

RESEARCH
ARTICLE

Preliminary study of non-touch hand healing on human skin explants

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HIGHLIGHTS

This study investigates whether the healing practice of Non-Contact Hand-Based Energy Healing (NCH) can influence the early stages of wound healing by measuring specific biological markers (inflammation-related and tissue repair indicators) in human scarred skin samples. Based on these indicators, together with a morphological analysis, the researchers found signs of inflammation and early wound repair in NCH-treated skin samples, which were absent in untreated samples. These findings suggest that NCH may promote healing. This study supports the need for further research to explore NCH's potential role in integrative medicine.

ABSTRACT

Non-Contact Hand-Based Energy Healing (NCH) is a healing practice with deep roots in various cultures. NCH applied to wound healing still remains a controversial topic due to limited scientific evidence. Nevertheless, it has a strong potential for integrative medicine practices and requires further studies like the present one to provide additional results with more precise measurement techniques. This preliminary work was inspired by the experiments of Grad (Grad et al., 1961, 1963, 1965) and Souza (Souza et al., 2017). The primary goal of this study is to replicate and validate previous findings by applying non-touch NCH on human skin explants. This research investigates the biological effects of NCH on scarred skin explants, with the main objective of determining whether NCH influences early-stage wound healing through specific biomarkers linked to inflammation and tissue repair. Human scar-wounded skin explants were selected as the biological model. Based on preliminary tests, key wound repair markers were chosen for analysis, including erythema-like morphological changes, Ki67 protein expression, and IL6 cytokine concentration. This comparative study involved dividing the skin explants into two groups: a treated group exposed to NCH techniques and a control group with no treatment. The process was performed under standardized conditions to ensure comparability between groups. Both groups underwent three analytical methods: macroscopic morphological inspection via photographs, immunohistochemical analysis for Ki67 and K17 proteins, and ELISA tests for cytokine markers, particularly IL6.

The primary outcome measures included the presence of erythema-like changes (indicating inflammation) in the photographs, the expression of Ki67 protein (a marker of cell proliferation), and IL6 cytokine levels (an inflammatory marker). These indicators were selected for their known relevance to early wound healing and inflammatory

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responses. In the treated group, erythema-like changes were observed, along with low levels of Ki67 and elevated levels of IL6. These responses were absent in the control group. The presence of these markers in the treated group suggests an inflammatory response, indicating the early stages of wound repair. This study supports the hypothesis that hand healing induces a biological response in the treated skin explants, characterized by inflammation and early markers of wound healing. These results align with prior studies and provide a basis for further investigation into the efficacy of traditional hand healing practices.

KEYWORDS

laying-on-of-hands, hand healing, therapeutic touch, biofield, skin explant, Ki67, IL6, cytokine, wound repair, wound healing, inflammation, scar, immunohistochemistry, ELISA.

INTRODUCTION

Non-Contact Hand-Based Energy Healing (NCH) is part of complementary and alternative medicine (Benor, 2001). It is sometimes called “healing touch” or “therapeutic touch” (Krieger et al, 1979; Benor, 2001, p. 43), but actual touching of the physical body is not necessary for NCH. Indeed, throughout the present study, NCH was performed without any direct contact with a patient’s skin.

There are two main ways to study NCH: 1) to design and conduct an experimental evaluation of a measurable effect; 2) to develop a theory based on initial, generally accepted principles and to derive the observed effects from these principles.

The latter approach is speculative and could easily be dismissed by arguing that it is useless to elaborate a theory on a hypothetical phenomenon. Opponents of the former approach say that we cannot assume the existence of something that we cannot explain with a proper theory. This reasoning contradicts the scientific methodology, which states that we must first observe a phenomenon, then test it under various conditions, and finally elaborate a theory according to these tests’ results. As Einstein wrote: “Logical thinking is necessarily deductive; it is based upon hypothetical concepts and axioms.[...]. The most satisfactory situation is evidently to be found in cases where the new fundamental hypotheses are suggested by the world of experience itself.” (Einstein, 1936). Examples of such a methodology abound in science, *e.g.*, superconductivity, photosynthesis, and cancer studies. Moreover, the absence

of a complete theory does not prevent us from using the observed natural phenomena, as in, *e.g.*, superconductivity and quantum physics in general.

In the present study, we have taken the first path: our experimental study described here aims to assess the existence of an objective effect of NCH on human skin, assuming that hands can have a biological influence on organic tissues.

Either the placebo effect, through conditioning or expectation (Kaptchuk & Miller, 2015), or a psychological effect such as suggestion (Bedford, 2012) are sometimes cited to explain the results obtained. The former placebo effect is emphasized in either human or animal studies. To the authors’ knowledge, it is not recognized as effective in plants, as it is a psychological effect. Note here that acknowledging placebo effect in animals implies the existence of consciousness in animals.

Hence, we carefully designed our experimental protocol so that the placebo effect could not be used to explain our results. We made use of skin explants to avoid this effect in this study and based the rest of our design on previous experiments with mice (Grad et al., 1961; Souza et al., 2017).

After describing the historical and scientific context that helped us design the current experiment, we will describe both the biological processes involved here and our experimental protocol. Finally, we will present the results along with our interpretation and conclusions.

HISTORICAL AND SCIENTIFIC BACKGROUND

Historical background

Mentions of hand healing can be found in:

- Ancient Egypt’s Ebers papyrus (1600 BCE): “Put your hand on the pain, and say that the pain goes away,” and in The Book of the Dead: “I place my hands upon you, Osiris, for your good, to make you live.” (Jagot, 1974).
- Ancient Greece by Solon (638 -559 BCE): “But he makes at once healthy the one who is wrecked by some evil and difficult diseases, by touching [him] with both hands” (Tipei, 2001, p. 87).
- Greece by Pline (23-79 CE): “Crates of Pergamus relates, that there formerly existed in the vicinity of Parium, in the Hellespont, a race of men whom he calls Ophiogenes, and that by their touch they were able to cure those who had been stung by serpents, extracting the poison by the mere

imposition of the hand.” (Pline the Elder, Vol. VII, II.16).

- Hippocrates: “the force which flows from many people’s hands. We can find comparable accounts by Roman authors like Celsius and Plautus.” (Rubik et al., 2015).
- Assyrian/Babylonian tablets: “When I approach the sick person, when I enter his house, when I lay my hand on his head...” (Tipei, 2001, p. 92)

The distant action of the hands was associated by analogy with another distant action known at that time: the one of Magnesia stone from Greece, referred to by Thales of Miletus, Anaxagoras, and Aristotle (Thouret, 1784).

The medieval Europe rediscovered Greek knowledge concerning magnets through Arabic invasions, notably with Avicenna, Aetius of Amida, and Alexander Trallianus (Thouret, 1784). Certain authors, like Paracelsus, as well as Digby, attribute the distant action of the hands to a vital or universal fluid/spirit, while some others establish a link with stone magnetism: Cabée, Kircher (1641), Glocecius, Van Helmont, Fludd, and William Maxwell (Thouret, 1784). Kircher believed that “all phenomena are linked together by a magnetic chaining”.¹ (Thouret, 1784). Other scientists have the intuition of a correspondence between electricity and the nervous system, e.g., Newton (Newton, 1846, p. 507) relates the motion of the body’s members to electricity. They were followed by Le Noble, Laennec, and Maggiorani (Jagot, 1974). Mesmer made his own synthesis from these predecessors, calling the hand healing process “animal magnetism” (Mesmer, 1779). He gave an account of animal magnetism in which he underlines “the analogy of its properties with those of the magnet and electricity”. He also describes these properties, noting that it: “penetrated everything; that it could be accumulated and concentrated, like the electric fluid; that it acted in the distance [...]”.

In 1784, Mesmer was put under scrutiny by two different medical commissions that could not reach a common conclusion. But Lavoisier commission could have been charged with scientific wrongdoing by replacing the initially agreed “curing ability” criterion with the “fluid detection ability” (Belhoste, 2021; Conickx, 2023). Doing so, the curing tests on patients were replaced by tests of the healers’ ability to detect “magnetized” water in glasses and magnetized trees vs non-magnetized ones. While the conclusion of Lavoisier commission was negative, Mesmer also contributed to the science of his time by drawing attention to “somnambulism” and suggestion, now called “hypnosis”.

Doing so, he paved the way for future investigators like Charcot (Charcot, 1892) and Freud (Freud, 1891).

Even before that time, but especially during the 17th and 18th centuries, there were remarkable interactions, confusions, and influences between the fields of hand healing, hypnosis, electricity, and magnetism. The aforementioned quote by Mesmer testifies that these domains were seen as related. The then recent discovery of electrical influences on biological organisms by Galvani (Guéron, 2009) could have influenced Mesmer for merging the older concept of a vital fluid with the new one of electricity. The subsequent exploration of this field later gave birth to electrophysiology by du Bois-Reymond in 1842 (Finkelstein, 2015) and electrotherapy (Bonney, 1882). Furthermore, the need to understand the behavior of people reacting to “magnetic” treatments from Mesmer and colleagues led to the scientific study of nervous diseases (Charcot, 1892) and then to psychoanalysis (Lakhdari, 2007). Indeed, Freud and Jung recognized hypnosis as a useful tool for psychology (Freud, 1891; Hartmann & Zimberoff, 2013). On the physics side, J.C. Maxwell explained how electricity and magnetism are deeply related. As knowledge progressed, these fields specialized and took separate paths. In spite of this separation, some connections seem to exist, e.g., physical healing can be attributed to psychological suggestion (Bedford, 2012). Hence, the need to properly address the placebo effect in such studies.

Scientific background

The first half of the 20th century was troubled by two wars and the advent of chemical medicines, which held back research in the NCH field. As of 2015, there have been “over 30 published clinical trials reporting effects of biofield therapies for pain in ambulatory and hospitalized patient populations with chronic pain, arthritis, and movement restriction.” (Jain et al., 2015). One of these pioneering works was held in the 1960s by Bernard Grad, who applied modern scientific methodology to this field by performing experiments on mice and plants (Grad et al., 1961, 1963, 1965) in Canada.

The Grad experiment (Grad et al., 1961) with wounded mice needs to be detailed here as it was one of the main sources of inspiration for our own experiment described hereafter. Grad cut out a part of the skin from 300 mice put to sleep, the area of each wound was then precisely measured at four different moments in time: the day of the wounding (day 0), then on day 1, day 11, and day 14. Before each trial, ten mice were placed in individual boxes within

cages and trained to remain calm. The mice were divided into three groups: two control groups and one treatment group. One of the control groups received heat to simulate the temperature increase caused by hands during treatment. The treatment group received hand healing sessions from healer Oscar Estebany twice daily for 15 minutes each, five times a week. On day 11 and day 14, the wounds of the treatment group were significantly smaller compared to both control groups. The wound areas of the two control groups were not significantly different from each other.

Grad then came up with the idea to conduct an experiment using “wounded” plant seeds (Grad et al., 1963, 1965). The procedure involved watering barley seeds with a salt solution on day 1 (wounding step), followed by drying them in an oven at 38-40°C, and then watering them again with regular water. The plant pots, each containing 20 seeds, were not directly hand-treated, but NCH was applied to the watering solution poured over the seeds. Two parameters were measured: the number of seedlings emerging from the soil and the heights of the plants. Statistical analysis revealed that the treated group performed better than the control group in terms of both criteria. This experiment was repeated in a double-blind manner, yielding the same statistically significant results.

Grad and others (Barry, 1968) inspired Dolores Krieger, a nurse, and Dora Kunz, a natural healer, to develop their own therapeutic method (Krieger, 1979). The NCH was renamed “Therapeutic Touch” (TT) by Krieger, which is confusing, as it implies a physical contact between hand and body, while there is not. She emphasized the fact that anybody can heal with their hands, having the proper mindset.

It should be noted that the need for a specific mindset has not been established as a mandatory requirement, either through the daily NCH practice or the training of other practitioners by one of the authors. We rather suspect an underlying mechanism of a physical nature, *e.g.*, an electromagnetic interaction.

The Grad experiment was replicated in 1984 in the Villejuif hospital near Paris with healthy seeds (no salt solution used to “wound” the seeds) by Yves Lignon (statistician), Léon Schwartzberg (experimenter), and Jean-Marie Le Gall (hand healer) (Lignon, 1989; Raoul, 2021; Lignon & Danier, 1984). The originality of this experiment is that it was done under the scrutiny of a legal officer (Maître Djian) to ensure no trickery could be performed. Sixteen plant pots were used for the results to be statistically significant, eight being watered with treated water and eight with ordinary water. Pouring water volume was precisely measured.

The NCH treatment duration was 10 minutes per bottle of water. The plant height measurement occurred two weeks after the start of the experiment. Both peas and radish seeds were used. Another identical, two-week experiment was performed after the first one. The results of the second experiment show statistically significant higher heights in the case of radish plants ($p < 0.001$). The experiments by Grad and Lignon both suggest that there is a statistically significant effect of the NCH on plants.

Souza et al. (Souza et al., 2017) performed an assessment of NCH on 24 wounded rats. Fibroblast count and measure of wound size were performed on day 1, 4, and 7 after the wounding. On the 7th day, fibroblast counts were significantly higher in the treated group than in the control group. The wound shrinkage was also more pronounced in this group. These data support the Grad hypothesis made with mice (Grad et al., 1961, 1965), that NCH may accelerate wound repair, presumably by increasing fibroblast activity, among other potential processes.

Our study design was inspired by these past works: we chose to focus on NCH effect on human skin explant in order to limit any type of psychological influence (placebo effect) on the outcome of the experiment.

Biological processes involved in wound repair

Wound repair mechanism

Wound or tissue repair occurs after a cutaneous injury has occurred, which also induces bleeding. It is usually described as a process involving four main stages (Laurent et al., 2017): hemostasis, inflammation, proliferation, and dermal remodeling (Wilkinson & Hardman, 2020). It should be noted that these stages have no clear-cut separations between them: they overlap as tissue repair is a continuous process, well-orchestrated and synchronized, involving many actors present through the above-mentioned stages.

The hemostasis stage. This stage is aimed at forming a clot, which prevents both blood from leaking and bacterial entry by re-establishing the barrier function of the skin. The hemostasis stage includes a coagulation step occurring through platelets and mediated by the thrombin enzyme. Platelets are activated by contacting the damaged vascular subendothelial matrix. Platelet receptors, like, *e.g.*, glycoprotein VI, react with extracellular matrix (ECM) proteins, *e.g.*, fibronectin or collagen, to enable

their adherence to the blood vessel wall (Wilkinson & Hardman, 2020).

Moreover, platelets release:

- cytokines attracting leukocytes to the injury site;
- growth factors that stimulate the migration of skin constituents like fibroblasts and keratinocytes. Fibroblasts are cells dedicated to the formation of tropocollagen, the precursor of collagen. Fibroblasts are also involved in the secretion of all the components of the ECM through synthesis of the gel-like ground substance. Fibroblast transform itself into fibrocyte, its stable state. As mesenchymal cells, fibroblasts express a "major type III intermediate filament (IF) protein", namely: vimentin. Vimentin can thus be used as a biomarker for fibroblast, in addition to playing "an important structural and functional role in forming and regulating the cytoskeleton" of the cells (Dave & Bayless, 2014). Keratinocytes, on their side, are the cells forming the normal skin's outermost layer (epidermis).

Thrombin is central to the coagulation process as it is involved in a lot of reactions occurring during this step. The primary thrombin reaction takes place with fibrinogen, converting it into fibrin. Indeed, fibrinogen is depleted as the thrombin concentration increases (Dashkevich et al., 2012). According to this latter study, thrombin propagates in the form of an impulse-like wave of constant peak amplitude and spatial velocity. As the thrombin wave propagates, the fibrin clot also extends at a constant spatial velocity. The thrombin excitation wave propagation is made possible by:

- a positive feedback loop leading to an autocatalytic thrombin production;
- a clotting protein: plasma thromboplastin antecedent (factor XI).

Blood can thus be compared to an excitable medium that supports thrombin wave propagation and functions as a "biological nonlinear dynamic system" (Dashkevich et al., 2012), in close analogy with what is known from neural impulse propagation. This phenomenon enables "Rapid and steady spatial signal propagation across significant distances in a heterogeneous system" (Dashkevich et al., 2012). It was also found by the same authors that this thrombin excitation wave stops propagating as soon as it comes into contact with thrombomodulin, which is abundant in healthy endothelium.

The inflammation stage. Inflammation is an immune response against pathogenic wound invasion. It involves mainly two types of leukocytes (neutrophils and monocytes), pro-inflammatory proteins, and macrophages (Laurent et al., 2017). During the inflammation stage, pro-inflammatory proteins such as cytokines and chemokines are released through "a variety of cell types, but the most important sources are macrophages and monocytes at inflammatory sites" (Gabay, 2006). These proteins attract moving leucocytes, notably neutrophils, to the wound to prevent infection. In turn, these neutrophils release additional cytokines. Cell adhesion molecules, such as selectins, are also expressed through pro-inflammatory molecules during the inflammation process. Selectins have been shown to play an important role in immune cell recruitment (Wilkinson & Hardman, 2020). The most notable cytokines released at the cutaneous injury are: tumour necrosis factor (TNF)- α , interferon (IFN)- γ , transforming growth factor (TGF)- β , interleukin (IL) 1, 6, and 8 (Jiang et al., 2012). Lipopolysaccharide (LPS) also plays a role in the inflammation process by triggering the release of cytokines. (Wilkinson & Hardman, 2020; Gabay, 2006). Neutrophils are mostly destroyed by adhering to the fibrin scab. The remaining neutrophils are eliminated by macrophages originating from monocytes that migrate to the wound tissue. Two types of macrophages are involved in the inflammation: the pro-inflammatory phenotype macrophages (PIPM) and anti-inflammatory (AIPM) ones. The PIPM replace neutrophils as the main inflammatory agent, through the phagocytose of the latter. The AIPM results from either a phenotype switch from the PIPM type or from newly coming monocytes (Wilkinson & Hardman, 2020). Moreover, B, T, and mast cells have also been shown to play an anti-inflammatory role during the inflammation process (Nosbaum et al., 2016; Weller, 2006).

At the end of the inflammation stage, foxp3-expressing regulatory T cells (Tregs) accumulate in wound tissue, inducing a decrease in IFN- γ and PIPM production, thus attenuating the inflammation. This step facilitates "wound repair through epidermal growth factor receptor (EGFR) pathway" (Landén et al., 2016).

During the transition from inflammation to proliferation, plasmacytoid dendritic cells (pDCs) produce type I interferons (IFN- α/β) via toll-like receptors (TLR)7- and TLR9-dependent mechanisms, which induce "early inflammatory responses and re-epithelialization of injured skin" (Landén et al., 2016): the wounded epidermal layer starts to reform. Langerhans cells are also concentrating at

the wound site, though their role is not fully understood (Landén et al., 2016).

The proliferation stage. Proliferation encompasses re-epithelialization (granulation tissue formation through keratinocytes), fibroblast migration and proliferation, and synthesis of extracellular matrix (ECM) (Prabhu et al., 2022). These are stimulated by wound-related signals, some being synthesized by macrophages, e.g., nitric oxide.

The proliferative phase also involves $\gamma\delta$ T cells eliminating damaged keratinocyte, releasing fibroblast growth factor (FGF)-7, keratinocyte growth factor (KGF)-1, and insulin-like growth factor (IGF)-1. This, in turn, stimulates the proliferation of healthy keratinocytes. $\gamma\delta$ T cells also produce IL-17 and express host-defense molecules in epidermal keratinocytes, promoting wound healing (Landén et al., 2016). Of particular interest in relation to NCH potential mechanism: “keratinocytes are activated by changes in mechanical tension and electrical gradients” (Wilkinson & Hardman, 2020). Keratinocytes upregulate some keratin (K) IF proteins, notably K17. K17 is not normally expressed in the suprabasal layers of the epidermis but are rapidly induced by stressed keratinocytes upon wounding (Werner et al., 2007; Russo et al., 2020). The release of K17 is ongoing through the following remodelling stage, until the barrier function of the skin is restored, “suggesting these keratins play important physiological roles during repair” (Zhang et al., 2019).

Mast cells not only contribute to the proliferation of keratinocytes too, but are also involved in the proliferation of fibroblasts and endothelial cells (Weller et al., 2006).

Plasmin, a protease, enhances keratinocyte migration by eliminating the provisional fibrin-rich wound tissue. Fibroblasts have a key role in replacing the fibrin-rich temporary matrix with a more substantial new connective tissue and microscopic blood vessels (both forming the granulation tissue). Proliferating cell nuclear antigen (PCNA) and Ki67 are common markers of cellular proliferation (Landén et al., 2016): “Ki67 is a nuclear protein associated with the cell cycle, synthesized by all proliferating cells of the active cell cycle and deficient in resting cells” (Prabhu et al., 2022).

Blood vessels form through the angiogenesis mechanism triggered by hypoxia, which also expresses other factors like, e.g., hypoxia-inducible factors (HIFs), cyclooxygenase 2, and vascular endothelial growth factor (VEGF). Macrophages are also known to participate in this process by structuring the newly created vessels (Wilkinson & Hardman, 2020).

The granulation tissue is mainly constituted of fibronectin, immature collagens, and proteoglycans. Fibroblasts are triggered by signalling molecules released through platelets, endothelial cells, and macrophages, along with such growth factors as, e.g., TGF- β and platelet-derived growth factor (PDGF) (Wilkinson & Hardman, 2020).

To rebuild sensory receptors, an innervation step then occurs to regenerate the nerve fibers.

The dermal remodeling stage. The remodeling of the ECM is either seen starting with the initial fibrin clot deposition (Wilkinson & Hardman, 2020) or at the end of the granulation tissue formation (Landén et al., 2016). This phase ends in a matter of years with mature collagen presence in the scar.

Mechanical force and cytokines enable fibroblasts to differentiate into myofibroblasts, which in turn express an actin that contracts the wound. Myofibroblasts then disappear through apoptosis. In parallel, the collagen III produced in the ECM during earlier stages is replaced by collagen I, which is a stronger type, though it takes longer to form. At this stage, fibroblasts replace the remaining fibrin clot with hyaluronan, fibronectin, and proteoglycans. The above-mentioned collagen evolution is promoted by proteoglycans. It requires a fine tuning between collagen degradation and synthesis, through temporal synchronization of metalloproteinases (MMPs), expressed by AIPM, fibroblasts, and keratinocytes. Elastin, formed from tropoelastin, enables the reformation of elastic fibres to retain skin elasticity (Wilkinson & Hardman, 2020).

A decrease in the blood flow and the number of blood vessels is observed (Landén et al., 2016).

The wound healing process ends when macrophages, endothelial cells, and fibroblasts disappear through apoptosis or migrate out of the injury site (Wilkinson & Hardman, 2020).

Wound repair markers elected for this study

The description of the wound healing process given above is synthesized in the overview Table 1, highlighting (bold font) the markers chosen for this study.

According to Table 1, Aquiderm –the contracted biological laboratory- selected the following seven markers of inflammation and proliferation: IL6, IL8, TNF- α , IFN- α , IFN- β , Ki67, and K17 (Rambert, 2019). Their concentration or count was measured to assess the presence of a wound repair mechanism at play in human skin explant samples.

Table 1. Overview table showing the cells involved in the wound healing process at each stage and the cells selected for subsequent analysis in this study (bold font).

Wound repair stage	Cells involved	Markers chosen in our experiment
Hemostasis	Thrombin, Platelet, fibrinogen, fibrin, fibronectin, glycoprotein VI, collagen III, fibroblast, vimentin, plasma thromboplastin antecedent (factor XI).	
Inflammation	Neutrophils, monocytes, cytokines (IL6 , IL1, IL8 , TNF-α , IFN- γ , TGF- β), chemokines, selectins, LPS, PIPM, AIPM, Tregs.	IL6, IL8, TNF- α
Transition from inflammation to proliferation	IFN-α , IFN-β .	IFN- α , IFN- β
Proliferation	Nitric oxide, γ 8T cells, FGF7, KGF1, IGF1, IL17, K17 , fibroblasts, plasmin, PCNA, Ki67 , HIFs, cyclooxygenase2, VEGF, BCL2, TGF- β , PDGF.	Ki67, K17,
Dermal remodeling	Myofibroblasts, actin, collagen I, hyaluronan, fibronectin, proteoglycans, and elastin.	

METHOD

The experiment described here was carried out at Aquiderm (within Inserm U1035 laboratory), Bordeaux, France. It took place from 26 August 2019 until 30 August 2019 (5 days). Below is a summary of their report (Rambert, 2019) with additions by one of the authors (ARC).

Experiment preparation and conditions

Materials

Thirteen tissue explants (NativeSkin® models) were provided by Genoskin company (Toulouse, France) and delivered by the TNT carrier. They originated from a twenty-one-year-old Caucasian woman with no skin pathology. The culture of these explants and the measurement of wound healing through molecular markers were performed by Aquiderm (Rambert, 2019).

Among the thirteen explants, six were dedicated to the hand healing treatment, while the seven left formed

the control group. Each skin explant was wounded by a scar in its center (Figure 1).

Culture of skin explants

The NativeSkin® models were placed in a maintenance medium (provided by Genoskin company) in an air/liquid interface, ensuring that the dermis was submerged in the culture medium while the outer layer of the Stratum Corneum was exposed to the air. The culture plates were kept in an incubator at 37°C, 5% CO₂, and 95% humidity. The maintenance culture medium was changed daily throughout the study.

Macroscopic Photographs

Every day during the study, the skin explants were observed, and macroscopic photographs were taken to assess morphological changes.

**Figure 1.** Photograph taken on the fifth day of the experiment. Control group.

Hand Healer Preparation and Method

One of the authors, (ARC), a hand healer, conducted an NCH experiment on scarred skin explants for five consecutive days at the Aquiderm facility (Rambert, 2019). Two sessions of NCH healing, each lasting 1.5 hours, were performed every day: one in the morning and one in the afternoon. In total, each treated sample received 15 hours of NCH healing throughout the entire experiment. The NCH was applied according to the method described in Durville (Durville, 1900).

Mental fatigue had to be accounted for, as the mental attitude required was a mix between relaxed meditation and focused concentration on the hands above the skin explants, to ensure proper positioning over a small surface area (3 cm of radius).

To prepare for this intense work and avoid physical and mental fatigue during the experiment, ARC underwent a two-month training regimen, which included:

- Reviewing previous protocols (e.g., Souza et al., 2017; Grad et al., 1961).
- Acquiring biological knowledge about the stages of wound healing.
- Abstaining from consuming any alcoholic beverages.
- Daily walks and jogging sessions covering a distance of 10 km per day.
- Daily Qi-Gong sessions to promote mental calmness and induce an "alpha wave" (8-10 Hz) state of mind, as well as facilitate the circulation of bodily energy.
- Regular sleep schedule with waking up at 7:30 AM and going to bed at 10:00 PM.

During the sessions at Aquiderm, ARC performed a "magnetic" pass known as palmar imposition (Durville, 1900, p.73; Durville & Jagot, 1914) without making contact with the open 6-well culture plate or the skin inside it. This pass was conducted by orienting the palm side of the hand towards the sample, while maintaining a distance of one to three centimeters.

The right and left hands were alternated over the samples during the NCH healing sessions. Once located over a sample, the hand remained static above it. The palmar imposition technique accounted for 80% of the total NCH healing duration of this experiment.

Palmar imposition was also done by placing both hands, with fingers extended, in a concave "dome" shape over the whole six culture plates containing the skin samples in their maintenance culture medium.

Another technique called finger projection ("projection digitale") or digital imposition (Durville, 1900, p. 74; Durville & Jagot, 1914) was employed, using only the fingertips that pointed towards the skin explants. This technique allows the practitioner to target a specific area of treatment by spreading the fingers apart (for larger areas, e.g., lower back pain) or bringing them together in "bundles" (for smaller areas, e.g., warts), based on the practitioner's assessment.

The third technique used is termed "heteronomic position" (Durville & Jagot, 1914): the object to be treated is placed between the palms of the hands facing each other, thus enveloping the object. This technique is traditionally used for deep organs, such as the intestines or liver, or any other organ located between the anterior and posterior sides of the abdomen. Enveloping or sandwich imposition allows for a more intense and extensive overall action on the anatomical areas.

This technique, through the alleged transverse field it produces, and the associated enhanced effects, seemed appropriate here to compensate for the lack of blood vessels in the skin explants, while the living organisms used in other experiments (Grad et al., 1961; Souza et al., 2017) were all vascularized. In the latter case, the placebo effect could have been invoked to explain the results, contrary to our experiment with unconscious skin explants. Furthermore, this technique, in combination with palmar imposition, replicates the real-life practice of wound care sessions with patients in a hand healer's office.

Finger projection and heteronomic position accounted for approximately 20% of the total NCH healing time in this experiment, with palmar position accounting for the remaining 80% of the time.

Methods of analysis and data exploitation

Sample preparation and preservation for analysis

At the end of the five days of NCH treatment:

- Submergents were collected and frozen at -20°C for subsequent measurement of inflammation mediator cytokines (IL6, IL8, TNF- α , IFN- α , IFN- β).
- Skin explants were also collected and cut into two parts. Half of these parts were frozen at -80°C, and the other half was embedded in paraffin to perform immunohistochemical staining of wound healing markers (Ki67 and K17) on tissue sections.

Methods of analysis used

Measurement of Ki67 and K17 proliferation markers by immunohistochemistry. The skin explants were cut in half at the center of the scar and perpendicular to it. The samples reserved for immunohistochemical analysis were fixed in a 4% paraformaldehyde solution for 24 hours. The explants were then embedded in a paraffin block following standard procedures. Thin sections of 4 μm thickness were cut using a microtome and mounted on glass slides.

After deparaffinization of the sections, antigen retrieval was performed using a pH 9 citrate buffer solution (Dako, Les Ulis, France). The sections were then incubated overnight at 4°C with either the anti-Ki67 antibody (Abcam, Paris, France) or the anti-K17 antibody (Clinisciences, Nanterre, France). For immunofluorescence staining, after incubation with the secondary antibody conjugated to Alexa-Fluor-488 (Invitrogen, Courtaboeuf, France), the nuclei were counterstained with DAPI.

Measurement of inflammation markers (cytokines) by ELISA immunological assay. The levels of cytokines IL6, IL8, TNF- α , IFN- α , IFN- β were measured using an Enzyme-Linked ImmunoSorbent Assay (ELISA). Maxisorp Immuno 96-well ELISA plates (Nunc) were coated with either anti-IL-6, anti-IL-8, anti-IFN- α , anti-IFN- β , or anti-TNF- α antibodies in PBS overnight at 4°C.

After washing the plates with a low-salt wash buffer (10 mM Tris, pH 7.2, 25 mM sodium chloride, 0.05% Tween 20), the plates were blocked with blocking buffer (PBS with 1% BSA) at room temperature for two hours, followed by additional washes with wash buffer. The culture media from the skin explants were then tested undiluted or diluted 1/100 (IL-6) in low-salt wash buffer containing 1% BSA, and the plate was incubated at room temperature for two hours. After extensive washes, either anti-IL-6, anti-IL-8, anti-IFN- α , anti-IFN- β , or anti-TNF- α antibody (diluted in wash buffer containing 1% BSA) was added and incubated at room temperature for one hour.

Then, the secondary antibody conjugated to horseradish peroxidase (1/200) (ECL) was added and incubated at room temperature for twenty minutes. After extensive washes, the plates were incubated with TMB substrate at room temperature for approximately twenty minutes, after which a stop solution (2N H_2SO_4) was added, and the absorbance was measured at 450 nm using an ELISA plate reader.

Data analysis

The null hypothesis (H_0) in the statistical analysis posits that: “the control and treated groups have the same mean value for the concentration of a specific biomarker”. In contrast, the alternative hypothesis (H_1) asserts that: “the biomarker concentration mean values differ between the two groups, indicating that the treatment has an effect on biomarker concentration compared to the control condition”.

RESULTS

Macroscopic Morphological Aspects

In Figure 1, skin explants from the control group (not treated by NCH), maintained in culture for five days, can be observed. In the center of the skin explant, the induced scar can be seen. No morphological evolution was observed compared to the initial morphology of the scarred skin explants.

In Figure 2, on the fifth day of NCH, erythema appears to be present at the scar site. This type of erythema is typically associated with the presence of a local inflammatory reaction. This erythema is not observed in the scarred skin explants from the control group.

Measurement of proliferation markers Ki67 and K17 by immunohistochemistry

For each skin sample and each analyzed marker, a fluorescence image of the epidermis is captured using NIS Element software (Nikon®). Visual counting of positive cells was then conducted by Aquiderm for each sample and each marker (Rambert, 2019).

During the early stages of wound repair, the Ki67 marker is typically low (Prabhu et al., 2022). Accordingly, the fluorescence images clearly show a lower presence of Ki67 in the case of treated explants compared to untreated ones (Figure 3). This suggests that NCH treatment has contributed to the initiation of the wound repair process.

Unlike Ki67, Aquiderm did not measure a significant difference in the number of K17 markers between treated and untreated skin explants (Rambert, 2019). The same level of K17 expression was found in both cases (Figure 4).

Measurement of inflammation markers (cytokines) using the ELISA immunological test

The concentrations for the following cytokines, IL8, TNF- α , IFN- α , and IFN- β , were below the detection limit. Only IL6 showed a sufficiently high concentration to be



Figure 2. Photograph taken on the fifth day of the experiment. NCH-treated skin explants.

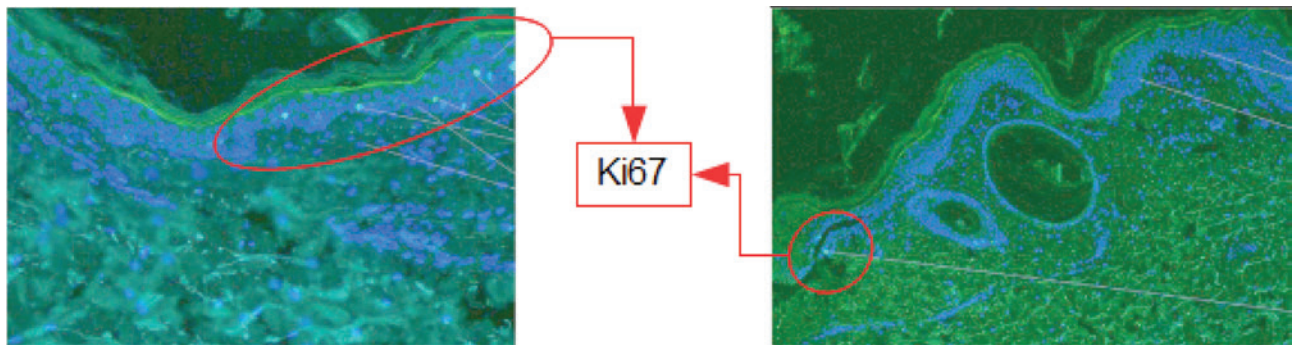


Figure 3. Fluorescence image for the detection of Ki67 on a control sample (left) vs treated (right) scarred skin explants. The bright green dots indicate Ki67 markers.

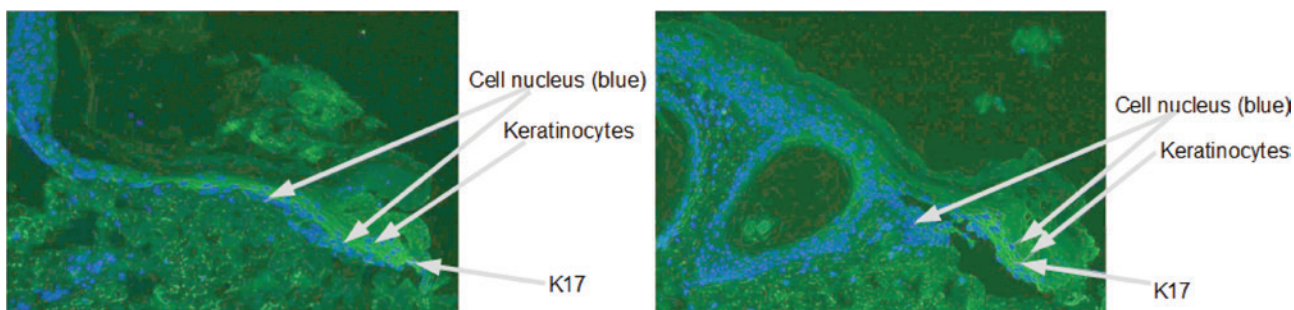


Figure 4. Fluorescence image for the detection of K17 in control sample (left) vs treated sample (right) of scarred skin explants.

measured in all scar tissues, treated or not, as shown in Figure 5.

The high levels of IL-6 measured in the supernatants of NCH-treated skin explants indicate a stronger local inflammation compared to untreated ones, which is consistent with the initial phase of the wound repair process described earlier. The concentration of IL-6 suggests a correlation between NCH treatment and the manifestation of

wound repair in treated samples, while this manifestation is absent in untreated samples.

Since the sample sizes differ (six samples in the treated group and seven in the control group), and assuming unequal variances in IL-6 concentrations inside each group, a Welch’s t-test—a variant of the Student’s t-test that accounts for differences in group sizes and variances—is applied. By performing Welch’s test on the IL-6

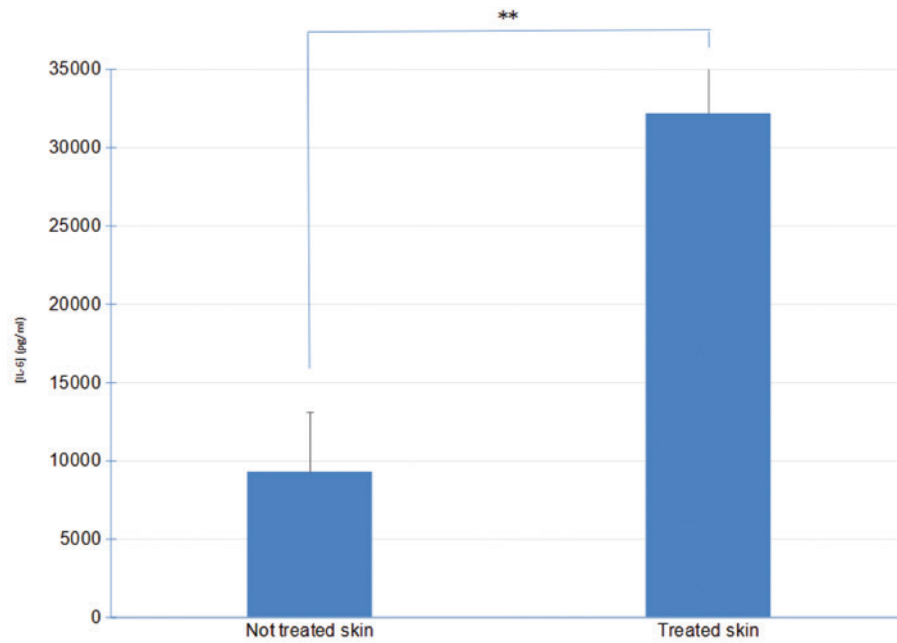


Figure 5. IL6 concentrations in cut skin explants. A p-value of $p < 0.05$ is considered statistically significant; in this case, $**p < 0.01$.

concentration values from the treated and control groups, we obtain a p-value of 0.00407. This value is well below conventional significance thresholds, such as $\alpha = 0.05$ or even $\alpha = 0.01$, indicating that the probability of observing such a result under the null hypothesis (H_0) is very low (~0.4%). We therefore reject H_0 at both the 5% and 1% significance levels. This result provides strong statistical evidence of a difference in mean IL-6 concentrations between the treated and control groups, suggesting that the NCH treatment has a measurable effect.

DISCUSSION

The results of the NCH on skin explant experiment described above reveal two indicators that support the manifestation of a wound repair process in the treated group compared to the control group. The reduced presence of the Ki67 marker and the higher concentration of the IL6 cytokine are both indicative of the initial phase of the wound repair process. Therefore, this suggests that, most likely, the NCH-treated skin explants have undergone a more advanced wound repair process compared to the untreated skin explants.

During the course of the experiment, one of the authors held the 6-well culture plate in his hands to apply the heteronomic treatment. This holding is thought to have no consequences as there is no obvious reason why only Ki67 and IL6 would have been affected by it, contrary to

other markers. It is worth noting that the 6-well culture plate for the two groups were also manually moved in and out of their CO_2 incubator.

While the results of this experiment are promising, they also highlight the challenge of selecting the most relevant markers to assess the wound repair process. In this case, the concentrations of IL8, TNF- α , IFN- α , and IFN- β were not measurable. If only these markers had been chosen, the experiment would not have yielded conclusive results. It is worth noting that to avoid this limitation, Aquiderm initially measured a broader set of inflammation markers, including 13 different cytokines, using multiplex assays (Rambert, 2023). These results were then confirmed through ELISA tests on selected cytokines.

Furthermore, this experiment shows that assessing the existence of NCH effects on biological organisms is facing many practical difficulties:

- our knowledge of external physical influences on biological samples is currently rather poor. Systematic trials should be done to characterize the sources of biological effects (electric or magnetic field, electromagnetic radiation, sound and ultrasound, etc.) and the magnitude of these effects in relation to the properties (frequency, power, etc.) of these sources. Hence, it is difficult to design an experiment that will isolate the samples from such unknown influences.

- This difficulty is further increased by the fact that these experiments are located at the frontier between biology and physics and require a good knowledge of both fields and their interactions.

Taking a skeptical position, we could require that one precisely measure physical influences around the skin samples, *e.g.*, static and variable electric, magnetic, and electromagnetic fields, including thermal and microwave radiations, potentially emanating from ovens, cell phones, refrigerators, extractor hood vibrations, etc. Adopting the same viewpoint, we could also require that the samples be isolated from these influences.

Although this seems to be a sensible proposal, it is not relevant here for the following reasons:

- Both the treated skin samples and control group were located in the same room, so both types of samples underwent the same influences from external fields;
- hand healers operate in their daily practice without isolating their patients from these influences;
- Furthermore, it may be possible that hand healers act as catalysts of surrounding fields that they are able to manipulate in order to cure. Within such a hypothesis, it may be counterproductive to isolate the samples from any such external physical influences.

The above-mentioned limitations underline the need for a close cooperation between physicists and biologists to design such experiments, ensuring any influence other than the one being assessed is effectively discarded, be it, *e.g.*, during the handling of the samples, their storing, or the experimental trials. For this type of experiment, the biological laboratory should ideally seek assistance from one or more physics advisors.

IMPLICATIONS AND APPLICATIONS

Although it seems too early at this stage to make assertions about the exact mechanism at play in the wound repair process through NCH, we can report some non-exhaustive hypotheses found in the scientific literature. Some studies (Baerga-Ortiz et al., 2000; Finkelstein, 2015; Bonnefoy, 1882; Burr, 1972; McCraty et al., 1998; Zhao et al., 1999; Oliveira et al., 2019; Becker, 2000) suggest a connection between electricity and biological mechanisms in the human body, notably wound repair. McCraty et al. (McCraty et al., 1998) explain how a small electric stimulus

may have large biological effects through stochastic resonance (Benzi et al., 1981; McCraty et al., 1998). One of the authors initiated this study after measuring a low-intensity electric field from his hands that could be involved in the wound healing process. Such an electromagnetic cause is also supported by Rubik et al. (Rubik et al., 2015) and McCraty et al. (McCraty et al., 1998). Other studies (Hisamitsu et al., 1996; Moga & Bengston, 2010; Muehsam & Pilla, 2009a; Zimmerman, 1985) explore the influence of magnetic fields. Another potential explanatory mechanism could be biophoton emission by the human body (Popp & Chang, 1998; Hossu & Rupert, 2006; Rubik et al., 2015; Ives et al., 2014). Future studies of the underlying mechanisms may include: biochemical factors, notably other specific proteins, or assessment of changes in cell or tissue morphology. The variety of potential explanations (Markov, 2015) highlights the need for further experiments and theoretical advancements to establish the appropriate mechanism underlying the observed phenomena.

CONCLUSION

The results of the NCH experiment on human skin explants described here show two indicators of the initial phase of wound repair, linked to Ki67 and IL6 responses. This supports the hypothesis that this phase of wound repair occurred in the treated skin explants group, contrary to the control group.

This experiment points to a seemingly successful NCH application on human scarred skin and the possibility to perform such studies without being influenced by the placebo effect. Our results are detailed here to encourage further inquiry to better understand the parameters involved in the NCH mechanism.

In order to confirm, extend, and study the repeatability of our own findings, additional unbiased studies similar to ours need to be conducted and evaluated by peers. Some directions for extending this study could be, *e.g.*, working on more skin explants; using skin explants coming from different human races and/or donors; using vascularized skin explant (Chen et al., 2021); analyzing other wound repair markers (notably fibroblasts); isolating the sample and/or the hands from physical influences; analyzing other skin layers (notably the dermis, where fibroblasts are involved during the wound repair process). As NCH was historically associated with magnetism, it could also be interesting to isolate the sample from the influence of magnetic fields in a future study, in order to assess if traditional statements are scientifically grounded. Comparative studies may also

be undertaken to analyze the differences between NCH-based wound healing and other wound healing processes, such as, *e.g.*, drug treatments or physical therapies. Other further studies may explore individual variability of NCH wound healing in relation to: age, gender, or health parameters such as stress or fatigue. Another extension of this study may focus on double-blinded clinical trials to assess the NCH efficiency under various conditions. The corollary of these trials may be to investigate potential adverse effects of this practice or identifying adverse conditions giving no results. Psychological outcomes may also be considered, such as the impact of NCH on well-being and treatment quality perceptions.

It would be advisable to develop a standard protocol for NCH application and to conduct the aforementioned studies under these standardized conditions to ensure comparability.

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AUTHOR CONTRIBUTIONS

Arnault Richard de Chicourt: Conceptualization, Methodology, Investigation, Writing - Reviewing & Editing.

John Sadi: Methodology, Investigation, Writing - Reviewing & Editing.

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DECLARATIONS OF INTEREST

None.

ENDNOTES

¹ Recent discoveries also point to the existence of giant magnetic filaments connecting the

galaxies, stars, and planets throughout the whole universe (West, 2021).

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