BACKGROUND

Microneedling has evolved into one of the most effective and promising procedures to help reduce the appearance of scar tissue and aid in overall skin rejuvenation. Using tiny needles to make micro-injuries without significant damages in the epidermis and dermis, microneedling induces tissue remodeling such as thickening of the epidermis and dermis and inducing extracellular matrix (ECM) proteins like collagen and elastin through a natural wound healing process. All this occurs with minimal inflammation, which results in the eventual remodeling of the skin in a non-fibrotic (non-scar tissue) fashion without adverse events such as post-inflammatory pigmentation issues that are common with energy or chemical based devices used for skin rejuvenation.

SkinPen® is a leading microneedling device currently approved in the UK and Canada. While the results seen when using the SkinPen® device are often quite compelling, many needling experts would agree that how you take care of the skin post-needling is just as important as the needling procedure itself to obtain the best results. To ensure proper skin care following the SkinPen® procedure, Bellus developed the Skinfuse® Protocol. This Protocol contains a line of products formulated to support the skin during and after the SkinPen® procedure.

In order to assess the potential benefit of following the Skinfuse® Protocol after the SkinPen® procedure, an animal study was conducted to explore how the skin of a healthy rat would react to the SkinPen® treatment, the Skinfuse® Protocol, and a combination of the two. Each of the 30 male Sprague Dawley rats, weighing 350–375g, age 4 months, was randomized to one of five groups.

THE RATS THAT WERE GIVEN A SKINPEN® TREATMENT WERE TREATED EITHER ONCE (GROUP C) OR THREE TIMES AT THREE WEEK INTERVALS (GROUP D). ALL RATS MICRONEEDLED WERE GIVEN TREATMENTS USING A 1MM SETTING ON THE SKINPEN®. THOSE RATS GIVEN THE SKINFUSE® PROTOCOL (GROUPS B AND E) RECEIVED THE FOLLOWING:

<table>
<thead>
<tr>
<th>PROCEDURE GROUPS</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SkinPen® Procedure</td>
<td></td>
<td></td>
<td>1x‘</td>
<td>3x†</td>
<td>3x†</td>
</tr>
<tr>
<td>Skinfuse® Protocol</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

*Needle depth set at 1mm. †Treatments at 3 week intervals.
Results

EPIDERMAL EFFECTS

Measurement of epidermal thickness in haematoxylin-eosin stained slides showed an average epidermal thickness in control group (A) of 27.62µm (n=4). All groups except group C showed a significant increase in epidermal thickness compared to the control group (Fig.1, 2). In addition, thickness of both epidermis including stratum corneum and dermis were measured. The thickness of epidermis including stratum corneum showed similar pattern as epidermis alone. All groups except group C showed an increased thickness compared to the control group (Fig.3).

FIG.1.
INCREASED EPIDERMAL THICKNESS BY MICRONEEDLING AND SKIN CARE. (A CONTROL, B SKIN CARE ONLY, C SINGLE MICRONEEDLING, D MULTIPLE MICRONEEDLING (THREE TIMES), E MULTIPLE MICRONEEDLING (THREE TIMES WITH SKIN CARE), N=4; *P<0.05, **P<0.01

FIG.2.
REPRESENTATIVE SKIN SAMPLES WITH H&E STAINING FOR EPIDERMIS. ALL PHOTOGRAPHS SHARE THE SAME SCALE (BLACK BAR: 50ΜM)

FIG.3.
INCREASED EPIDERMIS THICKNESS INCLUDING STRATUM CORNEUM BY TREATMENTS. (A CONTROL, B SKIN CARE ONLY, C SINGLE MICRONEEDLING, D MULTIPLE MICRONEEDLING (THREE TIMES), E MULTIPLE MICRONEEDLING (THREE TIMES WITH SKIN CARE), N=4; *P<0.05, **P<0.01, ***P<0.001
Results

GENE EXPRESSION PROFILE

Research has shown that microneedling does cause tightening of the skin through the remodeling process which induces collagen expression (1). Indeed, collagen I expression level was increased in treated skin compared to control skin. It was shown a single microneedling could induce collagen I induction mostly (2). We also observed group C showed the highest increase of collagen I induction. In addition, the ratio of collagen I to III was increased in all treated samples (Fig.4). It may be noted that the degree of collagen induction spikes following the first microneedling treatment and lowers with each subsequent treatment (as seen in group D). This is caused by the skin’s acclimation to the injury and way in which it is responding. For this reason, perpetual treatment of the skin with microneedling is not recommended. Following a series of treatments, the skin should be allowed to fully heal/remodel which takes anywhere from 3 months to a year. After this, the skin may again respond properly to the microneedling procedure, and another series may commence if appropriate.

FIG.4.
INCREASED COLLAGEN I EXPRESSION BY MICRONEEDLING AND SKIN CARE. (A CONTROL, B SKIN CARE ONLY, C SINGLE MICRONEEDLING, D MULTIPLE MICRONEEDLING (THREE TIMES), E MULTIPLE MICRONEEDLING (THREE TIMES) WITH SKIN CARE). RELATIVE MRNA COLLAGEN I (BLACK BAR) AND III (GREY BAR) LEVEL TO GAPDH *P<0.05 **P<0.01

Results

The dermis loses its thickness during aging process, mainly caused by changes in fibroblasts which is responsible for production of collagen and elastin. [Not only is the collagen and elastin production critical, but shape and structure of fibroblast is also critical for skin.] The modulation of dermal fibroblast shape and mechanical force by disrupting the actin cytoskeleton, reduced fibroblast spreading, which is observed in aged skin (4,5). Based on this data, we tested whether microneedling indeed could induce beta actin and elastin expression. As expected, both beta actin and elastin were increased by skin care and microneedling (Fig.5). The results were similar to those of the collagen expression.
Results

Again, one of the main benefits of microneedling is the overall minimization of inflammation. Here we tested the expression of cytokines which can regulate inflammation after a medical needling treatment. Interestingly, pro-inflammatory cytokines such as TNF-µ and IL-1µ were decreased by SkinPen® treatment alone, but not with the Skinfuse® Protocol alone. However, the combination of treatments did again provide for a synergism. Also examined was the anti-inflammatory cytokine IL-10, which was seen to increase after SkinPen® treatment and the combination of SkinPen® and Skinfuse® (Fig.6). Here, again, we observe the beginnings of acclimation of the skin to injury by the third treatment.

**FIG.5.**
GENES REGULATION BY MICRONEEDLING AND SKIN CARE. (A CONTROL, B SKIN CARE ONLY, C SINGLE MICRONEEDLING, D MULTIPLE MICRONEEDLING (THREE TIMES), E MULTIPLE MICRONEEDLING (THREE TIMES) WITH SKIN CARE); N=4; *P<0.05, **P<0.01, ***P<0.001

**FIG.6.**
GENES REGULATION BY MICRONEEDLING AND SKIN CARE. (A CONTROL, B SKIN CARE ONLY, C SINGLE MICRONEEDLING, D MULTIPLE MICRONEEDLING (THREE TIMES), E MULTIPLE MICRONEEDLING (THREE TIMES) WITH SKIN CARE); N=4; *P<0.05, **P<0.01, ***P<0.001

0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
A B C D E
tnfa
il-1a
cytokine / GAPDH mRNA

0 2 4 6 8 10
A B C D E
il-10
IL-10 / GAPDH mRNA