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Percutaneous collagen induction. Scarless skin rejuvenation: fact or fiction?

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Summary

Photoageing is generally treated by ablative procedures that injure the epidermis and basement membrane, and lead to fibrosis of the dermis. Percutaneous collagen induction (PCI) therapy is an alternative treatment for photoaged skin that does not result in clinical signs of dermal fibrosis. In this study, the immediate effects of PCI on the skin were assessed, including the systemic inflammatory response and the production and gene expression of transforming growth factor (TGF) isoforms β1, β2 and β3. Eighty rats were split into four groups: group 1 (n = 24; PCI plus skin care); group 2 (n = 24; skin care only); group 3 (n = 24; PCI only) and group 4 (n = 8; controls). Microarray analysis showed that TGF-β3, an essential marker for preventing scarring, was upregulated and expressed for 2 weeks postoperatively. PCI might offer a regenerative therapy to improve skin appearance and quality and to improve or even prevent scarring.

The primary environmental factor that causes skin ageing is UV irradiation. There are numerous methods available to tighten skin such as laser resurfacing and deep peeling.[1,2] These treatments are ablative, injuring the skin and leading to postinflammatory fibrosis of the dermis.

Transforming growth factor (TGF)-β plays a crucial role in fibrotic scar formation. Previous research focusing on the TGF family of molecules has found that TGF-β3 elicits a scar-free or regenerative healing response, whereas TGF- β1 and TGF- β2 elicit a fibrotic scarring response.[3] Those authors have shown that percutaneous collagen induction (PCI) therapy (skin needling) can be used to treat photoaged skin without the risk of skin dyspigmentation.[4,5] These studies did not provide any determination of the acute phase of PCI, including the systemic inflammatory response, or production and gene expression of transforming growth (TGF) isoforms β1, β2 and β3.

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Report

This study was reviewed and approved by the Lower Saxony District Government (Hannover, Germany). Eighty male Sprague–Dawley rats were divided into four groups: group 1 (n = 24: PCI plus skin care, Fig. 1a); group 2 (n = 24: skin care only, Fig. 1b); group 3 (n = 24 PCI only) and group 4 (n = 6: controls).

Each rat in groups 1 and 3 received a single treatment with PCI with a medical PCI instrument (Environ® Medical Roll-CIT™; Vivida SA cc, Cape Town, South Africa) on an area of 30% of total body surface area to induce percutaneous collagen. The instrument was rolled vertically and diagonally with pressure over the treated area to create as many microscopic dermal

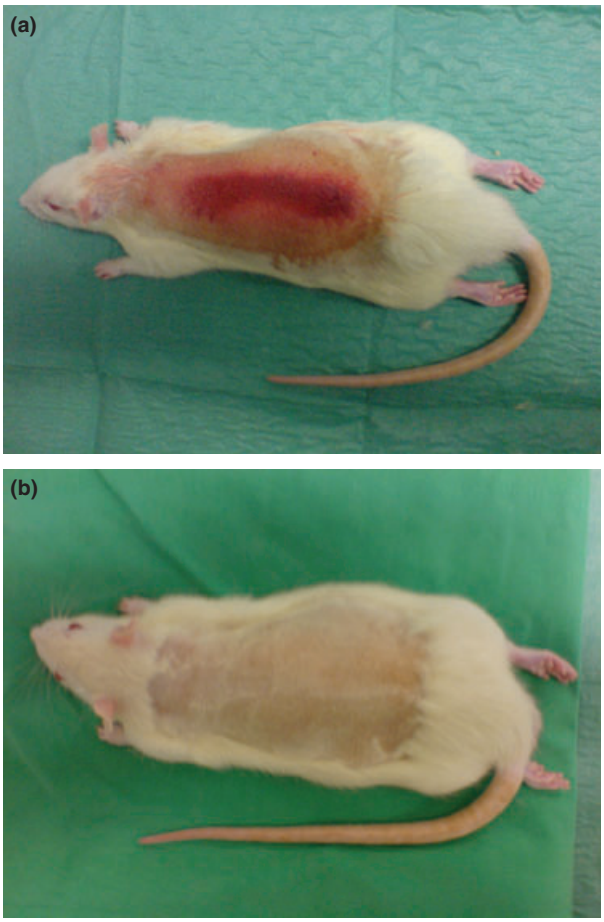

Figure 1 (a) Shaved and needled rat directly postoperative. (b) Rat with shaved and unneedled back.

wounds as possible within 10 min to achieve a confluent zone of superficial inflammation (Fig. 2).[6]

The rats in groups 1 and 2 were given skin care; they were treated with vitamin A cream (retinyl palmitate; Environ® Original) and vitamin C cream (ascorbyl tetraisopalmitate) (Environ® C-Boost) [both Environ Skin Care (Pty) Ltd, Cape Town, South Africa]. Both vitamin creams were applied topically once per day to both

Figure 2 Medical percutaneous collagen induction instrument (Roll-CIT; Vivida C.C. Renaissance Body Science Institute, Cape Town, South Africa).

maximize initial collagen production and to maintain the homeostasis between collagenesis and collagenolysis. Group C received no skin care. Group 4 served as untreated, time-matched sham rats to establish baseline levels for the study.

Using a commercial kit (Aurum Total Fatty and Fibrous Tissue Kit; Biorad, Munich, Germany), 5 μg of total RNA was isolated. During reverse transcription, RNA was converted into cDNA labelled with fluorescein (Fl) and biotin 2. Specifically bound labelled cDNAs were sequentially detected with a series of conjugate reporters, finishing with tyramide-Cy3 and tyramide-Cy5. The hybridized chips were scanned (Axon 400B™ scanner, Sunnyvale, CA, USA) six times with different settings. Primary image analysis was performed using the software tool Gene Pix Pro™ (version 6.0; Gene Pix, Sunnyvale, CA, USA).

The averaged values for each gene of gene expression analysis comprised nine replicates. Outliers were eliminated by the Nalimov outlier test. The independent Student's *t*-test was used to determine the significance of the differences between the staining patterns and the

Table 1 Comparison of needled rats against control animals at different time points.

	2 weeks				4 weeks				8 weeks			
Gene ID	Regulation	Ratio	SEM	*P*	Regulation	Ratio	SEM	*P*	Regulation	Ratio	SEM	*P*
TGF-β 1	0	1.16	1.62	0.271	0	1.36	0.81	0.180	1	−2.43	0.56	0.005
TGF-β 2	1	11.31	2.26	< 0.00001	1	1.97	0.71	0.001	1	−1.41	2.49	0.010
TGF-β 3	1	13.75	1.67	0.004	1	4.37	0.89	0.002	0	2.35	4.61	0.111

Gene regulation was calculated by *t*-test (*P*-value = 0.05): 0 means unregulated when comparing the relevant time point with untreated control rats. 1 means that the gene is regulated. The ratio gives the amount of regulation. The last column shows the standard error. If genes could not be detected because they were not expressed, all values are given as zero.

values for histological measurements in needled and untreated skin. All *P*-values were two-tailed, and $P \leq 0.05$ was considered significant. Summary data are expressed as the mean ± SEM.[7]

We have previously shown that PCI combined with skin care is a safe method for treating wrinkles. It was shown that 'PCI only' leads to an increase of collagen I in the dermis but PCI plus skin care maximizes these results.[4,5] The necessity for using vitamin A and C with PCI has been well described by Aust *et al.*[4,5]

We performed DNA microarray experiments to identify regulated genes in treated and untreated rat skin. The microarray used comprised 15 genes coding for cytokines and extracellular matrix proteins. We found upregulation of all three TGF-β genes 2 weeks after PCI and significant downregulation 8 weeks after PCI. Upregulation of TGF-β1 was low, but TGF-β2 and TGF-β3 were significantly upregulated. TGF-β1 and TGF-β2 were expressed after 2 weeks in treated animals, but only faint expression remained after 4 weeks. TGF-β3 remained upregulated after 8 weeks in treated animals compared with controls. TGF-β2 was significantly downregulated 8 weeks after PCI (Table 1). The dataset is available on the Platform ID GPL5462 in the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/).

Ageing skin is commonly rejuvenated by ablative procedures that injure the epidermis and its basal membrane, and lead to dermal fibrosis. Dermal scarring after injury may have adverse effects such as loss of function or restriction of movement.[8] In previous studies,[3,6,8] healing adult wounds were manipulated to mimic the scar-free embryonic profile, e.g. neutralizing TGF β1 and TGF-β2 or adding exogenous TGF-β3. These experiments resulted in scar-free wound healing in the adult. Such experimental approaches indicate that TGF-β3 may be a particularly important molecule for healing with improved scarring.

In the present study, we hypothesized that skin could be regenerated by PCI without scar formation. We found an initial upregulation of TGF-β1 and TGF-β2 at 4 weeks after treatment, followed by strong downregulation at 8 weeks after treatment. There was also strong upregulation of TGF-β3 at 2 weeks after PCI, without any downregulation at 4 and 8 weeks postoperatively.

Based on these results, we suggest that PCI offers a novel antiageing method to improve both skin appearance and quality by lessening or preventing scarring. PCI might be a useful treatment to alter the ratio of TGF-β isoforms in healing adult human wounds. PCI increased TGF-β3 levels for a period of 2 weeks after PCI while simultaneously reducing the levels of TGF-β1 and TGF-β2 until weeks 4 and 8 after PCI in this animal study.

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