# VISIBLE FACIAL PORES: NEW INSIGHTS FOR THEIR ASSESSMENT AND TIGHTENING TREATMENT

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## INTRODUCTION

Today, many people use close-up photos or videos in social media. However, these tend to reveal facial imperfections such as visible pores, complexion, wrinkles and fine lines. In the skin, there are two types of pores: sweat pores, and "visible" pores, where sebum secretion occurs. These small orifices are part of the pilosebaceous apparatus.

Intrinsic (genetic predisposition, aging, hormones, hyperseborrhea...) and extrinsic factors (UV, xenobiotics...) are described as being able to cause dilation or enlargement of the facial pores, making them visible to the naked eye [1]. This aesthetic imperfection generates in some people a phobia qualified as "poroxia" by dermatologists. The trend for selfies and the quest for an "Instagram Face" exacerbates this feeling. Partly for these reasons, treating visible pores has become a concern for both cosmetics and dermatology. Furthermore, the exact causes of appearance of visible pores are still widely debated in the literature [1,2].

During the aging process, the extracellular matrix (ECM) alteration causes a loss of skin elasticity and firmness which was described to be correlated with the down expression of microfibril-associated glycoprotein 1 (MAGP-1), a crucial component in elastic fibers assembly and in skin elasticity [3,4]. As a result, the dermis becomes less dense and disorganized for a thinner and wrinkled skin [5].

Facial pores are structures along the vellus. To support the whole structure, the pore is surrounded by concentric sheaths including the connective tissue sheath (CTS). This CTS is an ECM composed of fibers organized in circles around the follicle ostium thus providing it with a tight cylindric shape [6]. It thus suffers from the same age-induced alterations as the dermis: a sagging occurs. The pore is enlarged and therefore more visible [1,7].

In this study, we present an original approach for assessing the effect of a new silanol SiA (combination of adenosine and a core of organic silicium (MTS)) in the dermis and on the specific structures around the pore. We then present the resulting effects of a topical treatment with this silanol on skin biomechanical properties, on skin relief and on pore perception.

## MATERIAL & METHODS

***Immunohistological studies***

***Explant culture***: Human skin explants were obtained from an abdominoplasty and a face lifting from female Caucasian donors aged 47 and 64 respectively who underwent plastic surgery. The explants were topically treated for 7 days with the active ingredients (20 µl/punch, 1 or 2 applications/day).

***Classical transversal histological skin sections:*** 5-7 µm thick sections of paraffin-embedded biopsies were realized using a microtome.

***Longitudinal histological skin sections (transversal sections of the pore)***: 7-10 µm thick transversal sections of the pores were performed with a microtome for paraffin embedded samples, or with a cryostat and stored at -20°C until AFM measures on cryosections (Fig. 1).

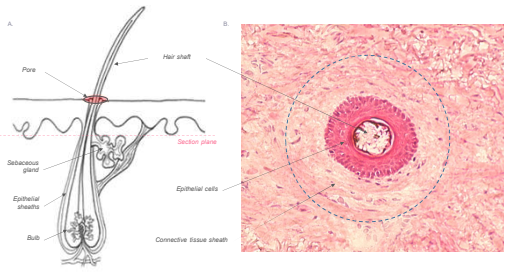


**Fig. 1 Transversal section of the hair follicle** *A) Schematic representation of a longitudinal section of a hair follicle. The pore is highlighted in red. B) Microphotograph of a transversal section of a pore according to the section plane (HE staining). The circled zone is the connective tissue sheath (CTS).*

***Quantification of total collagen content on transversal sections of the skin:*** The slides were stained with picrosirius red that stains in red all collagen fibers. Total collagen content, expressed in percent of the stained area of a region of interest, was quantified by image analysis.

***Quantification of procollagen-I positive cells around each pore:*** The number of procollagen I positive cells was assessed by immunofluorescence. The sections were counterstained with DAPI. 15 facial pores were selected and the DAPI positive nuclei and procollagen I positive cells were manually counted around each pore. The results are expressed as the ratio of procollagen I positive cells on total cells.

***Detection of elastin and MAGP-1 on transversal sections of pores:*** Elastin and MAGP-1 expression were assessed by immunofluorescence on 7 µm thick paraffinized sections. The sections were counterstained with DAPI. 8 to 15 facial pores were selected. Elastin and MAGP-1 were quantified by image analysis (signal intensity and/or staining area).

***Atomic force microscopy (AFM) measurements***

The AFM measurements were performed with a Bioscope Resolve (Bruker) coupled with an epifluorescence microscope (Leica DMi8).

***Viscoelasticity:*** Human skin explants were obtained from an abdominoplasty from female Caucasian donors aged 55 and 60 respectively who underwent plastic surgery. Punches of 12 mm were cultured and topically treated for 48 h days with the active ingredients (20 µl/punch, 1 or 2 applications/day). The explants were then left at room temperature for 30 min and the skin viscoelasticity was measured by AFM (contact force) on the whole explant surface.

***Young's modulus:*** Transversal cryosections of the pore structure were obtained from the 64 y.o. donor as aforementioned. The Young's modulus of fibers within the CTS was measured by QNM (Quantitative Nanomechanical Mapping) Peakforce® mode. Force measurements were performed in air and consists in the acquisition of force volume (FV) to an area of 15 µm x 15 µm. In this area, each measurement point corresponds to an indentation force curve from which the Young's modulus is then extracted. Finally, 16 FV per slide were performed for a total of 48 measurements per condition.

***Clinical study:***

40 Caucasian women aged 40-66 (mean age 55) received a daily treatment of placebo or SiA (5%) twice a day for 28 days. Pore perception was assessed by self-evaluation and by dermatological evaluation. Wrinkle depth and skin rugosity (Rz) were assessed by AEVA-HE®.

***Statistical analysis:***

Experimental values are represented as arithmetic mean +/- SEM. Statistical analyses were performed using JMP software. Normality was tested with the Shapiro–Wilk test. Homogeneity between groups at baseline was tested by ANOVA. Differences between treatment groups were calculated using Student's T-test, Welch test or Wilcoxon test. The statistical significance was considered as follow: non-significant (ns) for p-values>0.05, significant (*) for p-value<0.05, very significant (**) for p-value<0.01 and highly significant (***) for p-value<0.001.

## RESULTS & DISCUSSION

**SiA increases collagen expression in the dermis of skin explant**

Preliminary data on reconstructed full thickness skin (T Skin, Labskin) showed that a systemic treatment with SiA 5% strongly increases collagen I production detected by collagen I C-terminal propeptide quantification (data not shown). Therefore, the effects of a topical treatment of a human organotypic skin explant from an aged donor with SiA 5% for 7 days were assessed. The total collagen expression in the dermis was measured by histological analysis (Fig. 2).

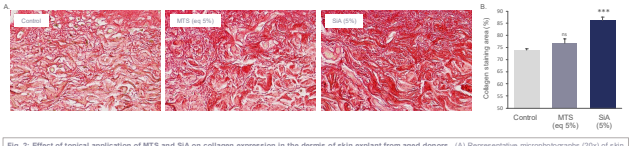


**Fig. 2: Effect of topical application of MTS and SiA on collagen expression in the dermis of skin explant from aged donors.** (A) Representative microphotographs (20x) of skin section from an aged patient treated with water (Control), MTS (eq 5%) or SiA 5%, for 7 days. Collagen fibers appears in red. (B) Quantification of the treatment effect on collagen expression from image analysis. Data are presented as means ± SEM. *** p<0.001 vs Control; ns: non-significant

The dermis of the control aged skin presents few thin compacted collagen fibers covering only 74% of the total dermis area. The same explant topically treated with SiA 5% presents thicker collagen fibers, denser dermis with lower interstitial space. Indeed, the treatment with SiA 5% leads to a 16% increase of the collagen fiber staining area compared to control. This effect could be explained by an increased synthesis and a better arrangement of the fibers. During aging, a decrease of collagen production in the papillary dermis is described [5]. Individually, silicium and adenosine increases collagen I production in skin fibroblasts and/or decreases the expression of matrix degradation enzymes [8,9]. The association of both actives strongly increase the dermis collagen content. The beneficial effect of SiA on this parameter may be explained by a synergy between silicium and adenosine.

**SiA improves the fiber network in the dermis and around the pore**

The ECM of the dermis and of the CTS is composed of a fibrous network within a GAG gel. This fiber network is composed of collagen (mostly I and III) and elastin fibers organized together by the glycoprotein MAGP-1 [4]. With age, this network becomes altered [5]. The skin becomes thinner and wrinkled. The pore slacks and becomes dilatated, more visible [7].

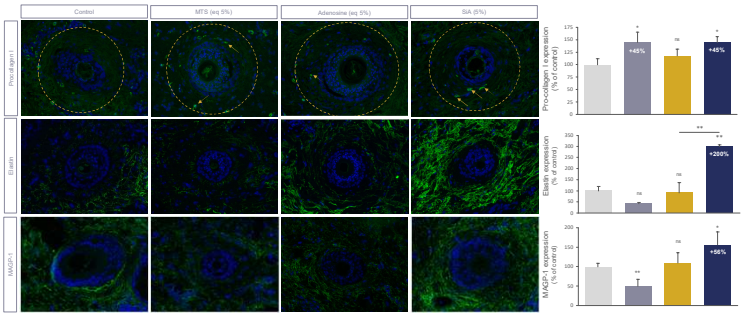


**Fig. 3 : SiA stimulates the expression of several selected constituents of the fiber network of the ECM.** Representative microphotographs (20x) of pore transversal sections of human skin explant topically treated with water (Control) or MTS (eq 5%), adenosine (eq 5%) and SiA (5%), twice a day for 7 days. Procollagen-I positive cells (top lane), elastin (middle lane) and MAGP-1 (bottom lane) were detected by immunostaining and quantified. Mean ± SEM. ns: non-significant; *p<0.05; ** p<0.01 vs control. The markers of interest appear in green and cell nuclei in blue (DAPI).

The treatment with SiA (5%) increases the expression of the selected constituents of the ECM near the pore structure.
The collagen production was significantly increased in the CTS (Fig 3, top lane) where the effect seems driven by the MTS.
Interestingly, a synergy between the MTS and the adenosine was observed. It allowed for a dramatic increase for both elastin and MAGP-1 expression (Fig 3, middle and bottom lanes) in the dermis and around the pore.
Aging alteration of the fibers network around the pore has been proposed as a possible mechanism for pore sagging [4].
Taken together, these data suggest that SiA could contribute to fighting against age-induced pore sagging by restoring the ECM fiber network.

**SiA improves the biomechanical properties of the dermis and of the CTS**

The skin biomechanical properties such as viscoelasticity and Young's Modulus are important parameters modulated by aging [11]. They are correlated with the ECM expanse and skin turgor, thus promoting the resistance to physical stress and the maintenance of its structures.

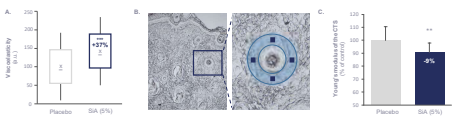


**Fig. 4 : SiA improves the biomechanical properties of the dermis and the CTS**
(A) Measure of the viscoelasticity of aged explants treated with SiA (5%) with AFM. (B) Microphotograph of a longitudinal section of the skin observed in visible microscopy. The blue zone highlights the connective tissue sheath (CTS). The dark blue squares are the FV zones where AFM measures were performed. (C) Explants from aged donor were treated with SiA (5%). The Young's modulus was measured in the CTS using atomic force microscopy. Mean ± SEM. ** p<0.01 vs control. n=3.

The treatment with SiA (5%) increases the viscoelasticity of whole skin explants from aged patients (Fig. 4A). Near the pore structure, SiA decreases the Young's modulus (Fig. 4C). The CTS fibers are therefore less rigid. By improving the fiber network of the ECM in both the dermis and around the pore, SiA is able to improve the age-altered skin biomechanical properties.

**SiA improves skin relief and decreases pore perception**

The *in vitro* and *ex vivo* observations were confirmed in a clinical study performed on 40 volunteers.

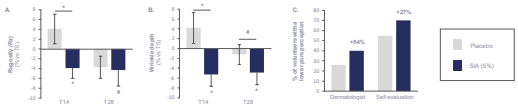


**Fig. 5 : SiA provides clinical benefits**
Clinical benefits of a 28 days treatment with SiA (5%) on skin rugosity (A) and on wrinkle depth (B). (C) Percent of volunteers with an improvement in their perception of visible pores. Mean ± SEM. *p<0.1; °p<0.05 vs T0.

SiA decreases the wrinkle depth (Fig 5A) and skin rugosity (Fig 5B). Because of the skin relief improvement, the pores are less perceptible as they seem smaller and less visible (Fig 5C). Taken together these clinical data suggest that the pore perception may be improved because of the filling effect of SiA on the dermis and specifically in the perifollicular area.

## CONCLUSION

This work presents an original approach for assessing pore sagging observed during aging and shows that collagen and its associated fibers play a key role in the pore supportive architecture. The biomechanical studies provided new insights for the comprehension of the pore enlargement mechanism. Although this topic still needs to be further studied.

Our results strongly suggest that a combination of adenosine and a core of organic silicium could have a synergistic beneficial effect on skin density, firmness and flexibility, in the dermis and around the pore. The silanol could therefore be a good candidate for reducing visible pores and more widely, for limiting the first clinical signs of aging.

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