

## Saponins: A New Generation of Hyaluronan-Stimulating Actives for Human Skin

S. Gallinat, F. Rippke, C. Keppler, J. Mergell, A. Bürger, F. Stüb, H. Wenck  
Beiersdorf AG, Hamburg, Germany

### Abstract

Biochemical and structural changes of the dermal connective tissue substantially contribute to the phenotype of skin aging and, in particular, to the development of deep wrinkles. Among the factors that most prominently affect the clinical appearance of aging skin is hyaluronan. This glycosaminoglycan exerts a great variety of effects including the binding of vast amounts of water leading to firm skin.

To further characterize the role of hyaluronan in aging skin we performed cDNA microarray studies *in-vitro* (primary human dermal fibroblasts, n=6) and *ex-vivo* (human punch biopsies from buttock skin) in young (18-35 yrs, n=10) and old (57-70 yrs, n=10) volunteers. Overall, several genes of the connective tissue metabolism involved in expression, processing and degradation were found to be significantly regulated. In particular, as demonstrated by ELISA on suction blister fluids obtained from young (aged 18-35 yrs, n=6) and old volunteers (ages n=6, >60 yrs), older subjects displayed a lower baseline level in hyaluronan levels. In order to counteract these age-dependent changes cultured fibroblasts were treated with a specific soy extract (10 µg/ml, n=9, 50-67 yrs) particularly rich in saponins. Most interestingly, this treatment resulted in pronounced increased hyaluronan synthesis by 256%. Taken together, our data indicate that reduced hyaluronan levels in human skin contribute to the phenotype of skin aging. Interestingly, application of a soy extract rich in natural saponins improves hyaluronan synthesis indicating beneficial effects for aging skin.

### Introduction

With increasing age, structural and functional changes occur in the epidermis and dermis resulting in a gradual decline in overall skin physiology. As a consequence of intrinsic and extrinsic factors, epidermal turnover is reduced, an epidermal atrophy develops, dermal collagen is reduced or degraded and hyaluronan levels are reduced. Clinically, these cellular and biochemical alterations are apparent by the development of wrinkles, an increased skin roughness and a reduction of skin elasticity, to name a few.

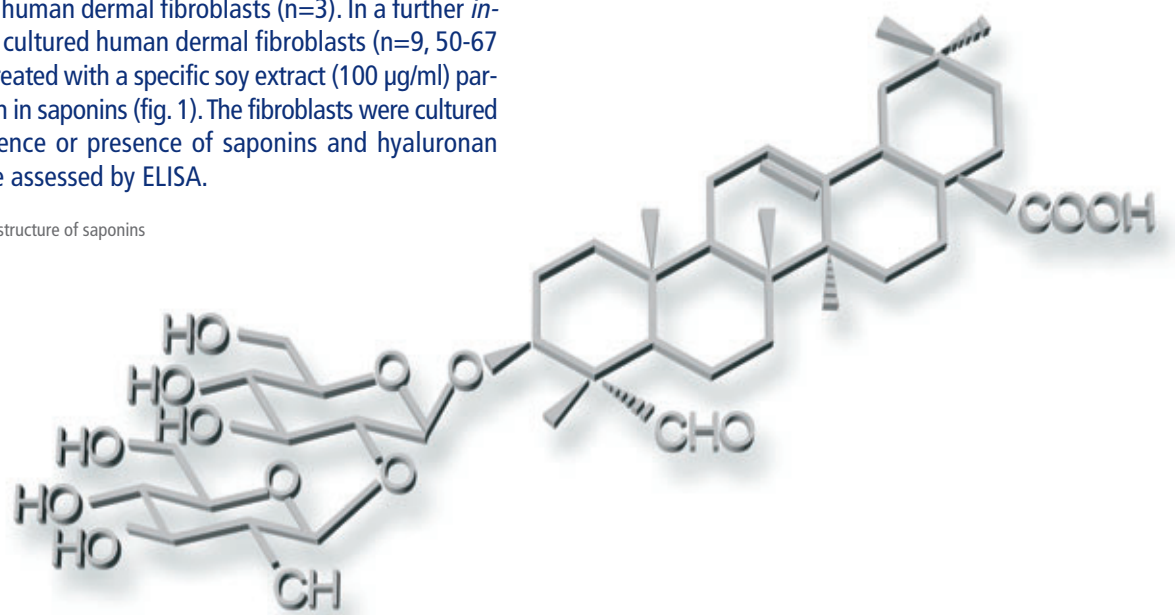
The studies presented here were undertaken to identify effective natural compounds that counteract the development of wrinkles. In particular, given the relevance of hyaluronan for the skin (i.e. skin hydration, suppleness and wrinkle reduction) the overall objective was to create a topical solution to effectively reduce wrinkles.

### Materials and Methods

cDNA microarray studies were carried out *in-vitro* (primary human dermal fibroblasts, n=6) and *ex-vivo* (human punch biopsies from buttock skin) in young (18-35 yrs, n=10) and old (57-70 yrs, n=10) volunteers. To determine hyaluronan levels in skin, specific hyaluronan antibodies were used.

In *in-vitro* screening studies, the effects of various natural compounds on hyaluronan synthesis were investigated employing human dermal fibroblasts (n=3). In a further *in-vitro* study cultured human dermal fibroblasts (n=9, 50-67 yrs) were treated with a specific soy extract (100 µg/ml) particularly rich in saponins (fig. 1). The fibroblasts were cultured in the absence or presence of saponins and hyaluronan levels were assessed by ELISA.

Fig. 1 Chemical structure of saponins



### Results

The cDNA microarray studies revealed that overall several genes of the connective tissue metabolism involved in expression, processing and degradation were significantly regulated. In particular, as demonstrated by ELISA on suction blister fluids obtained from young (aged 18-35 yrs, n=6) and old volunteers (ages n=6, >60 yrs), older subjects displayed a lower baseline level in hyaluronan levels (fig. 2).

In the *in-vitro* stimulation studies, Hyaluronan levels were significantly induced only by the saponin-rich extract but not by the other compounds (soy extract containing 30% isoflavones, purified isoflavone or genistein) (fig. 3).

Treatment of dermal skin cells with this particular plant extract resulted in a significant stimulation of hyaluronan levels by 256% (fig. 4), counteracting the age-dependent decline.

### Discussion and Conclusion

The data presented here demonstrates reduced endogenous hyaluronan levels in aged compared to young skin. Furthermore, our studies show that, contrary to various other phytochemicals, a specific soy extract rich in natural saponins effectively stimulates hyaluronan synthesis in human skin cells. Considering the biological role of hyaluronan and its decline in aging skin this data clearly indicates beneficial effects on aged skin and, in particular, on wrinkle appearance.

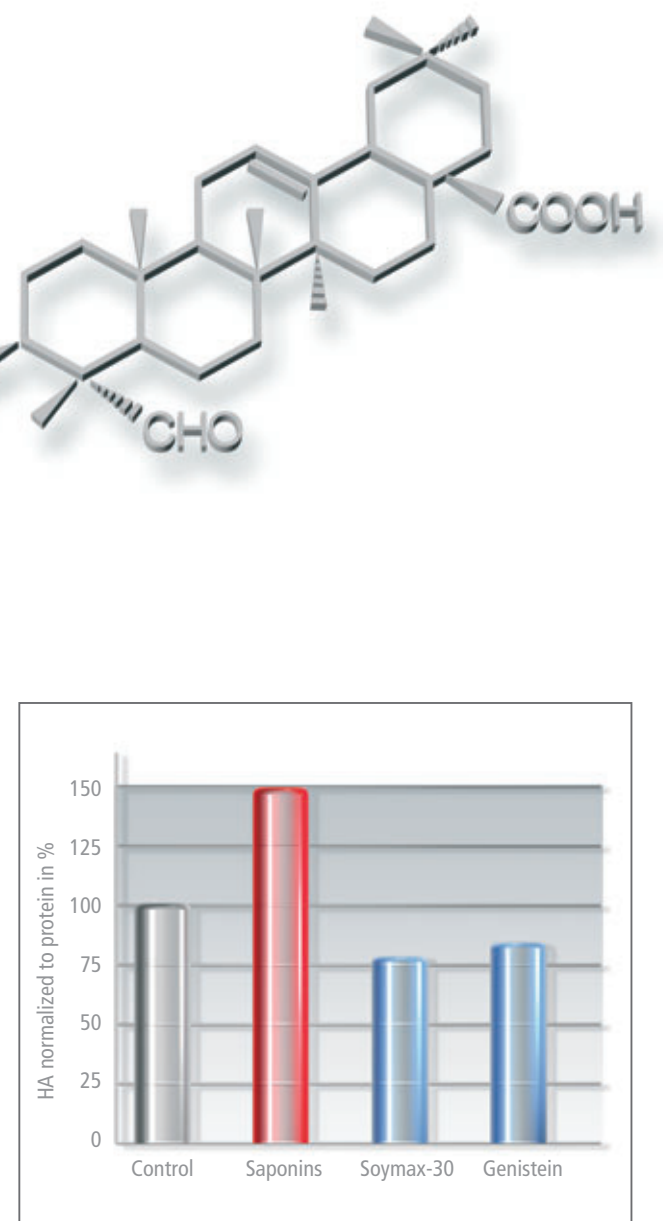


Fig. 3 Effects of natural compounds on hyaluronan synthesis in dermal fibroblasts (n=3).

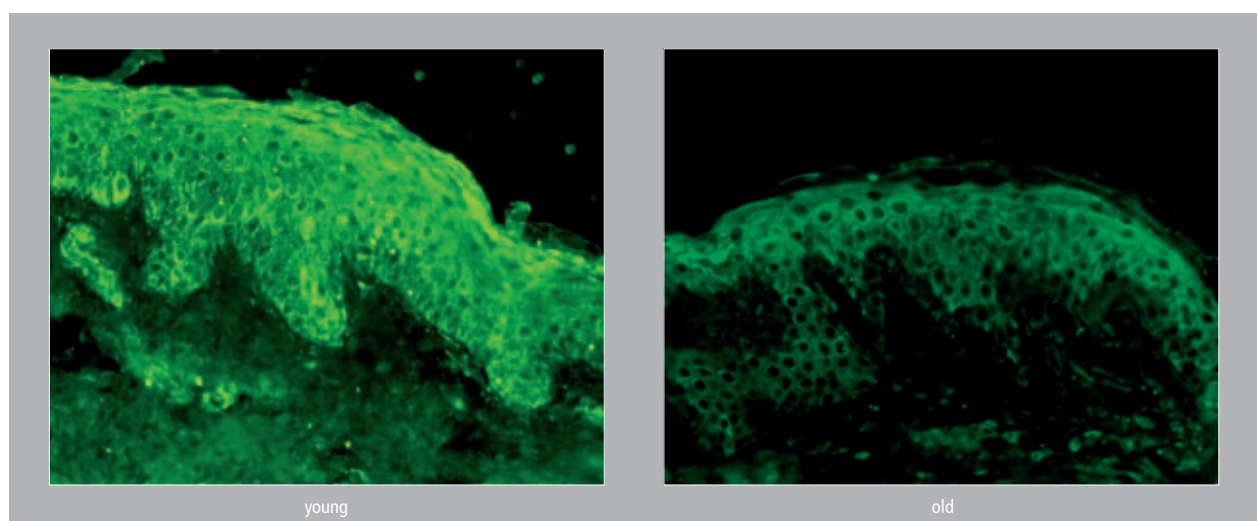


Fig. 2: Age-dependent hyaluronan concentration in human skin *ex-vivo*. Hyaluronan levels are substantially reduced in aged skin contributing to wrinkle development.

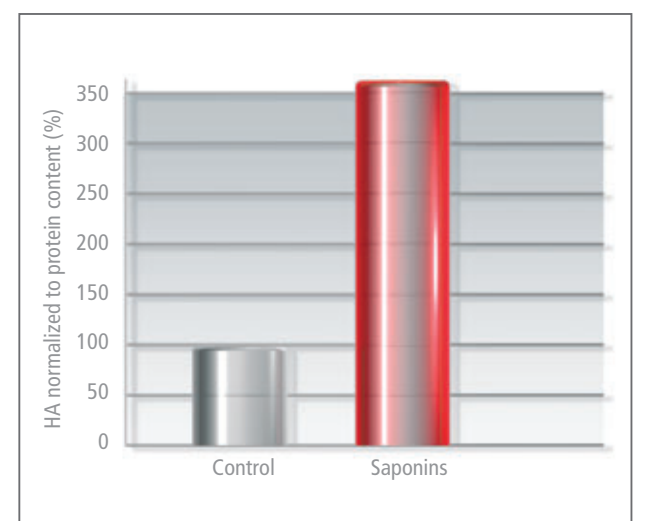


Fig. 4 Stimulation of hyaluronan synthesis by saponins.