Aline Hueber, Christine Saecker – DSM Nutritional Products, Switzerland

Formulating anti-ageing products with folic acid

If skin aged counter-clockwise when people turned 35 years, not only would consumers select their cosmetic products differently but also the whole cosmetic industry would have to offer completely different product categories. Of course this is just an attractive idea. Ageing proceeds in one direction and the skin unfortunately often shows the most obvious signs of ageing. The major telltale signs are reduced elasticity, poorer structure and appearance of wrinkles. Reduced turnover of skin cells and exposure to environmental factors (such as UV light), speeding up the process of skin ageing, are essential causes.

It is recognised that especially the more deeply penetrating UVA light triggers cutaneous photo-ageing by a pathway that starts in dermal fibroblasts with alterations of (mitochondrial) DNA.[1]

A strategy helping to slow down premature skin ageing would therefore involve stimulation of skin cells such as fibroblasts, while preventing DNA damage at the same time.

Several substances are known to help slow down premature skin ageing by antioxidant activity, by stimulating collagen synthesis or by other specific mechanisms. None of the vitamins or anti-ageing ingredients used so far in cosmetic formulations could demonstrate modulation of the DNA repair mechanism.

The B vitamins are a class of vitamins the cosmetic industry would not go without. The well-known vitamin B5 (panthenol), vitamin B3 (niacinamide) and vitamin B6 (pyridoxin HCl) have been used for years in personal care products because of their benefits for skin and hair.

Only vitamin B9 (folic acid) remained the least tapped for cosmetic applications.

Essential substance

Folic acid is a substance that consists of a pteridine core, p-amino benzoic acid and L-glutamic acid (Fig. 1). The naturally occurring forms are referred to as folate and can be found in several food sources

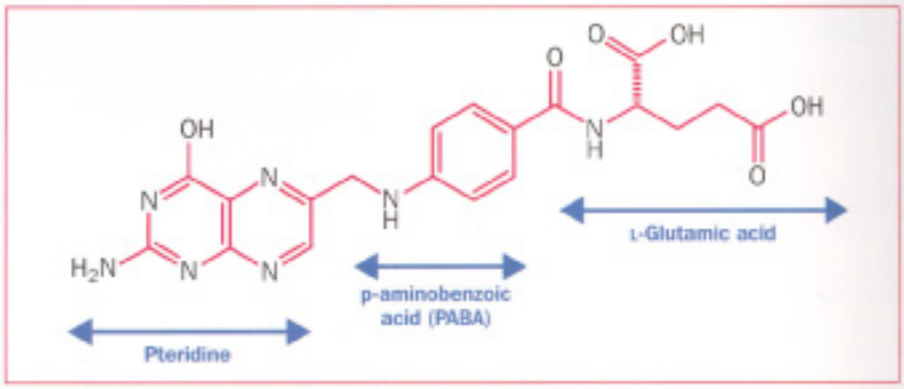

Figure 1: *Structure of folic acid (pteroylmonoglutamic acid).*

and green leaves and may contain up to 9 conjugated glutamic acid molecules. Folic acid is also known as vitamin B9, vitamin M or vitamin Bc. The commercially used pteroylmonoglutamic acid is the most stable and most bioavailable form. The name folic acid is derived from the Latin word *folium* for leaf.

Folic acid is crucial for many biochemical processes and acts as a coenzyme.

The reduced form of folic acid, tetrahydrofolate, plays a key role in the methylation cycle and in DNA biosynthesis as donor or acceptor of one-carbon (C1) groups.

Its deficiency might result in DNA damage as well as aberrant patterns of DNA methylation.[2,3] Many studies showed

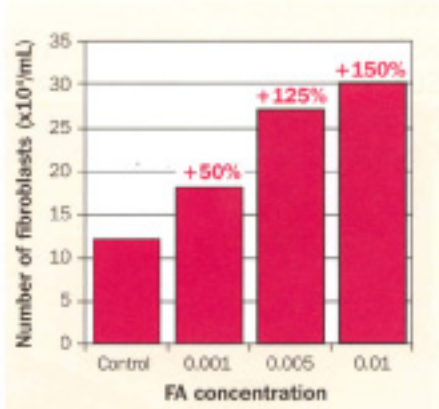

Figure 2: *In vitro study with mature human fibroblasts cultivated for 7 days without foetal calf serum and different concentration of folic acid (FA).*

that background level of DNA damage is elevated under folate deficiency.

Folic acid is absolutely required for normal development and growth during gestation. It is recognised that neural tube defects such as spina bifida and anencephaly are caused by a shortage in folate and that an adequate intake of folate can largely prevent these defects.

Folic acid has a long and successful history of application in dietary supplements and for food fortification. The fortification of cereals with folic acid even became mandatory in the USA and Canada after 1998.

Fibroblasts

Fibroblasts in ageing skin are reduced in their number as well as in their biosynthetic capacity, which consequently leads to a gradual reduction of collagen and elastin fibres synthesised in fibroblasts and their respective structural organisation. Studies with cultivated human fibroblasts showed[4] that folic acid promotes the growth of fibroblasts. Folic acid stimulated the division of both young and mature fibroblasts growing either in standard medium or under nutrient deficiency (Fig. 2). The fibroblast cells are morphologically heterogeneous depending on their location and activity. The microscopic observation of the test culture further revealed that the fibroblasts were in presence of folic acid more regular in shape (spindle-shaped), indicative of a high proliferation capacity (Fig. 3).

Apoptosis

Apoptosis can occur when a cell is damaged, infected with a virus, or undergoing stress conditions such as starvation or radiation. DNA damage from UV radiation can also induce apoptosis if no repair mechanism is in place.

A study conducted by Debowska *et al*[5] showed that 0.01% folic acid increased the viability of fibroblasts after UV exposure by 58%. The UV-protective effect on keratinocytes was even higher (Fig. 4).

The results indicate that the folic acid treatment prior to UV exposure can increase the survival rate of skin cells after exposure to UV radiation and prevents thus photo-ageing.

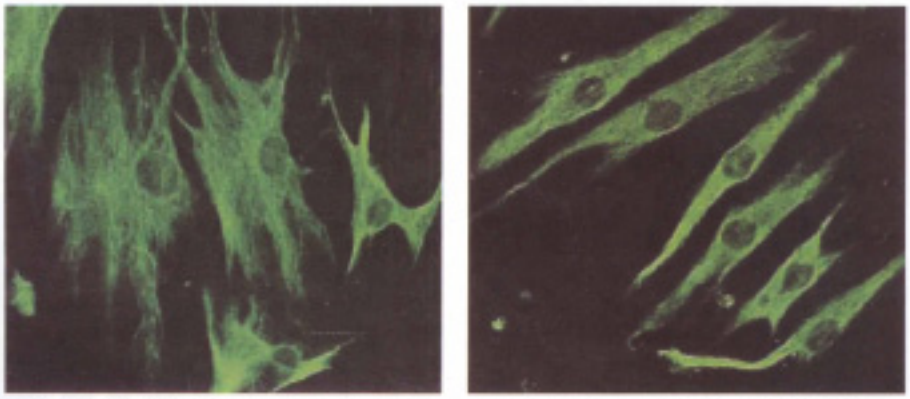

Figure 3: *Fibroblasts cytoskeleton under confocal microscopy.* **Left:** *Fibroblasts cultivated for 14 days without folic acid. The fibroblasts are broad, attached to each other and irregular in shape. Mitotic divisions only sporadically observed.* **Right:** *Fibroblasts cultivated for 14 days with 0.01% folic acid. The fibroblasts are regular and spindle-shaped with a small cytoplasma volume. High mitotic division rate.*

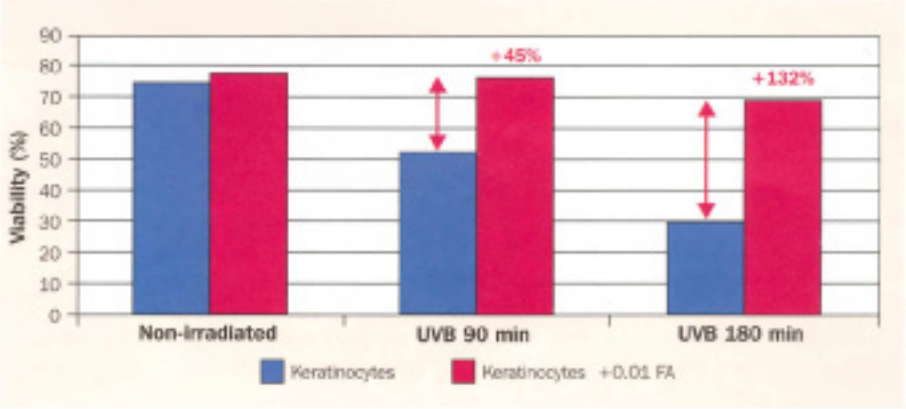

Figure 4: *Increased viability (reduced apoptosis) of keratinocytes by folic acid (FA) treatment (0.01% during 16h prior to UVB radiation). Viability was determined 90 minutes and 180 minutes after UVB exposure.*

DNA repair

For many years it has been acknowledged that skin ageing is a process comprising chronological (intrinsic) ageing superimposed by ageing triggered by environmental influences. Among all environmental factors, frequent and cumulatively prolonged solar UV radiation is the most relevant factor in premature skin ageing, referred to as photo-ageing.

Most, if not all age-accelerating environmental factors damage DNA either directly or indirectly, often through oxidation. The rate and fidelity of DNA repair is further inversely correlated with the age of cells in humans but also in other species.[6]

A skin cell that has accumulated a large amount of DNA damage, or that no longer effectively repairs damage incurred to its DNA, can undergo unregulated cell division, which finally can lead to the formation of cancerous skin cells.

Therefore studying the DNA repair mechanisms of photo-damaged DNA and positive influences by topically applied substances is a very fascinating topic as the DNA repair ability of a cell is vital to the integrity of its genome and thus to its normal functioning.

A recent study[5] suggests that folic acid treatment increases the repair rate of UV-damaged DNA by the NER (nuclear excision repair) mechanism, which involves transient DNA breaks (Fig. 5). NER is the principle pathway for repairing UV-induced lesions.[7] The study conducted by Debowska *et al* was done with primary human fibroblasts treated for 13 days with folic acid and then exposed to UV light. An alkaline comet assay was performed to visualise the DNA strand breaks. In the presence of folic acid, the comet assay shows a higher migration of DNA fragments (DNA damage). This is indicative of the NER process promoted by folic acid (Fig. 6).

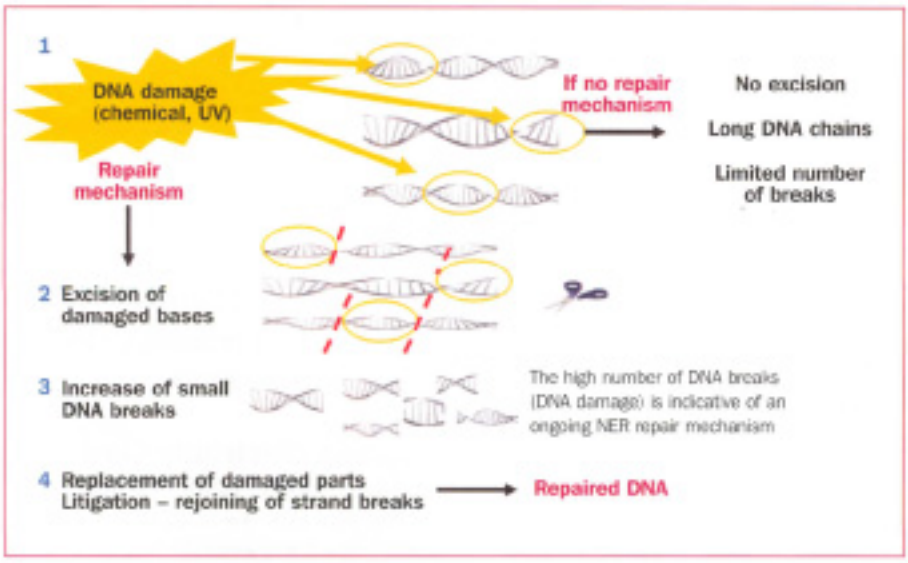

Figure 5: *Principle of the repair of DNA damage by NER mechanism.*

Dermal cells

Both topical application and cell culture tests could demonstrate that folic acid is available in dermal cells.

The uptake of folic acid is dose-dependent and is further increased under UV light. The folic acid content in dermal fibroblasts (from skin biopsies) was 185% higher in UV exposed cells.[8] The *in vivo* skin penetration was demonstrated with volunteers who had applied a cream with 0.03% folic acid for 4 weeks.[8]

Topical application of cosmetic products enriched with folic acid help to ensure a sufficient supply in the dermis.

Formulating anti-ageing products

Intrinsic ageing is partly due to reduced turnover in skin cells, whereas photoaging is partly due to a pathway triggered by UV light that damages DNA in dermal fibroblasts.

As folic acid can slow down premature skin ageing by stimulation of skin cells such as fibroblasts while repairing DNA damage at the same time it is a powerful active for cosmetic products designed to fight ageing as well as photo-ageing. The following examples highlight some product concepts with folic acid.

- **Anti-pollution cream** – day cream to protect from environmental stress including UV radiation.
- **Anti-wrinkle cream** – with regenerating and protective folic acid helping to prevent signs of ageing and photo-ageing.
- **Night eye serum** – with folic acid to boost the cell turn-over to help reducing tired-looking skin.
- **Cell activator men's facial cream** – anti-ageing day cream to keep the attractive smile.
- **After-sun treatment** – to prevent premature ageing linked to UV-induced damage.

The recommended use level of folic acid is 0.05%-0.2%. For intensive treatment up to 0.5% could be used. Detailed formulation guidelines and reference formulations with folic acid are available from DSM. PC

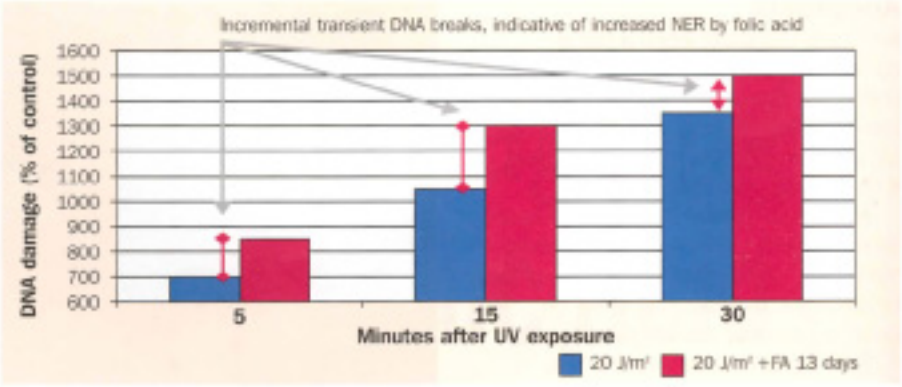

Figure 6: *DNA fragments migration associated with incomplete NER mechanism after exposure of primary human fibroblasts treated with folic acid (FA) to UV light.*

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