Strontium hexaferrite nanomagnets suspended in a cosmetic preparation: a convenient tool to evaluate the biological effects of surface magnetism on human skin

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\textbf{Background/purpose:} Magnetic therapy has been popular for ages, but its therapeutic abilities remain to be demonstrated. We aimed to develop a homogeneous, stable dispersion of magnetic nanoparticles in a skin-care preparation, as a tool to analyze the biological and physiological effects of superficial magnetism in skin.

\textbf{Methods:} SrFe\textsubscript{12}O\textsubscript{19} nanoparticles were generated by ultrasound, dispersed in glycerol, stabilized in Dermud cream and permanently magnetized. The magnetic cream was applied on the epidermis of human skin organ cultures. The effects on UV-induced cell toxicity, apoptosis and inflammatory cytokine expression were analyzed. A clinical test was performed to check skin moisturization.

\textbf{Results:} Nanomagnets were found to be homogenously and stably dispersed. After magnetization, the preparation generated a magnetic field of 1–2 G. Upon cream application, no cytotoxicity and no impairment of cellular vitality were found after 24 and 48 h, respectively. The anti-apoptotic and anti-inflammatory properties of Dermud\textsuperscript{TM} were not modified, but its long-term effect on moisturization in vivo was slightly increased.

\textbf{Conclusion:} Nanomagnetic Dermud\textsuperscript{TM} cream can be used as a tool to analyze the biological effects of nanomagnets dispersed on the skin surface at the cellular and molecular levels, thus allowing to explore the possible therapeutic uses of superficial magnetism for skin care.

\textbf{Key words:} nanoparticles – magnetotherpay – UV damage – inflammatory cytokines – skin care

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\textbf{Cosmetic products} are usually defined as topical applications that are legally limited to affect upper skin layers (1). To be eligible for a commercial career, however, a new product must prove substantial skin care efficacy. The cosmetic challenge therefore consists in designing strategies to elaborate efficient products without the need for dermal penetration. In this direction, surface magnetism provides one avenue for exploration. Here, we present the development of a preliminary work by Ahava-Dead Sea Laboratories (2, 3), which consisted in incorporating and stabilizing strontium hexaferrite (SrFe\textsubscript{12}O\textsubscript{19} or SHF) nanomagnets into a cosmetic cream, in order to analyze the biological effects of a superficial, static magnetic field on human skin.

Magnetic forces as a therapeutic tool have been attractive for centuries, despite the absence of established scientific evidence. Today, magnetic therapy is considered as ‘complementary and alternative medicine’ (CAM) (4). The use of permanent magnets for the treatment of various pathologies has steadily increased, and in 1999, the sales of therapeutic magnets reached US$350 million in the United States and US$4 billion worldwide in 1999. Commercial magnets were evaluated as safe by the National Center of Complementary and Alternative Medicine (NCCAM) (5).

In the last decade, significant efforts were invested in investigating the biological mechanisms potentially involved in the physiological properties of magnetic fields (6). Low-frequency magnetic fields in the picotesla range were claimed to reverse the symptoms of Parkinsonism (7) and multiple sclerosis (8) in humans, and to affect morphine-induced EEG in rats (9).
Pulsed electromagnetic fields affect bone and cartilage growth in vitro, with potential benefits in bone (10) and cutaneous (11) wound repair, and in arthritis treatment (12, 13). Static magnetic fields displayed anticonvulsant (14) and analgesic (15) properties in mice, reduced inflammation and pain in a rat model of arthritis (16) and accelerated the rate of bone repair in guinea-pigs (10). Moderate static magnetic fields reduced edema formation (17), decreased the plasma levels of nitric oxide metabolites, angiotensin II and aldosterone (18) and enhanced the vasodepressor effects of nicardipine (19) in rats; they also decreased blood pressure in rabbits (20). Permanent magnets showed therapeutic activity against chronic pelvic pain (21), pain in postpolio subjects (22), pain in postoperative healing of suction lipectomy patients (23), and they were used as an analgesic treatment for symptomatic diabetic neuropathy (24). Magnetic fields have complex effects on blood flow and blood vessels in the microvasculature (25). Static magnetic fields were shown to inhibit normal angiogenesis (26), to reduce blood flow in tumor vessels and to retard tumor growth in a hamster model (27). As former data had also shown that static magnetic fields cooperate with vitamin D to reduce the proliferation of breast cancer cells in vitro (28), there is consistent evidence that magnets may be useful in cancer therapy.

Nanotechnologies open new fields to the use of magnets for therapy. Thus, magnetite nanoparticle-containing liposomes have been injected into rat tumor in vivo to induce local hyperthermy under an alternate magnetic field, and promote tumor immunity via Hsp70 protein expression (29). Our approach aimed to investigate the effects of a superficial magnetic field on skin biology, and consisted in stabilizing a cosmetic preparation enriched with 1% SHF nanoparticles (2). The suspension was subsequently magnetized, and applied uniformly to constitute a static magnetic field on the skin surface. The properties of the ‘nanomagnetic cream’ were analyzed on human skin in organ cultures and in vivo.

Materials and Methods
Preparation of a cosmetic cream containing hard magnet strontium hexaferrite nanoparticles
The SrFe$_{12}$O$_{19}$ (SHF) dispersion procedure was adapted from earlier work (2). Briefly, 100 mg SHF powder was mixed with 50 mL propylene glycol acetyl-methyl ether in an ultrasound bath (D-80, 43 kHz, 80 W) for 2 h. After precipitation of primary particles, the solvent was removed by decantation and the residue was dried under vacuum at room temperature for 24 h. Then it was added to 1 mL glycerol and treated as above in an ultrasound water bath for 4 h. Finally, the suspension was mixed with 10 g of Dermud™ cream (Ahava-Dead Sea Laboratories, Holon, Israel) in order to obtain a cosmetic preparation containing 1% (w/w) of SHF nanoparticles.

Physico-chemical characterization of nanomagnetic Dermud™ cream
The particle size and morphology in the cream were analyzed by electron microscopy, using a JEOL-JEM-100 transmission electron microscope (TEM; JEOL, Tokyo, Japan). Particle dispersion in glycerol was checked by dynamic light scattering (DLS), using a Malvern Zetasizer Nanoseries device (Malvern Instruments, Malvern, UK). The morphology and chemical composition of suspended particles were investigated by scanning electron microscopy energy-dispersive X-ray spectroscopy (SEM-EDS), using an ESEM Quanta 200 (FEI Company, Hillsboro, OR, USA) equipped with EDS (EDAX-TSL, Mahwah, NJ, USA) operating at 5–20 kV in the low vacuum mode.

Human skin organ culture model, UVB irradiation and biological tests
The methods for skin organ culture, UVB irradiation, mitochondrial (MTT) assay, caspase-3 assay and measurements of cytokine secretion have been described elsewhere (30).

Clinical evaluation of the moisturizing effect
Clinical tests were performed on 10 volunteers in the 18–60-year range. Two days before the measurements, the subjects were asked to refrain from using skin care products but a bar of soap for regular washing. 30 min before testing, they were isolated from physical and psychological stress. A test area of $6 \times 18$ cm was marked on each forearm in its flexor region and divided into equal subareas, following study requirements. A dose of cream (0.4 mL), corresponding to a calculated thickness of 37 μm, was spread after randomization over the test area of one forearm, while the second forearm served as a control.

In order to evaluate moisturization of upper epidermal layers, electrical capacitance was measured using two different devices: a Corne-
ometer CM 825 (Courage and Khazaka, Köln, Germany), which probes the upper 10–20 μm of the stratum corneum by applying a probe made of two finger-type metal plates close to each other, and a MoistureMeter-SC (Delfin Technologies, Kuopio, Finland), which generates an electromagnetic field in an open-ended coaxial probe that penetrates through the skin, and allows to measure the dielectric constant of the stratum corneum over its entire depth (about 30 μm) (31).

The probe head was applied on the skin surface at a constant pressure. Initial values were recorded on the distal and proximal parts of the forearm, immediately before application of the test products. Subsequent measurements were performed 0, 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min after application. All measurements were performed symmetrically on the two forearms at 20–23 °C under a constant humidity.

**Statistical analysis**

Measurements of the biological effects of magnetic and non-magnetic Dermud cream in skin in organ cultures were repeated at least 10 times. The average values are given here, with the corresponding standard error of the mean (SEM). The significance of differences between average values, measured under different conditions, was evaluated using the unpaired Student t-test, and the significance threshold was fixed at $P<0.05$.

**Results**

*Dispersion and stabilization of hard magnet SrFe$_{12}$O$_{19}$ nanoparticles*

In line with our previously described method to form and disperse nanomagnets in a cosmetic preparation (2), we prepared SHF nanoparticles and monitored their dispersion in glycerol under a light microscope and TEM. The suspension was found to be homogeneously distributed, with particles with an average size between 80 and 100 nm (Fig. 1a). Analysis by DLS showed that the particle diameters followed a Gaussian distribution between 30 and 110 nm (Fig. 1b), in accordance with TEM pictures. DLS was also used to examine the stability of particle size in glycerol: the mean diameter was 77.7 nm ± 2.5 after 2 h and 85.7 nm ± 4.0 after 20 h at room temperature, with no evidence of aggregation.

The glycerol suspension of SHF nanoparticles was incorporated into Dermud cream, an oil-in-water emulsion containing 2% (w/w) Dead Sea mud, up to a final SHF concentration of 1% (w/w). The preparation was investigated by optical microscopy immediately and after 10 months storage at room temperature. No appreciable change was observed (not shown).

The size, distribution and chemical composition of suspended particles in the SHF containing cream were investigated by back-scattered electrons imaging (BSE), which reveals the presence of heavy elements as bright areas, and EDS. Regular Dermud appeared as a dark continuum, with a few dispersed bright particles in the 80–130 nm range enriched in Zn, the only heavy element in the preparation (Fig. 2a–c). The SHF-containing cream displayed the same general texture with a higher density of bright nanoparticles and occasional larger bodies (up to 1 μm diameter), the former and the latter containing Fe and Sr as well as Zn (Fig. 2d–f). This suggests that SHF particles associated with Zn-containing nanostructures, in major part remaining dispersed in the cream and in minor part forming micron-range aggregates.

![Fig. 1. SrFe$_{12}$O$_{19}$ nanoparticles dispersion and size distribution. (a) This transmission electron microscopic picture represents a suspension of SHF nanoparticles in glycerol, prepared as described in the Methods section. (b) This bar diagram represents the size distribution of SHF nanoparticles in glycerol, measured by dynamic light scattering.](image)
Fig. 2. Back scattered electrons imaging (BSE) – energy-dispersive X-ray spectroscopy (EDS) analysis. Samples of regular (a-b) and SHF-containing (d-e) Dermud™ cream were observed by BSE. Bright areas represent matter with heavy element components (essentially metals). Defined sample areas were analyzed by EDS, which chemically characterizes X-ray emitting atoms in response to charged particle hits. Comparison of EDS profiles acquired from large areas of regular (c) and SHF-containing (f) Dermud™ demonstrates the presence of Fe and Sr in the latter, while the only heavy element in the former is Zn.

Fig. 3. SrFe$_{12}$O$_{19}$ nanoparticle magnetization curves. Magnetization cycles (hysteresis loops) were recorded at constant temperature (± 0.1 K) by sweeping the external magnetic field between 0 and 2 T in five quadrates, using a MPMS-XL SQUID magnetometer (Quantum Design). The experiment was repeated at various temperatures, ranging from 273 to 343 K (0 – 70 °C). Sample magnetization (M) was plotted vs. applied external field (H), and results obtained at different temperatures were superimposed. (a) 100% SrFe$_{12}$O$_{19}$ nanoparticles. (b) 1% SrFe$_{12}$O$_{19}$ nanoparticles in Dermud™ cream.
Magnetization of the SrFe$_{12}$O$_{19}$-containing cream

The magnetic field required for full magnetization of the cream containing 1% SHF nanoparticles was determined in the temperature range of 273–343 K (0–70 °C), which covers ambient and body temperatures. Magnetization curves were recorded, using in parallel magnetic cream and pure SHF, and the results were compared in order to check for a possible interference of cream with nanoparticle magnetism (Fig. 3).

Clearly, saturation magnetization of the magnetic cream scales with that of pure SHF particles in a 1–100 factor, equal to the dilution factor. This shows that SHF particles can be fully magnetized in suspension in Dermud™ cream. It can be seen that the external magnetic field needed to reach magnetization saturation (about 0.9 T) is independent of the SHF particle concentration. Remnant magnetization values also scale with the nanoparticle concentration, in a temperature-independent manner. While the magnetization curves of magnetic cream and magnetic particles are qualitatively similar, the hysteresis width of the magnetic cream decreases with increasing temperatures. This effect might result from a decrease in viscosity, which may enable individual particles embedded in the cream to rotate more easily and align parallel to the external magnetic field, thus allowing a ‘softer’ response to the magnetic field.

Magnetization stability

In order to check the stability of cream magnetization with time, remnant magnetization was measured at 90-s intervals over 14 h, after switching off an external magnetic field of 5 T. This experiment was repeated at different temperatures as above, as shown in Fig. 4a. The initial relaxation rate was relatively high at all temperatures; it slowed down significantly after 1–2 h, and further slowed down at later times, as shown by Fig. 4b at 293 K (20 °C). This behavior qualitatively resembles a ‘stretched exponential’ behavior (32).

We have used the ‘stretched exponential’ model to fit magnetic Dermud™ relaxation data, and to evaluate the lower limit of cream magnetic lifetime, using the formula:

$$M_t = M_0 + M_1 \exp\left(\frac{t}{\tau}\right)^n$$

where $M_t$ is the time-dependent remnant magnetization, $M_0$ the magnetization at $t = 0$, $M_1$ the amplitude of the relaxing moment, $\tau$ a time constant and $n$ the power of the stretch exponent.

An optimized fit was obtained for $n = 1.6$ and $\tau = 3340$ s, and is traced as a solid line in Fig. 4b. This implies that the magnetic moment is close to saturation, and its relaxation is almost over, after 12 h. Extrapolation to 1 year predicts a loss of cream moment of about 0.5% at 293 K (20 °C) and 55% at 343 K (50 °C). The cream is therefore expected to maintain its magnetic field for years when stored chilled or at room temperature.

Effects of regular and magnetic Dermud™ on cell toxicity and cytokine secretion in human skin organ cultures

We showed before that Dermud™ cream has antioxidant and anti-inflammatory properties that...
significant), and this trend was reversed after exposure to UVB.

can antagonize UVB effects in human skin (30). In this work, we have compared the action of regular and nanomagnetic Dermud™, when applied on human skin in organ cultures for 60 h under normal conditions, or 36 h before and 24 h after exposure to UVB.

Cell toxicity was investigated using two different methods: an MTT assay of MTT activity and an enzymatic assay of late apoptosis effector caspase 3. MTT activity was shown to decrease by 10% after UVB exposure (statistically non-significant), and this trend was reversed after topical application of regular or magnetic cream, which induced a 30% decrease (Table 1). The cream’s effect on UVB-induced apoptosis in epidermis was more drastic, with an increase of caspase 3 expression reaching 340% in untreated controls after UVB exposure, but only 17% and 43% after application of regular and magnetic Dermud™, respectively. The two creams also displayed an antiapoptotic effect in unirradiated samples, decreasing caspase 3 activity by 48% and 57%, respectively (Table 1).

We monitored the secretion of five different inflammatory cytokines into the culture medium using ELISA tests. UVB irradiation increased

### Table 1. Effects of magnetic and non-magnetic Dermud™ cream on cell toxicity and cytokin secretion in UVB-irradiated and non-irradiated skin organ cultures.

<table>
<thead>
<tr>
<th></th>
<th>No treatment</th>
<th>Dermud™</th>
<th>Magnetic Dermud™</th>
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<tr>
<td></td>
<td>No UVB</td>
<td>UVB</td>
<td>No UVB</td>
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<td>MTT test (mitochondrial activity)</td>
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<td>Average value</td>
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<td>102 ± 13</td>
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<td>P = 0.21</td>
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<td>P = 0.23</td>
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<td>t-test cream</td>
<td>P = 0.87</td>
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<td>P = 0.13</td>
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<td>t-test magnet</td>
<td>P = 0.54</td>
<td></td>
<td>P = 0.66</td>
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<tr>
<td>Caspase-3 (enzyme activity)</td>
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<td>Average value</td>
<td>100 ± 35</td>
<td>443 ± 54</td>
<td>52.4 ± 16</td>
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<td>P = 4.1 x 10^-4</td>
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<td>P = 0.012</td>
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<td>t-test cream</td>
<td>P = 0.24</td>
<td></td>
<td>P = 2.1 x 10^-4</td>
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<td>P = 0.0042</td>
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<td>P = 9.0 x 10^-5</td>
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<td>IL-1 (ELISA)</td>
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<td>Average value</td>
<td>100 ± 17</td>
<td>218 ± 24</td>
<td>88.5 ± 13</td>
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<td>35 ± 5.7</td>
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<td>t-test cream</td>
<td>P = 5.8 x 10^-5</td>
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<td>P = 4.9 x 10^-5</td>
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<td>44 ± 8.2</td>
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<td>P = 0.15</td>
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<td>t-test cream</td>
<td>P = 0.014</td>
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<td>P = 3.7 x 10^-4</td>
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<td>120 ± 21</td>
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<td>P = 0.57</td>
<td></td>
<td>P = 0.57</td>
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<td>TNF-α (ELISA)</td>
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<td>Average value</td>
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<tr>
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<td>P = 0.88</td>
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</table>

Statistical significance of changes: ‘t-test UVB’ designates Student t-test values obtained by comparing sets of data from UVB-irradiated samples with corresponding unirradiated samples; ‘t-test cream,’ by comparing Dermud™ or magnetic Dermud™-treated samples with corresponding untreated samples; ‘t-test magnet,’ by comparing magnetic Dermud™-treated samples with corresponding regular Dermud™-treated samples. Significance threshold was fixed at P < 0.05.

ELISA, enzyme-linked immunosorbent assay.
cytokine secretion to various extents, ranging from 20% for IL-10 to 520% for tumor necrosis factor α (TNF-α). Regular and magnetic Dermud™ reduced all cytokine levels drastically, limiting TNF-α increase to 126% and 176%, respectively, and decreasing the levels of other cytokines below their pre-irradiation values. These changes were statistically highly significant, but the differences between magnetic cream and regular creams remained insignificant. In unirradiated samples, the two creams also reduced the secretion of IL-6, IL-8 and IL-10 significantly (Table 1).

Moisturizing effect of regular and magnetic Dermud™ cream on human skin in vivo
In order to evaluate the moisturization effect, electrical capacitance was measured on the forearm of 10 volunteers up to 8 h after cream application. When the dielectric constant was measured using a ‘Corneometer CM 825’ (Courage & Khazaka, Köln, Germany), which probes the upper 10–20 μm of the skin (31), the two creams displayed a long-lasting (>8 h) moisturizing effect, with a slight advantage for the magnetic cream, statistically significant in the interval between 4 and 6 h (Fig. 5a). When using a ‘MoistureMeter’ (Delfin Technologies, Kuopio, Finland), which probes the entire depth of the stratum corneum (about 30 μm), a similar enhancement of the moisturization effect by nanomagnets was observed, and was statistically significant as of 5 h and later (Fig. 5b).

Discussion
In this work, we took advantage of emerging nanotechnologies to disperse a source of magnetic field (SHF nanomagnets) in a skin-care preparation (Dermud™), in order to apply a magnetic field uniformly at the skin surface. We succeeded in producing a stable nanomagnetic cream, displaying a field of 1–2 gauss at 2 mm from its glass tube. This value largely exceeds the natural earth field (about 0.5 gauss), but remains below the pacemaker safety limit (5 gauss).

The actual magnetic field on the skin surface after topical application is weaker, however, because the magnetic particles are then randomly oriented and the cream loses its global magnetization. Instead, the ‘operative’ magnetic field is that created by each individual particle, which ranges between 0.5 and 2 gauss at a distance of 1 μm. The effects can take place when nanomagnets are absorbed in the stratum corneum. They are expected to be limited to the epidermis and the uppermost dermis.

We monitored the effects of magnetic Dermud™ on MTT activity and on apoptosis-associated caspase 3 in normal and UVB-irradiated skin organ cultures. No evidence of toxic effect or of altered antiapoptotic properties associated with nanomagnets was found. Cream effects on UVB-induced cytokine secretion were also unchanged.
Moisturizing properties in vivo, however, were slightly but significantly improved, as shown by two different methods of electrical capacitance measurement.

This work is an attempt to provide a new route for the development of cosmetic products, which are subject to conflicting requirements because their composition is limited by law to non-penetrating materials while their raison d’être is to improve skin condition, and hence to deal with underlying dysfunctions. Use of ‘magnetic spread’ on the skin surface, in order to exert a physico-chemical action on biological processes in the skin without physical penetration, therefore seems to be a relevant approach. Indeed, a number of reports have shown measurable effects of magnetic fields in various instances as mentioned in the Introduction, and in particular on skin wound healing (11) and on blood flow and blood vessels in subcutaneous microvasculature (25). According to this, it is reasonable to speculate that a biological action of magnetic fields can be characterized in the skin. Topical application of a nanomagnet suspension seems to be appropriate to evaluate such a biological action in skin models in vitro as well as in vivo. Therefore, the nanomagnetic cream we describe may be a valuable tool to establish a rational basis for therapeutic use of magnetic fields in skin disorders.

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