetrating the skin, experimentation using extracted oils and iron powder in a water base was conducted on dermal fibroblasts and keratinocyte skin cells. Conclusively, the results found that the oil extracts with and without iron were able to sustain the cell environment of dermal fibroblasts and further support cell proliferation of keratinocytes. We propose that after application of the $SEACRET^{TM}$ Mineral-Rich Magnetic Mud Mask, existing air pollution particles will attach to the oil phase of the mask, due to their hydrophobic property, and will be removed magnetically along with the iron encapsulated in the oil of colloid.

Keywords: Pollution, Dead Sea Minerals, Cosmetic Mask

1. Introduction

In recent years, the connection between air pollution and premature aging has been intensively studied. Due to high levels of air contaminants around the world, particularly in Asia, cosmetic companies have been researching anti-pollution formulations. Air pollution is categorized into two types, primary and secondary pollution, where primary pollution consists of particulate matter (PM) and toxic gases. When primary pollutants react due to heat or UV radiation, secondary pollutants are formed [1].

Emissions from motor vehicles are one major source of concern for air pollution. The particulate matter emitted from motor vehicles, consisting of approximately 20% heavy metals and carbon-based materials, varies in size between 2.5 and 10 microns [2]. These metals, from greatest to least, include: calcium, iron, magnesium, zinc, chromium, nickel, lead, and barium [3]. For this reason, continuous urbanization has increased the demand for pollution removing cosmetic products. When metal pollutants encounter the skin, they can cause dryness, cellular damage, and pigmentation change, which indicate premature skin aging [4]. Skin pigmentation changes can include aggravation of acne as well [5]. Skin damage is caused by the ability of pollution to penetrate the skin [6]. Pollution removal is possible by washing with soap and water; however, the excessive use of soap can dry out skin because it is an astringent. Skin care products can aid in short-term skin health by forming a temporary shield against pollution. Research is ongoing for an anti-pollution cosmetic product that shields all types of skin. Varying skin type is a direct result of ethnicity differences, seasonal weather changes, differences in personal care, and so on [5].

The Mineral-Rich Magnetic Mud Mask from $SEACRET^{TM}$ is a colloid of oils, Dead Sea minerals, and iron listed with the following ingredients: propylene glycol, glycerin, dimethicone, isopropyl myristate, cyclomethicone, panthenol, capric triglyceride, grapeseed oil, jojoba seed oil, shea butter, fragrance, beeswax, phenoxyethanol, kaolin, rosehip oil, microcrystalline wax, vitamin E, and chamomile oil. The Mineral-Rich Magnetic Mud Mask claims to relax and rejuvenate the skin [7]. For centuries, Dead Sea minerals have been known for their therapeutic effects on human skin [8]. But, only a few cleansers currently on the market are capable of reducing pollution.

The use of existing magnetic mud masks, such as the Mineral-Rich Magnetic Mud Mask from $SEACRET^{TM}$, to remove pollution from facial skin have not been explored. Mechanistically, the mask is applied to facial skin in a thin layer and removed with a magnet after five minutes. The iron present in the mask allows for magnetic

removal. It is suggested to rub in the residue of oils left behind, allowing them to seep into the epidermis and dermis layers of the skin [8]. In this study, we demonstrate the successful removal of air pollution with the use of the $SEACRET^{TM}$ Mineral-Rich Magnetic Mud Mask. We propose a mode of action for the ability of applied colloid magnetic masks on polluted skin to dissolve the hydrophobic pollution particles by the lipid fraction. Pollutants are removed magnetically along with the iron-based mud masks.

2. Experimental

2.1. Materials

The Mineral-Rich Magnetic Mud Mask was obtained from SEACRETTM. The NaturaLamb collagenous membrane, manufactured by Church & Dwight Co., Inc., was used as a skin model. Car exhaust particles were collected directly from several tailpipes of cars to serve as model air pollution.

Primary keratinocyte and dermal fibroblast cell lines were supplied by the Skin Bank at the Dental School of Medicine (Isolated under IRB exemption 20076778). Keratinocytes were cultured in KGMTM-2 Growth Medium (Lonza). Fibroblasts were cultured in DMEM, 10% FBS, and Pen/Strep media (Gibco). $AlamarBlue^{TM}$ cell viability reagent was purchased from Thermo Fisher Scientific. Fine iron powder was acquired from Sigma Aldrich.

The X-ray Fluorescence (XRF) was detected using a Niton XL3t GOLDD+ handheld XRF from Thermo Scientific, for the pollution particles and mud mask on MYLAR thin 0.00024- 6.0 X-ray film.

The mud mask was analyzed using X-ray Diffraction (XRD) by the Philips Diffractometer with copper X-rays of 1.54184 .

2.2. Collagenous Membrane Treatment

To prepare the skin model, the collagenous membranes were stretched over 35 mm petri dishes and secured with Parafilm. The volume underneath was filled with DI water by a needle and syringe to maintain a damp construct. Air pollution was lightly patted onto the collagenous membrane surfaces. A thin layer of the mud mask was applied to all membranes and removed after five minutes using the magnet provided with the mask.

Images were taken of the collagenous membranes at 100x magnification before pollution, after pollution, and after application of the mud mask with an Olympus Light Microscope (BH2-UMA, f=180). Particle count and size identification were performed using ImageJ.

2.3. Keratinocyte and Dermal Fibroblast Cell Culture

A water-based extract of the mask was created in a 1:1 ratio of DI water: mud mask by vigorously mixing with a strong magnet for five minutes. Ultraviolet (UV) spectroscopy of the water-mask extract was obtained by the Thermo Scientific UV Spectrophotometer (Evolution 220).

The keratinocytes and dermal fibroblasts were plated on 96 well plates at a density of 104 cells/well, in their respective media, and incubated for 24 hours in a 37°C, 5% CO2 incubator. The water-mask extracts, with and without 1% iron powder, and DI water as a control, were applied to the cells in double dilutions from 10 to 160 times in media. The wells containing iron powder were incubated for five minutes before the iron was removed by a strong magnet and all the cells were incubated for another 24 hours. Afterwards, the media of each well was aspirated and replaced with 10% AlamarBlue in fresh media. After four hours of incubation in 37°C, 5% CO2 incubator, the BioTek microplate reader (FLx800; Excitation: 555 nm, Emission: 585 nm) was used to obtain optical density values of each well.

3. Results and Discussion

3.1. Pollution and Mask Characterization

Before applying the air pollution to collagenous membranes, they were examined by XRF. The elemental composition is presented in Table 1. The metals identified in our pollution model are comparable to the composition of car exhaust pollution determined by previous literature, including calcium, iron, zinc, and chromium [3].

Table 1: Elements Detected in the Collected Pollution Samples by XRF

Elements Found	Pollution (ppm)	Error (+/-)
Bal	932077	239
Fe	50120	239
Cr	4332	53
Ca	2766	215
W	249	30
Zn	188	7
Mn	131	41
Cu	79	8
V	54	28
Zr	49	2

To understand the content of the Mineral-Rich Magnetic Mud Mask, the elemental composition was tested by XRF. The most abundant element of the mask is iron, making up about 60% of the mud mask, as seen in Table 2, and is added to allow magnetic removal of the mud mask. All other metals identified are from the Dead Sea mud. Like the mud mask, the Jordan Dead Sea mud contains silicon in the greatest composition [9].

To identify the form of iron present in the mask, XRD testing was completed. It was concluded that the mud

Table 2: Elements Detected in the Mineral-Rich Magnetic Mud Mask by XRF

Elements Found	Pollution (ppm)	Error (+/-)
Fe	594768	6197
Bal	217548	7488
Si	177359	1708
P	5460	266
Co	2351	430
Ca	1529	303
Cr	230	58
Ba	217	90
S	188	64
V	127	60
Cu	98	50
Pb	64	28

mask contains pure iron rather than an ore of iron, such as the oxides magnetite and hematite. This is represented by the mud mask peaks, which occur at the iron ticks on the XRD spectra, as shown by Figure 1.

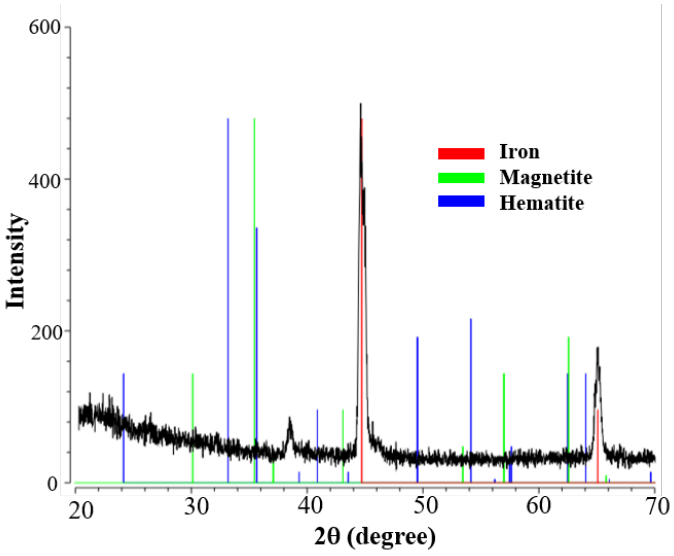

Figure 1: XRD Spectra of the Mineral-Rich Magnetic Mud Mask against Magnetite and Hematite Oxides

3.2. Pollution Removal Experiment

Collagenous membranes were used to study the efficiency of the mud mask to remove air pollution particles. Before pollution application, the original clean collagenous membrane did not contain particles, as seen by Figure 2(a). After the application of pollution, particles ranging from 5-50 micrometers (μm) in length were identified and are shown highlighted in yellow on Figure 2(b). Most of the pollution particles were 10-20 μm long, as shown by Figure 2(d). After proper application of the mud mask, the number of particles identified decreased, as shown by Figure 2(c). Pollution particles up to 50 μm in size were able to be removed.

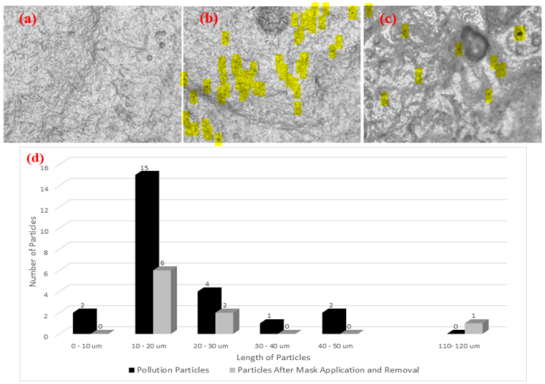

Figure 2: Light microscopy pictures (100X) of: (a) Clean Collagenous Membrane; (b) Collagenous Membrane with Pollution; (c) Collagenous Membrane after Mud Mask Application and Removal; (d) Number of Particles with Different Length Detected on Collagenous Membranes Measured by ImageJ

3.3. Effects of Mask on Keratinocyte and Dermal Fibroblast Cell Proliferation

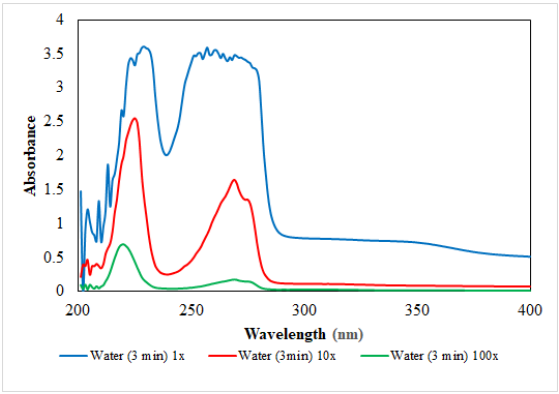

Figure 3: UV Spectra of Extract 1:1, Mud Mask: DI Water with 1x, 10x, and 100x Dilution

The experiment was done in triplicate and on average, the mud mask removed 65% of the air pollution particles originally identified. Of the 35% remaining, each trial contained one particle left behind that was greater than 50 m in length. These particles are most likely not part of the pollution applied because pollution particles this size were not detected before treatment with the mud mask. The particles could also be pollution particles encapsulated by lipids of the mud mask, making them appear larger, or a

component of the mud mask left behind, such as iron in the oils.

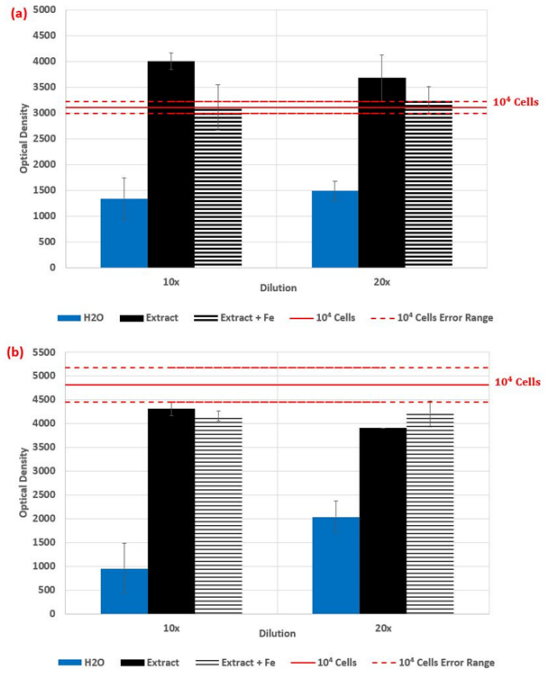

Figure 4: Cells Viability after Extract Application Measured as Optical Density (a) Keratinocytes and (b) Dermal Fibroblasts

Lipids are left behind after the removal of the mud mask with the magnet. To understand the effects of the lipids left behind by the mud mask, we tested the effect of the water-mask extracts on keratinocyte and dermal fibroblast cell proliferation. Because the mask cannot directly be applied on the cells, we began with the characterization of the water extracts of the mud mask using UV spectroscopy. The UV spectra, shown in Figure 3, has a large peak at approximately 220 nm and a smaller peak at roughly 270 nm. The large peak represents glycerol, one of the main ingredients of the mud mask [10]. The smaller peak was most likely caused by cera alba (beeswax), another ingredient the mask contains [11]. The UV spectra of the dilution shows oils of the product were successfully extracted.

Keratinocytes were chosen because the organics of the mask most likely have the greatest effect on the epidermis, the upper layer of the skin that is made up of keratinocyte cells. However, the lipids can penetrate through the keratinocytes to the dermis, made up of dermal fibroblast cells; consequently, we decided to treat these cells as well. To determine the toxicity of iron on the skin, if left behind, we also treated the cells with the water-mask extract with 1% iron powder added after extraction. The effects these water-mask extracts had on keratinocytes and dermal fibroblasts proliferation are shown in Figure 4(a) & (b).

As expected, the water control caused an inhibition of cell growth, due to the reduced osmotic pressure from the 10% diluted media with water, as shown in Figure 4(a) & (b). For comparison, the solid and dashed red lines represent cells plated at 104 and 0.5104 untreated in media, respectively. Treatment with the water-mask extracts stimulates the proliferation of keratinocytes, compared to untreated cells, as shown in Figure 4(a). Dermal fibroblasts that were treated with the water-mask extract and the extract with iron were able to maintain their viability at about 80-90%, as shown in Figure 4(b).

4. Conclusion

In this paper, we present a study on the Mineral-Rich Magnetic Mud Mask from $SEACRET^{TM}$. The capabilities of this mask, and masks of comparable composition, include the removal of air pollution and the support of keratinocytes and dermal fibroblasts cell proliferation by the organics remaining after product removal. The Mineral-Rich Magnetic Mud Mask was able to remove about 65% of pollution particles between 5-50 μm long, including the complete removal of particles less than 10 μm. We propose that the application of magnetic mud masks on polluted skin dissolves the hydrophobic pollution particles in the lipids within the five minutes of application. Then pollutants may be removed magnetically along with the iron-based mud mask. Minerals and oils deposited on the skin after the mud mask removal are contained in the water-mask extracts used to stimulate the keratinocytes proliferation. This suggests the capability of the mask to rejuvenate skin and improve dryness.

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