MONOGRAPHY

SKIN and SILICIUM (Silicon).
literature review

Courcelles – March 20 , 2020

1. INTRODUCTION

The human skin protects the organism from the external environment, most importantly from mechanical injuries, what is enabled by the mechanism of reversible deformation of the skin structure. All tissues, including skin, undergo deformation under the influence of external forces, particularly the weight. Human skin can be stretched to several times its original size and still maintains its original phenotypic properties. Such impressive expansion is possible because the skin is a highly specialized mechanical structure, responding through a network of interconnected cascades of chemical reactions, with the participation of extracellular, cytoplasmic and nuclear membranes.

The skin also has impressive functional plasticity which allows for its progressive adaptation to the environment. Changes in the skin tissue occurring during dermatological and surgical treatments initiate mechanotransducive paths that also increase the mitotic activity and the synthesis of collagen. However, if external stimuli such as mechanical stress reach sufficiently large values, they may cause irreversible deformation and damage to the skin, resulting in a loss of its mechanical properties.

Human skin is a complex living material, composed of several heterogeneous layers: epidermis, dermis and subcutaneous tissue, which is sticky and soft. Skin thickness varies depending on its anatomic location, fluid content and age. From the mechanical point of view, the skin is a very complex structure. Despite the fact that it consists of three layers with different mechanical properties, in medical tests the skin reacts as a homogeneous material and is treated as such in all indentation measurements. Analyses of skin morphology using quantitative methods of mechanical engineering are carried out in studies on skin ageing. This allows to determine the level of mechanical skin changes progressing with age. Physiological functions of the skin can be determined by mechanical parameters, which are subject to change, depending on the density and elastic components of the dermis.

Silicon based food supplements such as ortho-silicic acid and cosmetics containing bioavailable silicon source are known for their beneficial for skin repair and improving biomechanical properties of those.

In vitro, ex vivo and in vivo experiments suggest that silicium is important, on the skin, for optimal synthesis of collagen and for activating hydroxylation enzymes, improving skin strength, hydration, collagen content and elasticity of the skin. Although the exact mechanism is not completely understood, local application and Ingestion of bioavailable silicium have an impact on the activity and differentiation of fibroblast, stimulates collagen type I and elastin synthesis and participate to the reticulation of Glycosaminoglycan and collagen.

In this monography the effects of silicium on skin metabolism were examined.

REVIEW OF LITERATURE ON SKIN PARAMETRES

extract of Hamed Joodaki and Matthew B Panzer (J Engineering in Medicine 2018)

1. Importance

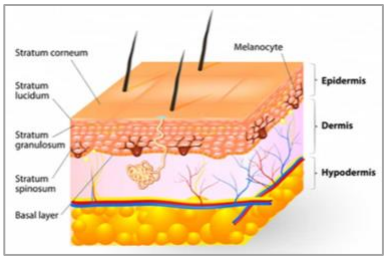
The skin, which is the largest organ in surface area, has several functions including the protection from environment, temperature regulation, and adaptation of contours of the body during movement. This tissue consists of several layers, each layer made of several components, which makes it mechanically complicated. The mechanical properties of the skin are of importance for various cosmetic, clinical, and biomechanical applications. Cosmetic applications include the efficiency assessment of cosmetic products in terms of emollience and hydration. Clinicians use the mechanical properties of skin to better understand the laceration process, techniques in plastic surgery and skin closure, treatments for skin diseases, and the formulation of scar tissue.

Finally, the biomechanical applications of skin material properties include the design and production of skin simulant materials for physical anthropomorphic devices such as crash test dummies and surgical simulators. In addition to use with physical biomechanical models, skin material properties are used in the development and improvement of computational human body models for biomechanical simulation.

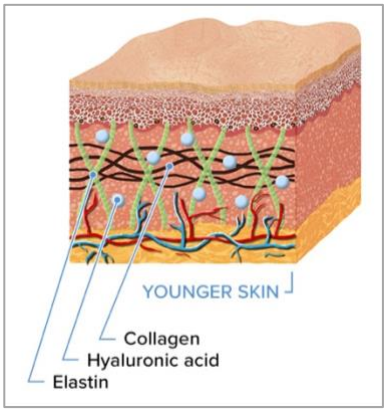
2. Structure of skin

To understand the mechanical behavior of skin, its structure needs to be considered. The skin is an organized structure consisting of three major layers, called the epidermis, dermis, and hypodermis or subcutis.

The topmost layer of the skin is called epidermis which is approximately 75–150mm in thickness and is made of four sub-layers, namely, stratum spinosum, stratum basale, stratum granulosum, and stratum corneum. The second layer is the dermis which is a dense fibrous tissue. The dermis and the epidermis together are also referred to as cutis, which has a thickness of 1.5–2.5mm. The dermis is a moderately dense connective tissue which comprises three fibrous proteins, collagen, elastin, and minute quantities of reticulin and a supporting matrix or ground substance.

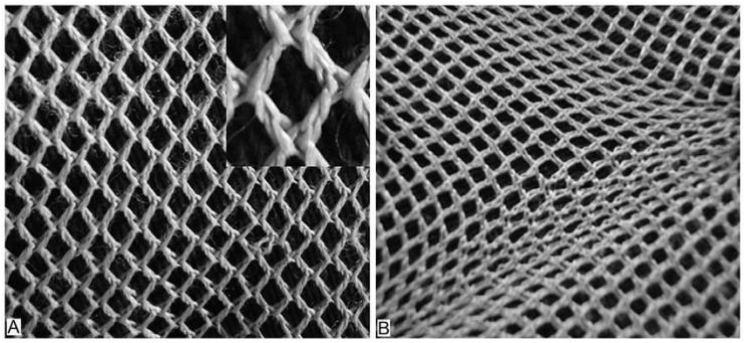


The third layer is the hypodermis (also called subcutaneous fat or subcutis), mainly made of adipose cells. The thickness of this layer varies notably over the surface of the body. It functions as an insulating and cushion layer and constitutes about 10% of the body mass.



The **collagen** fibers contribute to 75% of the fat-free dry mass and 18%–30% of the volume of dermis. The **collagen** fibers are within the ground substance and unconnected along most of their length. The elastin fibers comprise 4% of the fat-free dry mass and 1% of the volume of the dermis. **Elastin** dictates the mechanical behavior of skin at small stresses and strains. Especially, the elastin fibers are in charge of the recoiling mechanism after deformation. At tensile strains around 30%, the undulated collagen fibers are straightened.

3. Mechanical behavior of skin Nonlinear stress–strain relationship



A model of collagen organization in skin. An interpretation of how a collagen fiber network is interwoven (A), based on work from Gibson et al.]. The insert in A is a higher magnification of the intertwined fibers, also showing a 3D distribution of the fibers. When this network is stretched in any direction, the fibers become oriented parallel to the stretching direction (B)

The mechanical response of skin tissue is highly nonlinear due to the makeup of its microstructural constituents Under uniaxial tension, skin is relatively soft, and much of the structural response of the skin at low strain levels is carried through the elastin components as the **collagen** fibers are slack (sometimes referred to as wavy) and nonload- bearing. When the skin is stretched to higher strain levels the stiffness of the material increases rapidly as the collagen fibers are recruited, straightened, and begin to carry the major part of the load. In the third phase, all collagen fibers are straight, and the system has its highest stiffness. By comparing the results of testing on collagen and skin in high strains, it is shown that the **collagen is the main support structure** in the linear region of the stress–strain curve.

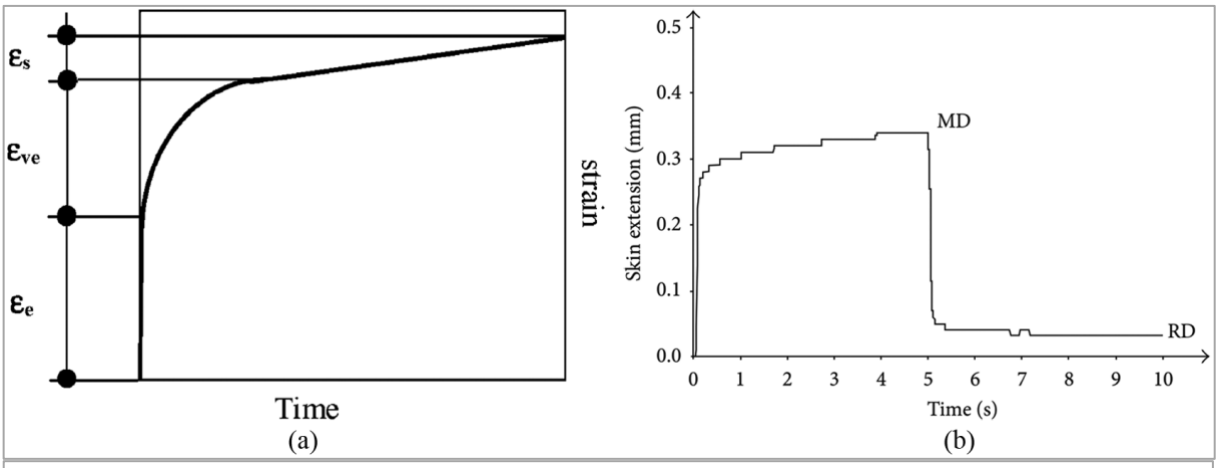
Several researchers tracked this behavior to higher strains and observed a decrease in the stiffness of tissue after the stage in which all fibers are aligned which is a result of gradual breaking of the collagen fibers. Some have used a simple model of the collagen fiber network in the dermis to calculate the strain level at which the stiffness of skin abruptly increases. The collagen network was modeled as consisting of small straight fibers of unit length attached to each other. They predicted that in high strain all fibers will become straightened. Nevertheless, it should be noted that this conclusion is based on the assumption that the directions of fibers are completely random which is inconsistent with many regions on the body due to Langer's lines.

4. Viscoelasticity

Several studies have shown the strain rate dependency of skin mechanical properties. Some researchers showed in a creep test that if a constant stress is imposed on the skin, the curve obtained can be decomposed into three phases (Figure (a)) : the first phase corresponds to a purely elastic deformation $\sum_e$, the second phase of variable creep corresponds to the viscoelastic phase $\sum_{ve}$, and the third phase corresponds to the constant creep phase $\sum_s$.

Other peoples conducted in vivo suction tests on human forearm skin by applying a 500-mbar suction for 5 s followed by a stress-off time of 5 s and measured the vertical skin deformation.

The results characterized the maximum deformation (MD) and the residual deformation (RD) under this testing condition (Figure (b)). Skin also exhibits the relaxation behavior characteristic of viscoelastic materials. When stretched and held to a constant strain level, the stress within the skin material will decay over time.



Skin creep curve under constant stress (a) schematic representation of three phases of skin creep test and (b) deformation versus time curve obtained under the creep mode procedure.

5. Anisotropy

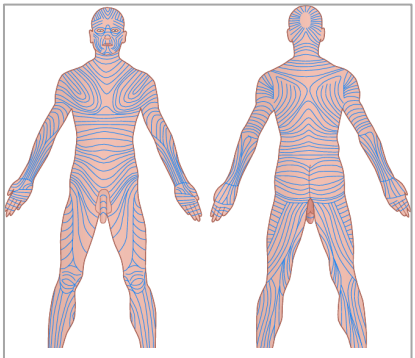


Figure 1 : Langer's lines

In vivo skin is normally in a state of tension. When a skin sample is excised from the body, it retracts, and the amount of retraction depends on the incision direction and site. This phenomenon was originally demonstrated by A.K. Langer. Langer identified lines of maximum skin tension over the entire human body, which have since been called Langer's lines (Figure 1). The higher tensile forces along the Langer's lines are due to the collagen fibers in the dermis being predominately oriented parallel to these lines. Those collagen fibers that are parallel to Langer's lines are stretched more than those across the lines in vivo. Hence, when the skin is stretched in the direction of Langer's lines, it will extend only a small amount before all fibers which are parallel to the Langer's lines orient themselves.

Nevertheless, when the skin is stretched across the Langer's lines, it will extend much further before those fibers, which are oriented across the Langer's lines, become straight and aligned in the direction of stretch.

This figure illustrates that the skin is stiffer when testing parallel to Langer's lines compared to the transverse direction. Moreover, by comparing the slopes of the two curves, it can be concluded that the tissue gets to the maximum stiffness at lower stretch ratios when testing along the Langer's lines compared to the transverse direction. Hence, the fibers become straight at lower stretch levels when stretching the tissue in parallel direction. It was concluded that skin is an anisotropic material on through-thickness plane as well. Preconditioning effect Connective tissues such as skin, ligament, and tendon which consist of elastin and collagen fibers show not only rate dependency but also load history–dependent behavior. Load history dependency means that the shape of the load–elongation curve will vary depending on the previous history of loading (irrespective of the viscoelastic effects).

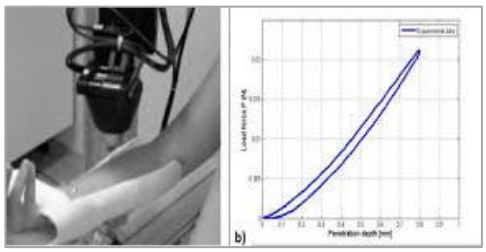
6. Failure properties

Several studies, mainly by in vitro uniaxial tension tests, have attempted to measure the failure properties of skin. Because of the viscoelasticity and anisotropy, the skin is expected to have different failure properties in different strain rates and loading directions. Strain rate is among the predominant factors which affect the soft tissue failure properties. Researchers stated that the total skin strength is the summation of the collagen fibril strength and the collagen–matrix interaction. At low rates of loading, the strength of the tissue is primarily due to the cohesive forces (i.e. crosslinks) between collagen fibrils themselves since the bonds between the collagen fibrils and the glycosaminoglycan matrix are broken early as the collagen fibrils deform along the axis of loading. However, the increase in tissue strength at high rates of loading was attributed to the viscous shearing effect between the matrix and collagen fibers. The dependency of failure properties on strain rate was observed in other studies consistently.

7. Skin testing In vivo

In vivo experiments provide data on tissue in its natural state, i.e., permeated with blood, and in a typical stress/ strain state. In some of the in vivo experiments, the measured behavior is attributed to the dermis. However, as a result of connections between the various skin layers, it is difficult to isolate the contribution of the dermis from that of the epidermis and subcutaneous tissues, such as muscle and fat. Four main methods used for in vivo testing of the skin are indentation, torsion, tension, and suction. In what follows, each method is described briefly.

Indentation : In indentation experiments, a rigid indenter (with a cylindrical, conic, or spherical indentation tip) is used to apply a known deformation force to the skin. Sub-micron atomic force microscopy indentation can be used to determine the mechanical properties of different layers



Torsion: In torsion tests, usually a constant rotation or torque is applied to the skin via an intermediary disk. The rotation of the skin resulted in an opposed moment and the coil moved until an equilibrium position was reached. At given times, the torsion angle was recorded by means of a light beam which was reflected by a mirror attached to the aforementioned bar.

Tension: In tensile testing, the skin is mainly loaded parallel to its surface using two tabs which are attached to the skin. The attachment of the tabs to the skin may significantly influence the results as many of the double-sided adhesive tapes exhibit creep deformation. Rapidly bonding cyanoacrylate adhesives are useful to avoid these effects.

Suction: In suction testing, the skin is elevated by applying a partial vacuum using a circular aperture and the deformation is quantified by optical or ultrasound devices. Two suction devices that are commercially available and used by the researchers are Dermaflex and **Cutometer**. Numerous studies are done on skin via suction method.

MECHANISMS OF SKIN REMODELING LINKED WITH SILICIUM (Silicon)

1. Silicon And Metabolism (Extract from Rodella L. & Al. - J Nutr Health Aging 2013)

Silicon (Si) is the second most common element in Earth's crust (28.9%), after Oxygen (45.5%). It represents the major trace element in the human body. In particular, highest Silicium concentrations are found in fast-growing cells such as hair, nails, bone and skin cells. The major and most important source of Silicium is the diet. Silicium daily intakes range from about 20mg/day to 50mg/day in Western countries. Higher intakes (104mg/day – 204mg/day), have been reported in China and India, where plant-based foods are the major components of the diet. There is an inverse relationship between silicium content and silicium availability was found in all foods. **About Silicium absorption, the main bio-available form, for human and animals, is the Ortho-Silicic Acid [O.S.A., $Si(OH)_4$].**

It is well-known that Silicium in the form of OSA existing only in liquid, like mineral water and beer but not in foods. Nevertheless, Silicium is hydrolyzed to OSA at the gastrointestinal level. Among foods, highest Silicium levels are found in grains, especially oats, barley, white wheat flour and some rice fractions. Silicium is also present in the form of synthetic compounds or silicates, but they are rarely found in the diet.

Orthosilicic acid $Si(OH)_4$ is the main Silicium species in man. After uptake, it is gastrointestinally absorbed and transported in the blood, mainly unbound. Only small amounts form complexes with Fe or Al at a neutral pH. In the human body, Silicium amounts to 1–2 g, which corresponds to 0.01% of body weight, i.e., lower than Fe and Zn. The concentration in serum ranges between 24 and 31 µg/dL.

Outside the blood compartment, it is mainly bound to glycosaminoglycans. It has been speculated that serum and tissue levels of Silicium might be regulated by responsive elements or transporters, since such transporters (SITs) have been identified in plants and silicified organisms, (e.g., in diatoms or sponges). Recently, a SIT named SLc34a2 was found in mammals.

Water channel aquaporins are homologous to SITs in rice and have been detected in the small intestine and renal epithelia, and also in the bones and joints of mice. Their expression seemed to be diet dependent: Under a Si-rich diet, certain aquaporins were upregulated in kidney and calvarial bones. However, how silicic acids reach their final site of deposition in the body still has to be investigated.

Silicium is excreted mainly renally after glomerular filtration and can be detected in the urine. It is possible that Silicium levels in urine could represent a parameter for Silicium bone metabolism, since reduced excretion could be associated with osteopenia. After uptake, most absorbed Silicium is excreted after 4–8 h in urine. In healthy human volunteers, the ingestion of soluble Silicium results in the excretion of the same quantity of Silicium within 24 h.

Silicium can be found in high levels in the extracellular matrix bound to different components, especially glycosaminoglycans.

The role of Silicium in connective tissue development and differentiation has been discussed, since Silicium can form complexes with polyols like hexosamines, which are components of glycosaminoglycans and mucopolysaccharides that form extracellular matrix components.

Additionally, Silicium plays a role as a cross-linking element in the bridging between proteoglycans and collagens. Silicium supplementation in the diet shows stimulatory effects on cartilage synthesis.

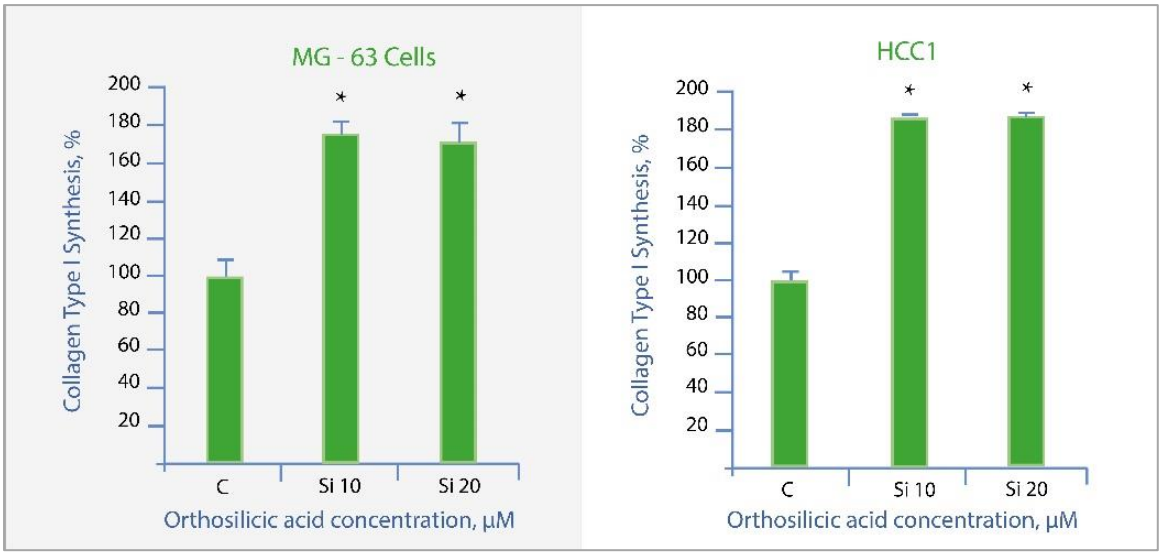
In the connective tissues of rats, Silicium concentration decreases with increasing age. Probably, Silicium is necessary in young animals for connective tissue and bone development.

This led to the suggestion that Silicium may play an important role in connective tissue metabolism especially in skin but also in bones and cartilages.

2. Silicium stimulates collagen synthesis in vitro

This hypothesis was tested in 2003 by Reffitt and collaborators(1). They examined the effects of soluble Silicium (OrthoSilicic Acid ; OSA) on collagen type-1 synthesis in 4 different cell types relating to bone, cartilage or skin : i) human osteosarcoma cell line MG-63, ii) primary osteoblast-like cells derived from human bone marrow stromal cells, iii) human bone marrow cell line (HCC1) which has been shown to differentiate along the adipogenic and osteogenic lineages with manipulation of culture conditions and iiii) human skin fibroblasts as type 1 collagen is also a major constituent of skin.

They demonstrated that collagen type-1 synthesis increased in all cell lines tested following treatment with orthosilicic acid. Comparable levels of collagen type-1 mRNA were detected in untreated MG-63 cells and cells treated with orthosilicic acid while the stimulatory effect of soluble Silicium on collagen type-1 synthesis was abolished in presence of prolyl hydroxylase inhibitors.



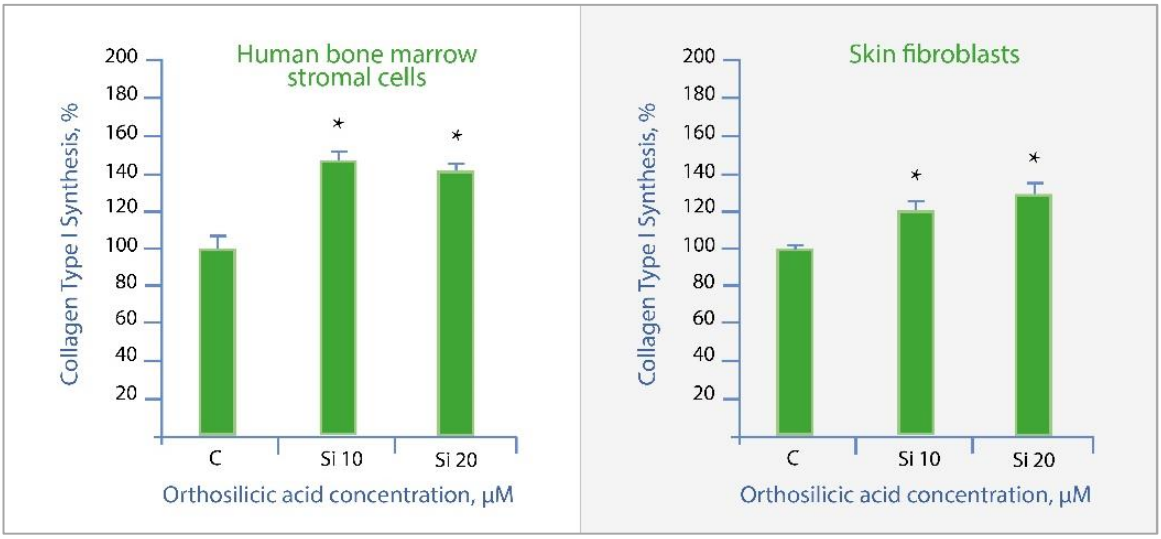


Figure 2 : Collagen I C-terminal propeptide (CICP) (ng/µg protein) in serum-free medium following addition of orthosilicic acid (10, 20 µM) expressed as percentage of control in MG-63 cells; HCC1 cells; Human bone marrow stromal cells ; Skin fibroblasts . *$P < 0.05$ compared to control

In this study, it seems that orthosilicic acid do not alter collagen type-1 gene expression but may modulate prolyl hydroxylase activity. The requirement of silicium for prolyl hydroxylase activity was already postulated by Carlisle many years ago(2) and this would be also compatible with the findings of Calomme and Vanden Berghe(3), who observed an increase in hydroxyproline concentration in the dermis of calves following supplementation with orthosilicic acid.

While Reffitt(1) only suggested a post-translational mechanism involving prolyl-hydrolylase for the increased production of collagen, another team suggested a mechanism involving increased transcription or mRNA stability for collagen type-1 (5,6).

These results have been confirmed by SIL'INNOV using EYTELIA Silicium Bio-activated. Human dermal fibroblasts (obtained from skin explants) were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin (100 IU/ml), ascorbic acid (50 µg/ml) and essential amino acids in 6-well plates. The amount of collagen in the medium and in the cell layer was assayed by immunoblotting.

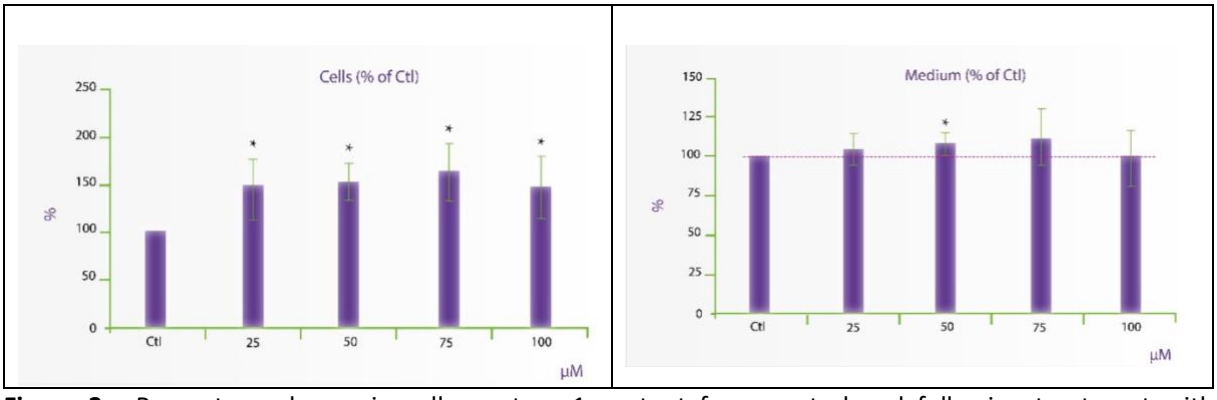


Figure 3 : Percentage change in collagen type-1 content from control and following treatment with orthosilicic acid at 25, 50, 75, 100 µM for 65 h. Contents were determined by Western blotting. The bands were scanned and quantified with Quantity One (BioRad). The results are presented as mean ± SEM of three separate experiments and 2 separate Western Blot for one sample. *P = 0.01 compared to control,

Collagen type-1 synthesis increased in human fibroblasts following treatment with EYTELIA Silicium Bio-activated at 25 to 100 µM. An increase of 10% of the concentration secreted in the medium was observed at 50 µM while an approximative 50% increase was observed in the cell layer at all concentrations tested (SIL'INNOV personal data, 2012, in collaboration with "Laboratoire de Biologie des Tissus Conjonctifs, Université de Liège, Belgique".

3. Silicium stimulates collagen and elastin synthesis ex vivo in a human skin model

In vitro experiments suggest that silicium is important, on the skin, for optimal synthesis of collagen and for activating hydroxylation enzymes, improving skin strength and elasticity. Based on this hypothesis, SIL'INNOV has investigated different skin parameters using living human skin explants following treatment with EYTELIA Silicium Bio-activated.

Living human skin explants were treated with UVA and UVB to mimic aging of the skin with decrease of fibroblasts metabolism and alterations of skin macromolecules. Aged skin explants were treated with EYTELIA Silicium Bioactivated and the effect of silicium was evaluated by skin histology and quantitative analysis of collagen fibers, elastin and glycoaminoglycans (GAGs) (SIL'INNOV personal data, 2006, in collaboration with GREDECO, Paris).

In an experimental model of photoaging that allows to obtain a skin deficiency in collagen and elastin, SIL'INNOV observed a protection and a repair of collagen and elastin

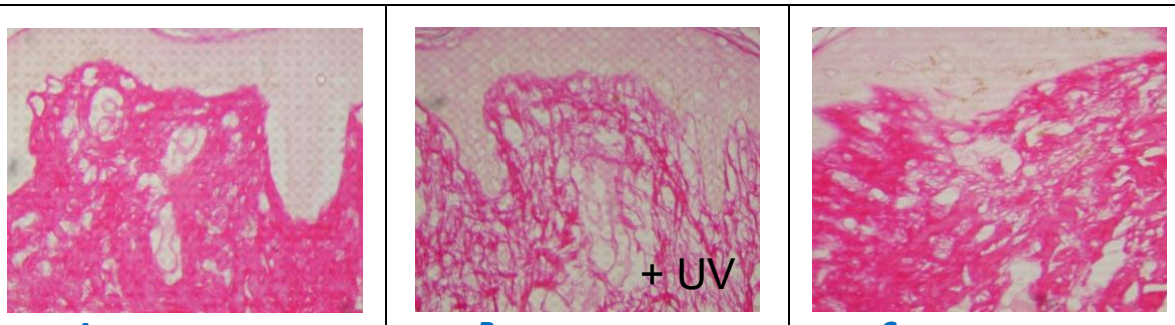


Figure 4 : The quantity of collagen is detected after ' A- Control normal skin explant without any treatment (UV or Silicium Bio-activated). B- Skin explant after UV treatment. C- Skin explant after UV irradiation and treatment with Silicium Bio-activated for 14 days.

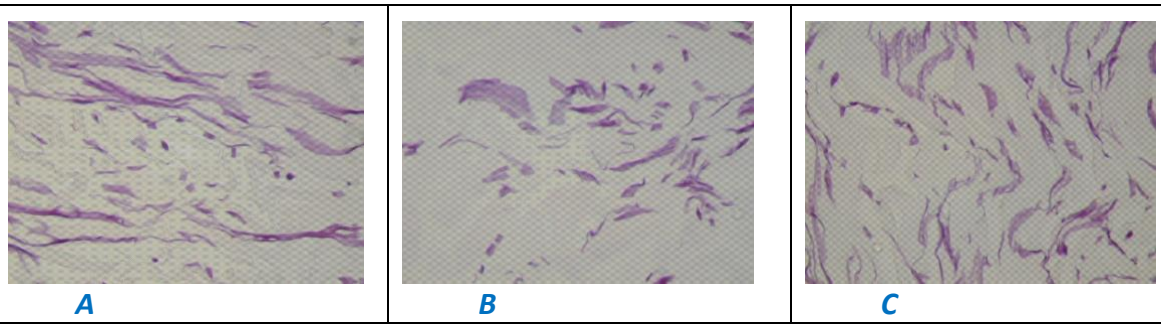


Figure 5 : The quantity of collagen is detected after **special coloration with (+) catechin**. A- Control normal skin explant without any treatment (UV or Silicium Bio-activated). B- Skin explant after UV treatment. C- Skin explant after UV irradiation and treatment with Silicium Bio-activated during 14 days.

fibers after treatment with EYTELIA Silicium Bio-activated (SIL'INNOV personal data, 2006, in collaboration with GREDECO, Paris)

4. Silicium increases parameters of skin hydration ex vivo in a human skin model

Silicium is important for optimal synthesis of collagen and for activating the hydroxylation of enzymes important in the formation of collagen network, improving skin strength and elasticity. But silicium is also associated with the synthesis of glycosaminoglycans (GAGs) improving skin hydration. Schwarz K in 1973[(6)] was the first to point out the involvement of silicium in the synthesis of glycosaminoglycans and to suggest that silicium could have a structural role as a cross-linking agent in connective tissues.

GAGs	µg/mg protein
Control skin	2.13 ± 1.2
Skin + Silicium Bio-activated	13.3 ± 7.74 (* p=0.006)

We have observed a significative increase of GAGs synthesis after treatment with EYTELIA Silicium Bio-activated (SIL'INNOV personal data, 2007, in collaboration with GREDECO, Paris).

Using the same skin explant model as previously, SIL'INNOV tried to evaluate the potential effect of EYTELIA Silicium Bio-activated on GAGs. The investigation was performed on the skin explant model but without experimental photoaging. After 14 days of treatment with Silicium Bio-activated, the total content in GAGs was quantified using a Blyscan Glycosaminoglycan Assay (Interchim). Results are expressed according to the total protein.

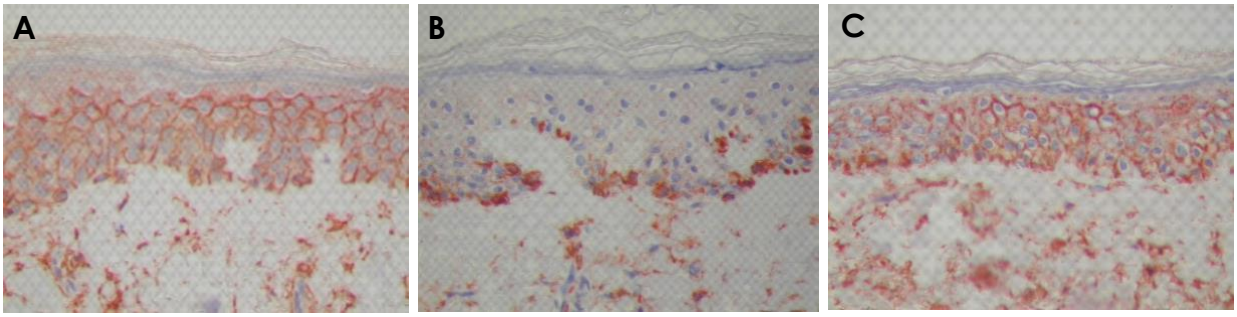


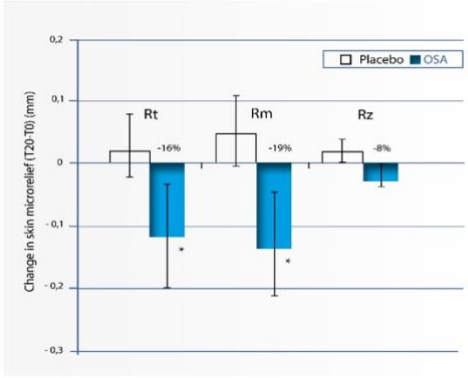
Figure 6 : CD44 is detected by immunochemistry. A- Control normal skin explant without any treatment (UV or Silicium Bio-activated). B- Skin explant after UV treatment. C- Skin explant after UV irradiation and treatment with Silicium Bio-activated during 14 days.

UV treatment induces a significant decrease of CD44 (topographic score of 0.91 in UV treated-explant vs 2.65 in control). Treatment with EYTELIA Silicium Bio-activated restore CD44 expression with a topographic score of 2.0 (p=0.046) after 14 days of treatment.

EYTELIA Silicium Bio-activated can improve skin hydration through the regulation of GAGs and CD44 synthesis (SIL'INNOV personal data, 2007, in collaboration with GREDECO, Paris).

5. Skin improvement by silicium – Human in vivo studies

In 1993, Lassus performed the first clinical study to evaluate the effect of silicium on skin [7]. In this open study, women with biologically aged skin and fragile or thin hair, or brittle nails were treated orally with silicium once daily for 90 days and applied silicium to the face for 10 min twice daily. 47 subjects were treated. Lassus observed a statistically significant improvement in the thickness and turgor of the skin, wrinkles and condition of the hair and nails. Ultrasound measurements did not detect any statistically significant change in the thickness of the epidermis or elasticity of the skin, but there was a significant increase in the thickness of the dermis. More recently, Barel et al., [8] conducted a randomized, double blind, placebo-controlled study on 50 females, aged between 40–65 years, with clear clinical signs of photoaging of facial skin. The supplementation was held for a period of 20 weeks with 10 mg of OSA taken daily or with a placebo. The skin microrelief (forearm), hydration (forearm) and mechanical anisotropy (forehead) were evaluated.



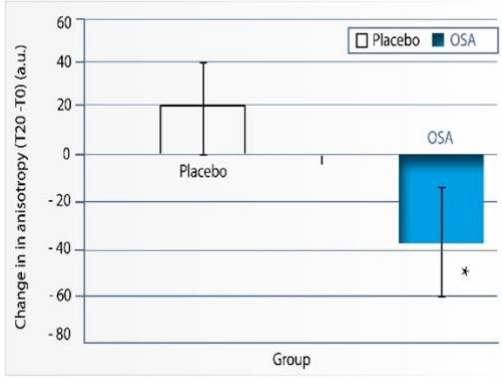


Figure 7. Change in skin microrelief parameters (Visiometer SV 600) from baseline, measured at the forearm, after supplementation with placebo (n=24) or OSA (n=24). Rt, depth of roughness; Rm, maximum roughness; Rz, mean depth of roughness. * P<0.05 vs placebo.	**Figure 8.** Change in mechanical skin anisotropy (Reviscometer MPA 5) from baseline, measured at the forehead, after supplementation with placebo (n=24) or OSA (n=24). Mechanical anisotropy was calculated as the difference between longitudinal and lateral shear propagation time. * P<0.05 vs placebo.

This randomized double-blinded placebo-controlled study illustrates positive effects of silicium, taken as an oral supplement, on skin microrelief and skin anisotropy in woman with photoaged skin. Skin roughness and the difference in longitudinal and lateral shear propagation time decreased in the silicium group, suggesting improvement in isotropy of the skin. In addition, silicium intake positively affected the brittleness of hair and nails. Oral supplementation with silicium had positive effects on hair morphology and tensile strengths, as shown in a randomized placebo-controlled double blind study by Wickett et al.(9).

These results have been confirmed by SIL'INNOV using EYTELIA Silicium Bio-activated.

We have conducted a biometrological evaluation of the anti-ageing effect of Eytelia Silicium Bioactivated used as a food supplement as well as in topical application.

Before and after 84 days, we have evaluated:

- ✓ the smoothing/anti-wrinkle effect in vivo of Eytelia Silicium Bioactivated using 3D Primos® Compact System,
- ✓ the smoothing/anti-wrinkle effect ex vivo by cutaneous replicas using Skin Image Analyser® (S.I.A®) with QuantiRides® software,
- ✓ the effect of Eytelia Silicium Bioactivated on the skin biomechanical properties,
- ✓ the effect of Eytelia Silicium Bioactivated on the cutaneous hydration rate,
- ✓ the redensifying effect by measurements of density of collagen using Siascope® TM Siametrics (MEDX HEALTH CORP).

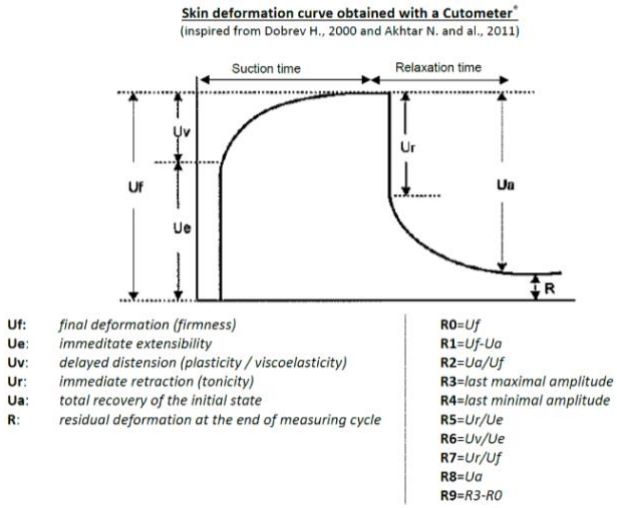
22 women completed the study. Participants took the silicium based food supplement once daily in the morning and applied the cream twice daily in the morning and in the evening.

Treatment took place on 84 consecutive days. Assessment of skin parameters was performed at baseline and after 28, 56 and 84 days of treatment in a controlled environment.

Five zones were defined on the face according to a randomization list. For each type of measurement, the location is the same for the duration of the study.

Effect on the skin biomechanical properties

MPA 580 Cutometer (Courage & Khazaka), an in vivo non-invasive method, was used to evaluate biological extensibility and elasticity variations. The technique consists on the suction of the skin in the orifice of a probe by a constant vacuum pressure and for a constant duration. The depth of penetration of the skin into the probe is measured, without friction and mechanical effects, by using two optical prisms located at the opening of this probe.



For cutaneous skin elasticity,
the measured parameters are expressed in Ue, Uf,... or R0, R1,…
In this study, cutaneous firmness/cutaneous tension and cutaneous elasticity parameters are studied. Firmness/cutaneous tension is evaluated measuring R0 (i.e. Uf) parameter. A decrease of R0 corresponds to a tensing effect of the product, to a firmer skin. Cutaneous elasticity is evaluated measuring R2 (i.e. Ua/Uf) parameter. A increase of R2 corresponds to a more elastic, younger-behaving skin.

	Parameters	Kinetics	Δ Dx-D0 (mean ± SEM)			Student t test p=	Student t test Significance	% of efficacy	% of subjects presenting an improvement
Firmness	R0 (Uf)	ΔD28	-0.048	±	0.020	**0.028**	Yes	+10%	64%
		ΔD56	-0.112	±	0.020	**<0.001**	Yes	+22%	86%
		ΔD84	-0.101	±	0.021	**<0.001**	Yes	+20%	82%
Elasticity	R2 (Ua/Uf) biological elasticity	ΔD28	0.083	±	0.030	**0.012**	Yes	+16%	68%
		ΔD56	0.077	±	0.034	**0.035**	Yes	+14%	64%
		ΔD84	0.078	±	0.032	**0.024**	Yes	+15%	64%

Table 1: Variation of the skin biomechanical properties (in comparison to the initial state)

After 84 days of daily use of Eytelia Silicium Bio-activated an improvement of the biomechanical properties of the skin was observed, characterized by:

- ***a significant decrease of the firmness parameter (R0) of 10% on D28, 22% on D56 and 20% on D84 on average.***
- ***a firmer skin was observed in 64%, 86% and 82% of the subjects, respectively.***
- ***a significant increase of the biological elasticity parameter (R2) of 16% on D28, 14% on D56 and 15% on D84 on average.***

Effect on the cutaneous hydration rate

Cutaneous hydration measurements are performed with a COURAGE & KHAZAKA CM 825 Corneometer®. The cutaneous hydration rate is studied before and after 84 days of once daily use of the food supplement containing OSA-VC and twice daily use of the cream.

				Student t test	
Kinetics	Variations in A.U. (mean ± SEM)			p	Significance
Δ D28	2	±	1	0.144	no
Δ D56	5	±	2	**0.008**	**yes**
Δ D84	4	±	2	**0.049**	**yes**

Table 2: Variation of the hydration rate of epidermis superficial layers after 84 days of use of the products (in comparison to the initial state)

Under these study conditions, 84 days of daily use of Eytelia Silicium Bio-activated, the cutaneous hydration rate was maintained on a stable level

Redensifying effect

Measurements are performed using **SIAscop (SiametricsTM)**. Each measurement is the result of an average of two acquisitions on close areas.

	Kinetics	Δ (mean ± SEM)	Δ% on the mean	Type of statistical test	p	Significance	% of subjects with expectef effect
Collagen density	ΔD28	28.1 ± 8.8	12%	Student t test	0.005	Yes	78%
	ΔD56	35.6 ± 8.5	15%	Student t test	0.001	Yes	89%
	ΔD84	25.7 ± 9.4	12%	Student t test	0.015	Yes	82%

Table 3: Variation of the collagen parameter in comparison to the initial state

84 days of use of Eytelia Silicium Bio-activated induced a redensifying effect by increasing the concentration of collagen in the papillary dermis of 12% on D28, 15% on D56% and of 12% on D84 on average. This effect was observed in 78%, 89% and 82% of the subjects, respectively.

Silicium and cutaneous rejuvenation

In 2017 Petersen & al.(10) have tested here the advantages of supplementation with silicon stabilized at a rate of 10 mg / day consumed orally (in vivo) on the biomechanical properties (firmness, texture, hydration) of the skin of patients. Silicon is documented as a collagen crosslinking agent. Collagen when it has a direct impact on the said properties.

Studies demonstrated that the therapeutic supplementation with silicon strengthens nail and hair, increases collagen and elastin synthesis, promotes maintenance of vascular elasticity and increases calcium fixation in bone tissue

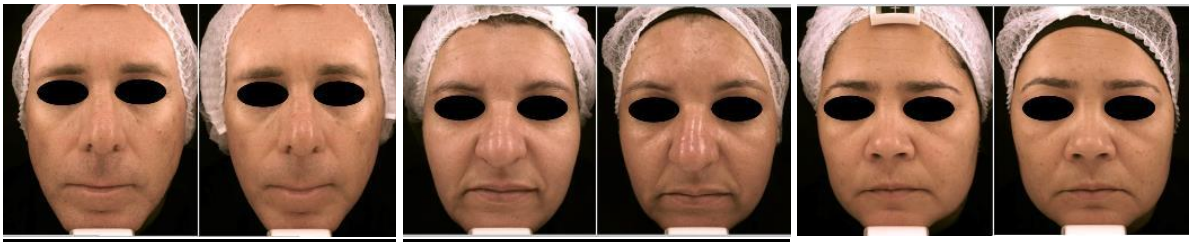


Figure 9 : Images of three selected patients at baseline (left) and 90 d post-treatment (right) with oral ortho-silicic acid stabilized by hydrolyzed marine collagen

Wound healing and silicium

In 2017, Pielesz & al (11) use a combination of orthosilicic acid and ascorbic acid as an hydrogel applied on burned skin samples (human, chicken). This combines the anti-radical power of ascorbic acid with the collagen structuring power of silicic acid due to hydrogen bonds formation

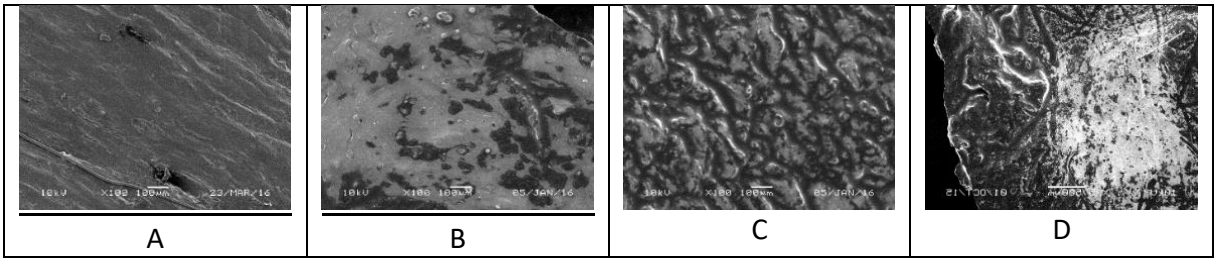


Figure 10 : Exemplary scanning electron microscopy images of the surfaces of skin samples: (a) burn injury human skin; (b) burn injury human skin incubated in presence of L-ascorbic acid solution; (c) burn injury human skin incubated in presence of 3.5% L-ascorbic acid solution and 7% H_4SiO_4-nH_2O hydrogels; (d) dry chicken skin heated to boiling point for 1 minute.

The beneficial effects of the above modifiers were highlighted by infrared (IR) and Raman spectroscopic analyses. Results confirmed changes in collagen molecular structure (from burn to native form) as well as the regeneration of the burn tissues. Topical use of orthosilicic acid in combination with antioxidant molecules to reduce oxidative damage from burns appears thus to be an ideal choice.

This can be related to the documented properties of silicium to stimulate collagen and connective tissue synthesis in general. It is important to note that only **low molecular orthosilicic acid demonstrates positive effects on the process of model burn wounds treatment, but that those effects are very promising and of major interest for more and more scientists.**

In 2018, Grotheer & al.(12) have examined the impact of orthosilicic acid-releasing spun fiber fleece (SIFIB) on wound closure in a porcine wound model in vivo as well as on wound healing-relevant parameters in vitro. They conclude that SIFIB exhibited strong anti-inflammatory properties, which based on SIFIB-dependent inhibition of expression and activity of NF-kB and/or concomitant enhanced expression of IkB, a NF-kB-inhibiting protein. Additionally, SIFIB significantly inhibited TGFb-induced fibroblast differentiation and collagen synthesis as well as effectively reduced TGF-b synthesis of activated fibroblasts. **They have**

demonstrated wound healing relevant biological activities of a silica-based bio-degradable material, which might represent a new therapeutic tool in the treatment of chronic wounds.

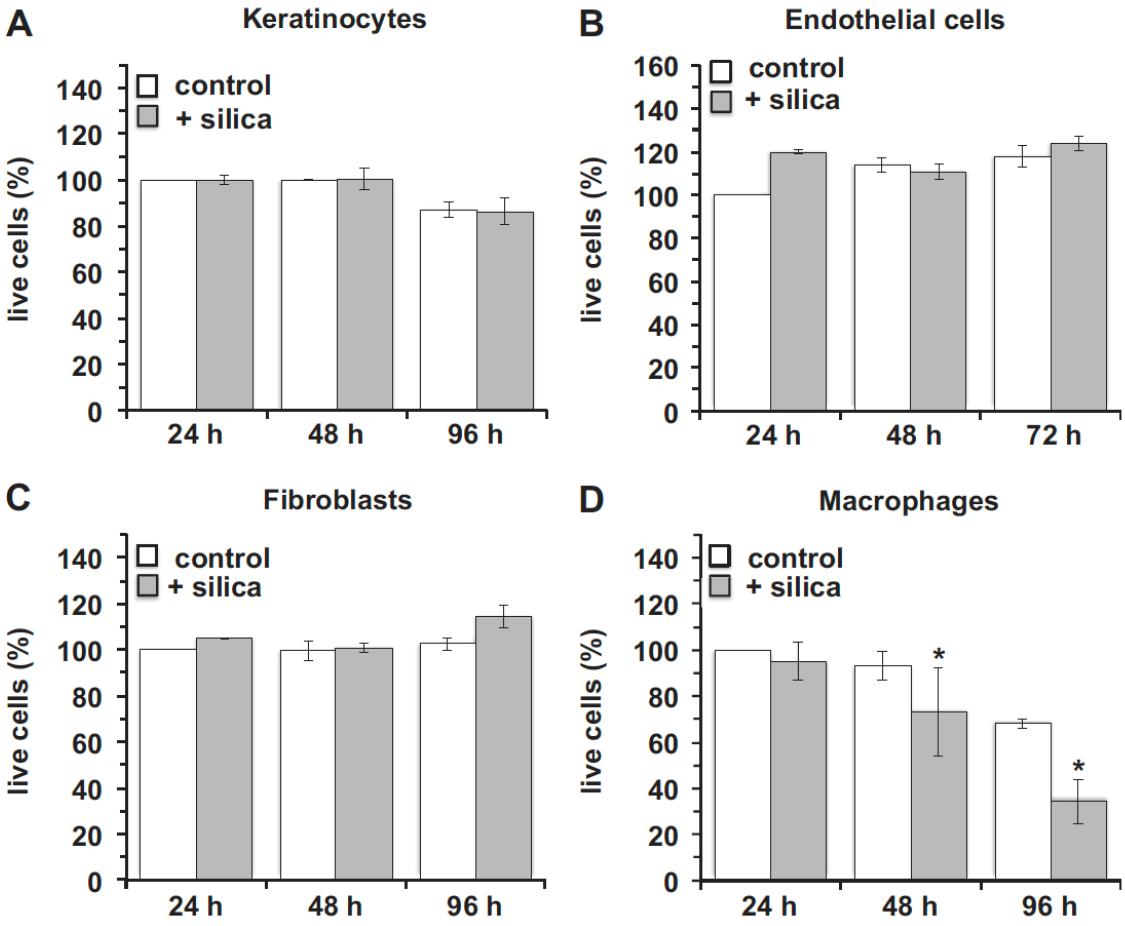


Figure 11 : Effects of silica fiber fleeces on cell viability. Cell types indicated were seeded at a density of 5x105 on 6-well culture plates and cultured for 24-96 h at 37°C with 5% CO2 (white bars). Alternatively, the different cell cultures were co-incubated with spun silica fiber fleece (75 mg) maintained in cell culture inserts with membranes PET track etched membranes (gray bars). The relative number of viable cells was calculated indirectly by using Cell Titer-Blue. A, human skin keratinocytes; B, human umbilical vein endothelial cells; C, human skin fibroblasts; D, human acute monocytic leukemia cell line THP-1. Values represent the mean # S.D of 6 individual experiments. *, $p < 0.005$

Wound Healing – A Use test with EYTELIA Silicium Bio-activated.

Two formulations based on silicium; orthosilicic acid (OSA) and monométhylsilanetriol (MMST) were tested in an experimental animal model of wound healing. The effect of both topical (MMST) and oral (OSA) administration of MMST and OSA are investigated.

16 hairless Skh-1 female mice weighing 20-25 gr were used and randomized in 4 experimental groups (n=4 animals in each group with 2 scarifications / animal) according to bodyweight.

Treatments with the different products began immediately after the induction of skin wounds and were as follow:

-Group1: topically treated with placebo ointment

-Group 2: topically treated with ointment containing MMST

-Group 3: orally treated with placebo solution
-Group 4: orally treated with solution containing OSA

Treatments were carried out daily from Day 0 (induction of skin wounds) until the complete wound healing of the skin wounds. The wound healing process was followed during the treatment's period.

Global macroscopic score of internal wounds at the complete wound healing.

For each mice, after complete wound healing of the 2 skin wounds, the skin from each back of mice containing healed scars was removed and the inner face of the skin was macroscopically scored for the visibility of the scar (0= clearly visible, 3= not visible), the presence of a mass of collagen and fibrin (0= no mass visible, 3= large mass) and the level of vascularization (0= important, 3= non-existent). The addition of the 3 scores defined the overall macroscopic score of internal healing (scores from 0 to 9).

Macroscopic evaluation of wound healing

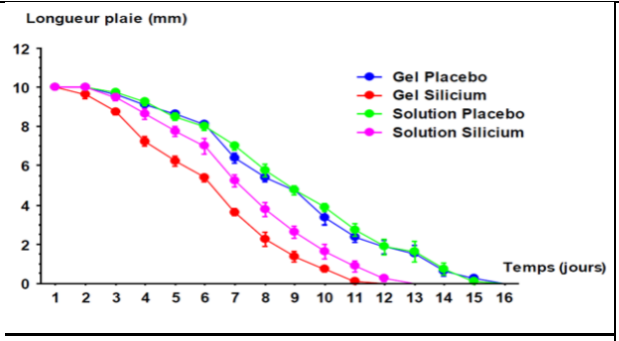


Figure 12 *: Mean length of skin wounds between day 0 and day 15 on mice treated by silicium containing products*

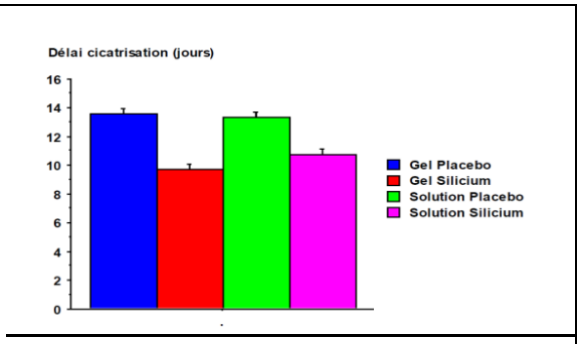


Figure 13 *: Mean number of days necessary to obtain complete wound healing of skin wounds*

The mean length of the skin wounds treated with silicium containing products were significantly lower than those from placebo groups.

The complete wound healing is observed after only 11 or 12 days in silicium groups while in placebo groups the complete wound healing takes 3 to 4 days more.

The mean macroscopic scores of internal wounds at the complete wound healing from silicium treated groups were significantly lower than that from placebo groups.

4. OTHER PUBLICATIONS ABOUT SILICIUM BASED PRODUCTS AND SKIN HEALTH

J Int Med Res. 1993 Jul-Aug;21(4):209-15.

Colloidal silicic acid for oral and topical treatment of aged skin, fragile hair and brittle nails in females.

Lassus A[1].

Abstract

In an open study, women with biologically aged skin and fragile or thin hair, or brittle nails were treated orally with 10 ml colloidal silicic acid (Silicol'") once daily for 90 days and applied colloidal silicic acid to the face for 10 min twice daily. Ofthe 50 subjects treated, three withdrew from treatment after 30 days because of excessive drying of the facial skin due to topical application. In the remaining 47 subjects there was statistically significant improvement in the thickness and turgor of the skin, wrinkles and condition of the hair and nails. The number of mottles also declined, but the change was not statistically significant. Ultrasound measurements did not detect any statistically significant change in the thickness of the epidermis or elasticity of the skin, but there was a significant increase in the thickness of the dermis.

J Int Med Res. 1997 Jul-Aug;25(4):206-9.

Colloidal silicic acid for the treatment of psoriatic skin lesions, arthropathy and onychopathy. A pilot study.

Lassus A[1].

Abstract

In a randomized, double-blind study, patients with chronic plaque-type psoriasis were either 15), or were treated identically with placebo gel (n =15) for 3 months. One stable psoriatic lesion on the knee or elbow was treated topically and followed throughout the study. Five patients in the treated group and seven controls had psoriatic arthropathy and 11 treated patients and 12 controls had psoriatic onychopathy. Three treated patients and six controls withdrew because of skin irritation or lack of efficacy. In the treated group there were clear improvements in scaling, induration and erythema after treatment. The nail changes were cured in five of 10 evaluable patients in the treated group and joint pain was reduced by almost half in the four evaluable patients with arthropathy. There were no such improvements in the placebo group.

Adv Ther. 2001 Mar-Apr;18(2):93-9.

The effects of Silicol Skin on moderate to severe acne: UK field survey.

Abstract

This field survey assessed the effect of the natural trace element silicon, the active ingredient in Silicol Skin, on moderate to severe acne. Members of the Acne Support Group used Silicol Skin for 6 weeks, completing identical questionnaires on the type and severity of acne before and after treatment. A statistically significant 86% overall improvement was reported, with most participants citing the chin as showing the greatest change. Total disappearance of acne was noted in some locations. Results did not differ significantly between the group that used Silicol Skin alone and the group that added it to their prescribed oral medication. Silicol Skin can help improve moderate to severe acne.

Arch Dermatol Res. 2007 Apr;299(1):41-5.

Remodeling of the human dermis after application of salicylate silanol.

Herreros FO[1], Cintra ML, Adam RL, de Moraes AM, Metze K.

Abstract

Recently, a controlled double-blind study in patients with photo-aged facial skin demonstrated the beneficial role of oral intake of silanol for skin, hair and nails. The aim of our pilot study was to investigate histologic alterations in human skin after injection of silanol. Seven healthy female caucasian volunteers with a moderate degree of photoaged skin received ten sessions of weekly injections of 0.1% salicylate silanol in the left ventral lateral forearm. The histologic features of punch biopsies of the treated area and the nontreated contralateral arm were compared and the collagen and elastic fibers quantified. Texture analysis was performed on digitalized microscopic images by analyzing the Sarkar fractal dimension or amplitudes (inertia values) after Fast Fourier transformation. The treated area revealed a statistically significant increase of the density of both collagen and elastic fibers. Texture analysis showed more compact and homogeneously distributed collagen fibers after silicon injection. Our results suggest that the application of silicon may stimulate the production of collagen and elastic fibers leading to remodeling of the dermal fiber architecture, which may explain the improvement of the skin surface observed in clinical studies.

J Wound Care. 2007 Oct;16(9):404-7.

A prospective analysis of the role of silicon in wound care.

Lansdown AB[1], Williams A.

Abstract

Silicon is an important micronutrient associated with the development of bone and connective tissue. This article discusses the properties of silicon, its absorption in the human body and its current and potential use in wound management.

In recent years, silicon-containing wound dressings and gel sheetings have become widely available for the treatment of open wounds and burns. In each case, the products exhibit minimal adhesion or obvious damage to injured tissue and are efficacious in advancing repair and regeneration in open wounds and burns as well as in reducing hypertrophic scars and keloids, with minimal discomfort to patients.

The importance of silicon as a trace element is often overlooked and its role in skin physiology is not fully appreciated. As new and more accurate analytical procedures become available, the opportunity is now available to investigate the biodegradation patterns of silicone wound dressings, gel sheetings and other medical devices, and to determine the biochemical value of silicon in critical phases of wound repair and regeneration. More information is urgently needed on the action of silicones and silicone fragments in isolated fibroblasts or keratinocyte cultures to evaluate the possible importance of silicon in biosynthetic pathways.

Aesthetic Plast Surg. 2008 Jan;32(1):82-92.

Evolution of silicone therapy and mechanism of action in scar management.

Mustoe TA[1].

Abstract

Silicone-based products are widely used in the management of hypertrophic scarring and keloids. This review discusses the range of products available and the clinical evidence of their

efficacy in preventing excessive scarring and improving established scars. Silicone gel sheeting has been used successfully for more than 20 years in scar management. A new formulation of silicone gel applied from a tube forms a thin flexible sheet over the newly epithelialized wound or more mature scar. Results from clinical trials and clinical experience suggest that silicone gel is equivalent in efficacy to traditional silicone gel sheeting but easier to use. The mechanism of action of silicone therapy has not been completely determined but is likely to involve occlusion and hydration of the stratum corneum with subsequent cytokine-mediated signaling from keratinocytes to dermal fibroblasts.

Korean J Nutr. 2009 Sep;42(6):505-515.

Effect of Vitamin C, Silicon and Iron on Collagen Synthesis and Break-Down Enzyme Expression in the Human Dermal Fibroblast Cell (HS27)

Kim JE , Lee J , Kim H , Kim J , Cho Y.

Abstract

Collagen is the major matrix protein in dermis and consists of proline and lysine, which are hydroxylated by prolyl hydroxylase (PH) and lysyl hydroxylase (LH) with cofactors such as vitamin C, oxygen, iron (Fe2+), ketoglutarate and silicon. The collagen degradation is regulated by matrix metalloproteinase-1 (MMP-1), of which is the major collagendegrading proteinase whereas tissue inhibitors of metalloproteinase-1 (TIMP-1) bind to MMP-1 thereby inhibiting MMP-1 activity. In this study, we investigated the effects of vitamin C, silicon and iron on mRNA, protein expressions of PH, LH, MMP-1 and TIMP-1. The physiological concentrations of vitamin C (0-100 μM), silicon (0-50 μM) and iron (Fe2+ : 0-50 μM) were treated to human dermal fibroblast cells (HS27 cells) for 3 or 5days. The expression level of mRNA and protein was increased in not only PH but also LH when cells were incubated with vitamin C. A similar increase in LH mRNA or protein expression occurred when cells were incubated with silicon. Our results suggest that treatment of vitamin C and silicon increased mRNA and protein expression of PH and LH in human dermal fibroblast.

Int J Cosmet Sci. 2012 Aug;34(4):311-7.

Evaluation of protective and restoring effects of a mixture of silanols on photoaging. Use of a device allowing the quantification of contractile strengths of human fibroblasts after UV Airradiation.

Robin S[1], Courderot-Masuyer C, Tauzin H, Guillon S, Gaborit J, Harbon S, Humbert P.

Abstract

Chronic sun exposure and especially UVA wavelengths are responsible for long-term clinical skin changes such as photoageing and photocancers. The objectives of the present study were to analyse the contractile activity of fibroblasts irradiated with several doses of UVA and to evaluate the preventive, protective and restoring effects of a mixture of monomethylsilanetriol mannuronate and dimethylsilanediol salicylate. The forces generated by fibroblasts in tense collagen lattices were quantified using Glasbox device before and after UVA irradiation and the addition of a mixture of monomethylsilanetriol mannuronate and dimethylsilanediol salicylate. The production of collagen was also evaluated before and after irradiation and with and without the presence of a mixture of monomethylsilanetriol mannuronate and dimethylsilanediol salicylate. A dose of 3 J cm(-2) of UVA showed more than 50% of mortality in fibroblast population after 48 h and significant decreases in contractile forces developed by irradiated fibroblasts and collagen I production.

One percentage of a mixture of monomethylsilanetriol mannuronate and dimethylsilanediol salicylate protected fibroblastsfrom UVA irradiation and made it possible to restore their capacity to the same level as fibroblasts that were not irradiated. It also tended to restore the capacity to synthesize collagen I. These results show that the use of the new device Glasbox makes it possible to evaluate a possible preventive and repairing effect of a cosmetic functional active on photoageing.

Biomaterials. 2013 Oct;34(30):7314-27.

The performance of an orthosilicic acid-releasing silica gel fiber fleece in wound healing.

Grotheer V[1], Goergens M, Fuchs PC, Dunda S, Pallua N, Windolf J, Suschek CV.

Abstract

In the present work, we have examined the impact of an inorganic orthosilicic acid-releasing spun fiber fleece (SIFIB) on wound closure in a porcine wound model in vivo as well as on wound healing-relevant parameters in vitro. In vivo SIFIB was completely bio-degradable and had no negative effects on wound closure or the wound healing process. In the in vitro experiments, SIFIB had no negative effects on proliferation of human skin fibroblast (FB) and endothelial cell (EC) cultures but strongly retarded the growth of the human monocyte cell line THP-1, and effectively inhibited human skin keratinocyte (KC) proliferation, which based on significantly enhanced KC differentiation. Furthermore, SIFIB exhibited strong anti-inflammatory properties, which based on SIFIB-dependent inhibition of expression and activity of NF-κB and/or concomitant enhanced expression of IκB, a NF-κB-inhibiting protein. Additionally, SIFIB significantly inhibited TGFβ-induced fibroblast differentiation and collagen synthesis as well as effectively reduced TGF-β synthesis of activated fibroblasts. We have demonstrated wound healing-relevant biological activities of a silica-based bio-degradable inorganic material, which might represent a new therapeutic tool in the treatment of chronic wounds.

Colloids Surf B Biointerfaces. 2017 Jul 1;155:530-537.

Silica nanoparticles as sources of silicic acid favoring wound healing in vitro.

Quignard S[1], Coradin T[2], Powell JJ[3], Jugdaohsingh R[3].

Abstract

There is good evidence that certain silicon-containing materials promote would healing and their common feature is the delivery of orthosilicic acid ($Si(OH)_4$) either directly or following metabolism. In this respect, amorphous silica nanoparticles (NP), which dissolve in aqueous environments releasing up to 2mM orthosilicic acid, may be appropriate 'slow release' vehicles for bioactive silicon. Here we studied the impact of silica NP suspensions (primary particles~10nm) in undersaturated conditions (below 2mM Si) with differing degrees of surface charge and dissolution rate on human dermal fibroblasts (CCD-25SK cells) viability, proliferation and migration in a cellular wound model. Silica was shown to be non-toxic for all forms and concentrations tested and whilst the anticipated stimulatory effect of orthosilicic acid was observed, the silica NPs also stimulated fibroblast proliferation and migration. In particular, the amine-functionalized particles promoted wound closure more rapidly than soluble orthosilicic acid alone. We suggest that this effect is related to easy cellular internalization of these particles followed by their intracellular dissolution releasing silicic acid at a faster rate than its direct uptake from the medium. Our findings

indicate that amorphous silica-based NPs may favour the delivery and release of bioactive silicic acid to cells, promoting wound healing.

J Cosmet Dermatol. 2018 Oct;17(5):814-820.

Evaluation of cutaneous rejuvenation associated with the use of ortho-silicic acid stabilized by hydrolyzed marine collagen.

Petersen Vitello Kalil CL[1], Campos V[2], Cignachi S[1], Favaro Izidoro J[1], Prieto Herman Reinehr C[2], Chaves C[3].

Abstract

Organic silicon plays an important role in dermal structure by promoting neocollagenesis. Thus, the supplementation of silicon in a highly bioavailable form can be used for skin rejuvenation.

AIMS:

This study aimed to evaluate skin changes associated with the use of ortho-silicic acid stabilized by hydrolyzed collagen.

PATIENTS/METHODS:

Patients were randomized to receive 600 mg of ortho-silicic acid stabilized by hydrolyzed collagen (group 1, n = 11) or placebo (group 2, n = 11) to be taken 15 minutes before breakfast for 90 days. Clinical, photographical, and patients' subjective evaluations were conducted.

RESULTS:

A total of 22 patients were included. Clinical evaluations demonstrated changes in skin texture, firmness, and hydration statistically superior in group 1. Brightness, firmness, and overall appearance showed trends for a difference favoring group 1 according to patients' subjective evaluations. Objective images showed no statistical differences. No side effects, hypersensitivity, or systemic symptoms were observed in group 1. Treatment satisfaction in group 1 reached 80%.

CONCLUSIONS:

Ortho-silicic acid stabilized by hydrolyzed collagen in a daily dose of 600 mg showed positive results in skin rejuvenation according to clinical evaluation in firmness, hydration, and skin texture. Further studies with larger and representative samples should be conducted to confirm our results.

Int J Cosmet Sci. 2019 Aug;41(4):405-409.

Topical monomethylsilanetriol can deliver silicon to the viable skin.

Polonini HC[1,2,3], Ferreira AO[1,2,4], Brandão MAF[1,4], Raposo NRB[1,4].

Abstract

OBJECTIVE:

Organic silicon has been linked to positive effects on the skin rejuvenation, mainly by the oral route. Thus, the main objective of the present study was to assess whether monomethylsilanetriol (MMST, a source of organic silicon) can deliver silicon to the epidermis and dermis, when applied topically in a cream. Once the hypothesis was confirmed, the present study also evaluated whether the product was toxic to keratinocytes; additionally, its possible antioxidant activity was assessed.

METHODS:

The ex vivo skin permeation profile was determined using human skin in Franz-cells equipment; cytotoxicity was assessed using HaCaT keratinocytes. Antioxidant capacity was

determined as scavenging activity, measured according to the 1,1-diphenyl-2-picrylhydrazil free radical method.

RESULTS:

The permeation percentage was almost 60% of the applied MMST, with a large quantity of drug found in the viable epidermis and dermis. The cell viability assay showed no significant difference in the percentage of viable keratinocytes among the treated groups at the doses used. In terms of antioxidant activity, the IC_{50} value obtained was 2400 $\mu g\ mL^{-1}$. Low antioxidant activity, negligible toxicity for keratinocytes and a significant percentage of permeation were observed.

CONCLUSION:

We provide evidence that MMST applied topically can deliver silicon to the skin in biorelevant levels for cosmetic purposes.

References :

1. Reffitt, D. M., Ogston, N., Jugdaohsingh, R., Cheung, H. F., Evans, B. A., Thompson, R. P., Powell, J. J., and Hampson, G. N. (2003) Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. *Bone* **32**, 127-135
2. Carlisle, E. M., Berger, J. W., and Alpenfels, W. F. (1981) A silicon requirement for prolyl hydroxylase activity. *Fed. Proc* **40**, 886
3. Calomme, M. R., and Vanden Berghe, D. A. (1997) Supplementation of calves with stabilized orthosilicic acid. Effect on the Si, Ca, Mg, and P concentrations in serum and the collagen concentration in skin and cartilage. *Biol. Trace Elem. Res* **56**, 153-165
4. Arumugam, M. Q., Ireland, D. C., Brooks, R. A., Rushton, N., and Bonfield, W. (2004) Orthosilicic Acid Increases Collagen Type I mRNA Expression in Human Bone-Derived Osteoblasts in Vitro. *Key Engineering Materials* **254-256**, 869-872
5. Arumugam, M. Q., Ireland, D. C., Brooks, R. A., Rushton, N., and Bonfield, W. (2006) The Effect Orthosilicic Acid on Collagen Type I, Alkaline Phosphatase and Osteocalcin mRNA Expression in Human Bone-Derived Osteoblasts In Vitro. *Key Engineering Materials* **309-311**, 121-124
6. Schwarz, K. (1973) A bound form of silicon in glycosaminoglycans and polyuronides. *Proc. Natl. Acad. Sci. U. S. A* **70**, 1608-1612
7. Lassus, A. (1993) Colloidal silicic acid for oral and topical treatment of aged skin, fragile hair and brittle nails in females. *J Int Med. Res* **21**, 209-215
8. Barel, A., Calomme, M., Timchenko, A., Paepe, K. D., Demeester, N., Rogiers, V., Clarys, P., and Vanden Berghe, D. (2005) Effect of oral intake of choline-stabilized orthosilicic acid on skin, nails and hair in women with photodamaged skin. *Arch. Dermatol. Res* **297**, 147-153
9. Wickett, R. R., Kossmann, E., Barel, A., Demeester, N., Clarys, P., Vanden Berghe, D., and Calomme, M. (2007) Effect of oral intake of choline-stabilized orthosilicic acid on hair tensile strength and morphology in women with fine hair. *Arch Dermatol Res* **299**, 499-505
10. Petersen C.L, Kalil V, Campos V, Cignachi S, Favaro Izidoro J, Prieto Herman Reinehr C. Evaluation of cutaneous rejuvenation associated with the use of ortho-silicic acid stabilized by hydrolyzed marine collagen. Journal of Cosmetic Dermatol. 2017; 1–7
11. Pielesz A, Biniaś D, Sarna E, Bobiński R, Kawecki M, Glik J, Klama-Baryła A, Kitala D, Łabuś W, Paluch J, Kraut M. Active antioxidants in ex-vivo examination of burn wound healing by means of IR and Raman spectroscopies-Preliminary comparative research. Spectrochim Acta A Mol Biomol Spectrosc. 2017 15: (173) 924-930
12. Grotheer, V., et al. (2013). "The performance of an orthosilicic acid-releasing silica gel fiber fleece in wound healing." Biomaterials 34(30): 7314-7327.