

Investigation of the mechanism of action of nonablative pulsed-dye laser therapy in photorejuvenation and inflammatory acne vulgaris

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Summary

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Key words

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Conflicts of interest

A.C.C. acts as a consultant for the device manufacturers. The other authors have no conflicts of interest.

Background Nonablative lasers are widely used for treatment of wrinkles, atrophic scars and acne. These lasers stimulate dermal remodelling and collagen production, but the early molecular stimulus for this is unknown. The mechanism of nonablative lasers in inflammatory acne is variously suggested to be damage either to sebaceous glands or to *Propionibacterium acnes*. Their effects on cytokine production are unknown.

Objectives To assess the *in vivo* effects of a short pulse duration nonablative pulsed-dye laser (NA-PDL) previously used for photorejuvenation and treatment of acne, on cytokine production, *P. acnes* colonization density and sebum excretion rate (SER).

Methods We examined the effect of NA-PDL (NliteV[®]; Chromogenex Light Technologies, Llanelli, U.K.) on *P. acnes* colonization before and after laser therapy using a scrub-wash technique and culture at 0 and 24 h ($n = 15$), on SER using absorptive tape at 0, 2, 4, 8 and 12 weeks ($n = 19$) and on cytokine mRNA using reverse transcription–polymerase chain reaction from skin biopsies at 0, 3 and 24 h ($n = 8$).

Results NA-PDL had no effect on *P. acnes* or SER. Transforming growth factor (TGF)- $\beta 1$ mRNA increased fivefold after 24 h and 15-fold in two subjects ($P = 0.012$).

Conclusions TGF- β is known to be a potent stimulus for neocollagenesis and a pivotal immunosuppressive cytokine which promotes inflammation resolution. Its upregulation by NA-PDL provides a possible unifying molecular mechanism linking stimulation of dermal remodelling in photorejuvenation with inhibition of inflammation in acne. Damage to *P. acnes* or sebaceous glands cannot explain the effect of this device in acne.

In the last decade nonablative laser therapies have been used increasingly for the aesthetic treatment of fine wrinkles, photoaged skin and scars, a process known as photorejuvenation.^{1,2} More recently they have also been used for inflammatory acne.^{3–8} In comparison with resurfacing lasers, nonablative lasers cause a relatively modest photothermal injury to the dermis, produce fewer side-effects and are considerably less inconvenient for patients.² Short pulse duration nonablative pulsed-dye lasers (NA-PDLs) produce light at a wavelength that is absorbed by haemoglobin, causing a photothermal injury to the dermis that induces a wound-healing response without causing vessel rupture. This process increases cutaneous collagen production and induces dermal remodelling.¹ The initial cytokine stimulus for dermal remodelling

and neocollagenesis in nonablative laser photorejuvenation is unknown.

Recently there has been renewed interest in physical treatments for acne. The beneficial effects of nonablative laser therapy for acne were probably first noticed in patients who received treatment for other indications and experienced coincidental improvement. In the last 4 years, several formal clinical studies of nonablative laser and light therapies for acne have been published, although most of these are observational and open in design, and some are conflicting.^{3–12} Nevertheless, the global use of these treatments is increasing and various nonablative laser and light devices have now received regulatory approval for acne treatment. We previously studied the effect of a single treatment with an NA-PDL on mild to

moderate facial acne in a double-blind randomized study, in which laser treatment was compared with a sham treatment.¹¹ Durable improvements were observed in the laser-treated group: 12 weeks after therapy there was a 53% fall in total lesion count in laser-treated patients compared with a 9% fall in sham-treated patients.

The mechanism of action of laser therapy in acne is unknown but has been proposed to be secondary to damage of either *Propionibacterium acnes* or the sebaceous gland itself.^{3,6,9,10} In this study, we investigated the mechanism of action of a short pulse duration NA-PDL that has been used widely for photorejuvenation and inflammatory acne, and report, for the first time, the *in vivo* effects of nonablative laser therapy on cutaneous *P. acnes* density, sebum excretion rate (SER) and immediate dermal cytokine expression.

Materials and methods

Device parameters

In all experiments, we used the NliteV[®] laser (Chromogenex Light Technologies, Llanelli, U.K.), a short pulse NA-PDL with the following characteristics: wavelength 585 nm, pulse duration 350 μ s, spot diameter 7 mm, mean \pm SD pulse-to-pulse fluence variation over 100 shots $2 \pm 1.5\%$. This device has been used for the treatment of wrinkles, atrophic acne scars and inflammatory acne.^{7,13–16}

Subjects and intervention

All subjects were healthy adult volunteers aged 18–55 years and were recruited at our institution (Hammersmith Hospital, Imperial College, London). For studies of the effect of laser treatment on cutaneous cytokine expression, a rectangular area of one buttock measuring 10 x 20 cm was treated using a single pass, with adjacent pulses and a subpurpuric fluence (2.0 J cm^{-2}). For studies of the effects of laser treatment on *P. acnes* colonization density and SER, the whole forehead was similarly treated (subpurpuric fluence at this site was 2.5 J cm^{-2}). All subjects gave informed consent before enrolment and our studies received prior approval from our institution's ethics committee. For practical reasons, the subjects involved in this study were asked to participate in only one type of experiment (assessment of effect on *P. acnes*, SER or cytokine expression), so that the cohort of subjects in each study was different.

Assessment of *Propionibacterium acnes* colonization density

To assess the effect of laser treatment on *P. acnes* colonization density, an established scrub-wash technique was used: an autoclaved metal ring was placed on each subject's forehead and 2 mL of sterile saline containing 0.1% Tween[®] 80 detergent (Sigma-Aldrich, Gillingham, U.K.) was applied. Skin was scrubbed with a flat-ended Teflon[®] (Dupont, Wilmington,

DE, U.S.A.) rod for 1 min to suspend surface microorganisms and the procedure was repeated. Sample fluid was serially diluted, and 20 μ L drop-plated on to Columbia agar with horse blood (Oxoid, Basingstoke, U.K.) and incubated anaerobically at 36.9 °C for 7 days, when *P. acnes* colonies were identified and counted. All experiments were done in duplicate. Sampling was done before and 24 h after laser treatment, from adjacent areas of the forehead. Later sampling was not attempted because *P. acnes* repopulates rapidly.¹⁷

Assessment of sebum excretion rate

We measured SER with a purposely designed lipid absorbent tape (Sebutape[®]; Cuderm, Dallas, TX, U.S.A.) which becomes transparent when lipid is absorbed.^{18,19} Residual lipid was removed from forehead skin with acetone and tape applied to the central forehead for 1 h. After the tape was removed, it was digitally photographed under standardized lighting and photographic conditions. Computerized photometric analysis of this digital image was used to measure the area of tape that contained lipid, the volume of lipid absorbed being calculated using the known tape thickness. We measured SER before and 2, 4, 8 and 12 weeks after laser therapy in patients with mild to moderate acne on no other treatment. These time points correspond to the time course of improvement of acne that we observed in our previous clinical study.⁷

Assessment of mRNA expression

Finally, we assessed effect of laser treatment on immediate production of selected inflammatory cytokine and receptor mRNA species. Punch biopsies (4-mm diameter) were obtained from the buttocks of volunteers, before laser treatment, and 3 and 24 h afterwards. To minimize the possible effects of previous biopsy sites on cytokine expression in subsequent biopsy sites, the control (nonlaser) biopsy was performed from the left buttock, whereas 3- and 24-h biopsies were performed on the right buttock and were 15 cm apart. Samples were homogenized and RNA purified by phenol-chloroform extraction using the RNeasy[®] kit method (Qiagen, Crawley, U.K.). cDNA was synthesized by adding the following reagents: 4 μ L of 5 \times first strand reverse transcriptase buffer, 2 μ L 0.1 mol L⁻¹ dithiothreitol, 2 μ L deoxyribonucleoside triphosphate (dNTP) DNA polymerization mix, 1 μ L random primers pd(N)6, 1 μ g sample RNA dissolved in 12.5 μ L double-distilled water (Promega, Southampton, U.K.) and 0.5 μ L reverse transcriptase (Invitrogen Life Technologies Reagent System[®], Paisley, U.K.). Reverse transcriptase-polymerase chain reaction (PCR) amplification was performed with oligonucleotide primers for the following molecules: interleukin (IL)-1 α , IL-1 α receptor antagonist (IL-1 α RA), tumour necrosis factor (TNF)- α , transforming growth factor (TGF)- β 1, melanocortin-1 receptor (MCR-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (see Table 1). A typical PCR reaction mixture contained the following reagents per 1 μ L of cDNA template: 2.5 μ L 10 \times magnesium-free buffer, 0.75 μ L

Species	Probe sequence	Product size (bp)	Annealing conditions
TGF- β 1	Forward: 5'-CCAGATCCTGTCCAAGCTG-3' Reverse: 5'-CCCTCAATTTCCCTCCA-3'	495	58 °C, 30 s
TNF- α	Forward: 5'-GCCCAATCCCTTATTACC-3' Reverse: 5'-ATACCCCGGTCTCCCAAATA-3'	492	57 °C, 30 s
IL-1 α	Forward: 5'-GTAAGCTATGGCCCACTCCA-3' Reverse: 5'-ACCCAGTAGTCTTGCTTTGTGCG-3'	644	60 °C, 30 s
IL-1 α RA	Forward: 5'-TGAAGAAGGAGGTGGAGGAG-3' Reverse: 5'-CCTTCGTCAGGCATATTGGT-3'	472	57 °C, 30 s
MCR-1	Forward: 5'-GCCAACCTCAGGCTCCTAAA-3' Reverse: 5'-AGTGCCCACTGTGAGCCTTC-3'	356	57 °C, 30 s
GAPDH	Forward: 5'-TGAGAACGGGAAGCTTGTC-3' Reverse: 5'-CAGTCTTCTGGGAGGCAGTG-3'	378	57 °C, 30 s

TGF- β 1, transforming growth factor- β 1; TNF- α , tumour necrosis factor- α ; IL-1 α , interleukin-1 α ; IL-1 α RA, IL-1 α receptor antagonist; MCR-1, melanocortin-1 receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 1 Probe sequences and reaction conditions for polymerase chain reaction. Conditions for denaturing and elongation were 94 °C for 60 s and 72 °C for 60 s, respectively

50 nmol L⁻¹ magnesium chloride solution, 1 μ L dNTP DNA polymerization mix, 1 μ L forward primer (10 pmol), 1 μ L reverse primer (10 pmol), 17.5 μ L double-distilled water (Promega) and 0.25 μ L Taq DNA polymerase (Invitrogen). The optimal PCR temperature cycling programme depended on the template and primers used. A typical cycle example is as follows: 94 °C for 60 s, 57 °C for 30 s, then 72 °C for 60 s. Completed samples were run on ethidium bromide/1% agarose gel. Bands were identified, quantified photofluorometrically and normalized to GAPDH (a housekeeping gene) to correct for loading error.

Statistical analysis

Parametric data are expressed as mean \pm SEM and nonparametric data as median with interquartile range in parentheses. Paired statistical analysis was done using t-tests for parametric data and Wilcoxon signed ranks tests for nonparametric data. Analysis of SER was performed by intention-to-treat, carrying forward last available values in the event of missing data. Per protocol analysis was also performed.

Results

Effect of laser therapy on *Propionibacterium acnes* colonization density

Propionibacterium acnes was isolated, cultured and identified in samples obtained from 15 of 19 subjects enrolled into this part of the study. Subject demographics were as follows: two men, 13 women, median age 37 (28–46) years. Mean \pm SEM *P. acnes* colonization density was 4.81 \pm 1.35 log₁₀ colony-forming units (c.f.u.) cm⁻² before laser therapy and 4.96 \pm 1.46 log₁₀ c.f.u. cm⁻² at 24 h after laser therapy. There was no significant decrease in *P. acnes* counts after laser therapy ($P = 0.780$).

Effect of laser on sebum excretion rate

SER was measured in 19 subjects immediately before laser therapy and was repeated at least once in 15. Subject demographics were as follows: six men, 13 women, median age 26 (24–33) years. Results were analysed on an intention-to-treat basis, by carrying forward last available values in an attempt to eliminate possible bias associated with nonattendance. Mean \pm SEM SER (expressed as nL cm⁻² h⁻¹) before laser therapy was 114.0 \pm 16.1. This is in keeping with other studies of SER measured using this technique. SER after laser therapy was 114.9 \pm 16.6 at 2 weeks, 128.6 \pm 17.6 at 4 weeks, 120.1 \pm 17.7 at 8 weeks and 118.1 \pm 18.0 at 12 weeks (see Fig. 1). Per protocol analysis yielded similar results. There was no significant change in sebum production following laser therapy ($P = 0.702$ baseline: 12 weeks).

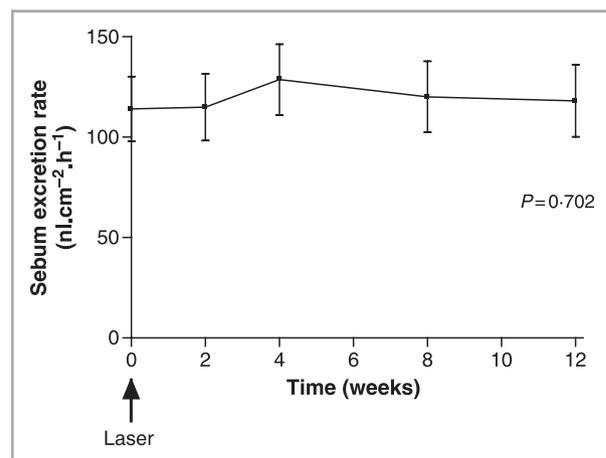


Fig 1. Effect of laser on sebum excretion rate measured by tape absorption of lipid (intention-to-treat analysis). Results are parametric and are expressed as mean \pm SEM.

Table 2 Effect of laser therapy on mRNA expression

mRNA species	Relative abundance at 3 h after laser therapy (% of baseline)	P-value ^a	Relative abundance at 24 h after laser therapy (% of baseline)	P-value ^a
TGF- β 1	152.4 (103.9–603.5)	0.093	510.1 (328.7–1542.7)	0.012
TNF- α	62.5 (52.8–155.9)	0.575	99.7 (70.7–143.0)	0.889
IL-1 α	173.8 (68.7–479.0)	0.753	136.4 (100.2–151.2)	0.249
MCR-1	61.0 (37.1–157.4)	0.176	91.4 (48.0–222.0)	0.575
IL-1 α RA	79.7 (42.4–505.0)	0.327	100.8 (52.2–791.1)	0.889

TGF- β 1, transforming growth factor- β 1; TNF- α , tumour necrosis factor- α ; IL-1 α , interleukin-1 α ; MCR-1, melanocortin-1 receptor; IL-1 α RA, IL-1 α receptor antagonist. Results are nonparametric and are expressed as median (interquartile range, IQR) percentage change from baseline. ^aStatistical tests of the null hypothesis that there is no change from baseline, performed on absolute values. n = 8: five men and three women, median (IQR) age 30 (26–39) years.

Effect of laser on cytokine mRNA production

Quantification of cytokine mRNA was performed in biopsies from eight patients (Table 2). The species of interest were identified in all samples. There was no significant change in levels of mRNA for IL-1 α , IL-1 α RA, TNF- α or MCR-1. However, levels of TGF- β 1 mRNA increased dramatically and rapidly: 24 h after laser therapy, TGF- β 1 mRNA increased to 510.1% of baseline ($P = 0.012$). A trend towards an increase in levels of TGF- β 1 mRNA (152.4% of baseline) was apparent as early as 3 h after laser therapy ($P = 0.093$) (Fig. 2). Levels of TGF- β 1 increased in all subjects following laser therapy and were 15 times greater than baseline levels in two of eight subjects at 24 h.

Discussion

Many different nonablative laser and lamp systems are used for photorejuvenation or for treatment of acne (see Table 3).

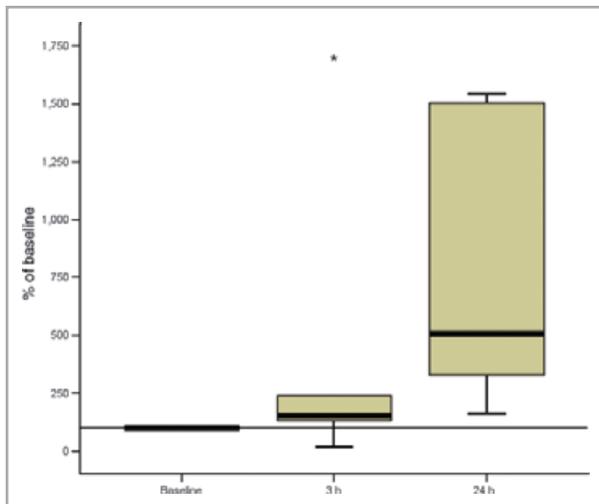


Fig 2. Boxplot showing the effect of laser therapy on transforming growth factor- β 1 mRNA concentration in human skin *in vivo*. Results are expressed as percentage of baseline, showing medians, interquartile range, outliers and extreme outlier (*). n = 8; $P = 0.093$ (3 h), $P = 0.012$ (24 h).

The process of dermal remodelling following NA-PDL laser treatment has previously been investigated at an ultrastructural level: several weeks after treatment, reorganization and increased linearity of collagen and elastin occurs, and fibroblast proliferation increases.^{1,20} The cytokines influencing this dermal remodelling presumably derive from laser-induced cellular infiltrates: a mixed monocyte, neutrophil, lymphocyte and mast cell infiltrate is observed 3 days after treatment, and a prominent mast cell and lymphocytic infiltrate occurs between 2 and 5 weeks.²⁰ Previous molecular studies of the effects of nonablative lasers have been restricted largely to the delayed measurement of collagen and related peptides: NA-PDLs increase procollagen I and III mRNA after 1 week and type III collagen protein (by 84%) after 72 h in human skin and stimulate production of collagens I and III in mice.^{14,21,22} However, the molecular stimulus for dermal remodelling after nonablative laser treatment is unknown.

The pathogenesis of acne involves not only changes in sebum excretion and *P. acnes* colonization, but also alteration of cytokine expression to initiate and maintain both the microcomedo and the inflammatory acne lesion: the development of the microcomedo, the precursor acne lesion, is stimulated by expression of IL-1 α and inhibited by IL-1 α RA.²³ The early inflammatory papule comprises an infiltrate of CD4+ lymphocytes expressing Th1-type cytokines.²⁴ In addition, *P. acnes* itself induces production of an array of proinflammatory cytokines by direct interaction with keratinocytes and monocytes.²⁵

We chose to investigate IL-1 α and IL-1 α RA because of their effects on comedogenesis.^{23,26} We also assessed TNF- α , an important proinflammatory cytokine and a stimulator of fibroblast proliferation, and MCR-1, which is expressed on the surface of healthy human sebocytes and is upregulated following exposure of skin to ultraviolet (UV) radiation.^{27,28} No alteration in expression of any of these species was seen after laser treatment.

In contrast, there was a rapid increase in TGF- β 1 transcripts in all subjects after NA-PDL therapy, amounting to a median fivefold increase after 24 h, with a 15-fold increase occurring in two subjects. There was a trend towards upregulation as

Light source	Photorejuvenation	Acne
532-nm KTP laser	+ ⁴¹	+ ⁸
585-nm PDL (short pulse 350 µs)	+ ^{13-16,42}	+ ⁷
595-nm PDL	+ ^{43,44}	+ ^{11,45}
810-nm diode laser (indocyanine green pretreatment)	+ ⁴⁶	+ ^{4,5}
980-nm diode laser	+ ⁴⁷	—
1064-nm Nd:YAG laser	+ ^{48,49}	—
1320-nm Nd:YAG laser	+ ⁵⁰⁻⁵²	—
1450-nm diode laser (cryogen spray cooled)	+ ^{43,53,54}	+ ^{3,6,11}
1540-nm erbium:glass laser	+ ^{55,56}	—
Intense pulsed light	+ ⁵⁷⁻⁶⁰	—
Blue light	—	+ ⁶¹⁻⁶³
Red-blue light	—	+ ⁶⁴

KTP, potassium titanyl phosphate; PDL, pulsed-dye laser; Nd:YAG, neodymium:yttrium aluminium garnet. + indicates a published study reporting efficacy.

Table 3 Nonablative laser and light devices used for photorejuvenation (e.g. treatment of wrinkles or atrophic scars) and inflammatory acne

Table 4 Biological effects of transforming growth factor- β

Stimulation of fibroblast proliferation
Stimulation of collagen synthesis
Stimulation of proteoglycan, glycosaminoglycan and fibronectin synthesis
Inhibition of metalloproteinases
Chemotaxis of lymphocytes, neutrophils and monocytes in resting state tissue
Potent inhibition of activated T and B lymphocytes
Inhibition of Th1 and Th2 cytokine production
Conversion of activated CD4+ lymphocytes to CD25+ regulatory cells
Suppression of inflammatory cytokine release during apoptosis and phagocytosis
Very potent inhibition of keratinocyte proliferation
Deactivation of macrophages
Inhibition of antibody synthesis
Suppression of cytotoxic T-cell activity

early as 3 h after laser therapy, although this did not reach statistical significance.

TGF- β plays a central role in the initiation of wound healing²⁹⁻³¹ (see Table 4). It stimulates fibroblast proliferation and dramatically increases the production of collagen, proteoglycans, glycosaminoglycans and fibronectin, while inhibiting degradation of these matrix proteins by reducing metalloproteinase synthesis.²⁹⁻³¹

In addition, TGF- β is a pivotal immunosuppressive cytokine that is involved in the resolution of inflammation (see Table 4). Its complex effects depend on the context in which it is produced: in resting state tissue, such as normal skin, TGF- β acts as a chemotactic factor for neutrophils, lymphocytes and monocytes.^{30,32,33} Its upregulation by this NA-PDL therefore explains previous findings of an infiltrate within 3 days of this therapy, that precedes neocollagenesis and fibroplasia.²⁰ The effects of TGF- β in inflamed tissue are contrastingly different: TGF- β is the most potent immunosuppressive molecule yet known and is a uniquely

important limiter of inflammation and modifier of innate and adapted immunity.^{30,33} Its effects include strong suppression of IL-2-mediated T- and B-lymphocyte proliferation, deactivation of macrophages, modulation of immunoglobulin synthesis and inhibition of cytotoxic natural killer cell activity.^{30,34} In addition, TGF- β is the most potent known inhibitor of keratinocyte proliferation, causing practical growth arrest.³⁵

TGF- β is also intimately involved in the development of immune tolerance, being produced by regulatory T cells, is suppressive of Th1 and Th2 immune responses and is able to convert CD4+ T lymphocytes to CD25+ regulatory T cells.^{30,32,33} It also inhibits release of proinflammatory cytokines during apoptosis and phagocytosis of inflammatory cells, thereby speeding up the resolution of established inflammation, and avoiding the proinflammatory stimuli that occur in necrotic cell death.³⁰ It was recently reported that this NA-PDL therapy caused reactivation of herpes simplex virus (HSV) infection in a patient with acne.³⁶ Interestingly, TGF- β production is known to be induced by HSV-1 itself during reactivation, and is thought to reduce host immune response to the virus.³⁷ The upregulation of this immunosuppressive molecule by laser treatment might therefore explain HSV reactivation in susceptible patients.

We suggest that marked and rapid upregulation of TGF- β provides a possible unifying molecular mechanism for this laser therapy in photorejuvenation and inflammatory acne, by linking stimulation of neocollagenesis with powerful inhibition of inflammation and keratinocyte proliferation, and possibly induction of immune tolerance. TGF- β would be expected to inhibit the CD4+ T lymphocyte-mediated inflammation that occurs in early acne lesions, and to speed the resolution of established lesions that contain a mixed infiltrate. TGF- β -induced keratinocyte growth arrest might also be expected to interfere with microcomedo formation, which occurs as a result of keratinocyte hyperproliferation at the pilosebaceous acroinfundibulum.

The mechanism of action of other nonablative lasers in acne has been suggested to be due to damage either to *P. acnes* or to

the sebaceous gland.^{5,6,9,10} *Propionibacterium acnes* contains small amounts of endogenous porphyrins, mostly coproporphyrin III and uroporphyrin III.³⁸ Photodestruction of *P. acnes* occurs most effectively at blue and near-UV wavelengths, which correspond to the porphyrin absorption maxima (approximately 415 nm).^{39,40} In this study we demonstrated that this 585-nm laser does not reduce *P. acnes* colonization 24 h after a single treatment of the face. We suggest that damage to *P. acnes* is unlikely to account for the mechanism of laser therapy in acne because even if it were completely eradicated from the face following treatment, this near-ubiquitous organism would repopulate very rapidly from other body sites, so that the observed prolonged therapeutic responses would be difficult to explain.¹⁷

Sebum excretion was similarly unaltered at any time point in a 12-week period after laser therapy, suggesting that alteration of sebum excretion is not an important mechanism of this laser. A previous study of the 1450-nm diode laser, which is also used for acne treatment, identified histological evidence of damage to sebaceous glands in a rabbit ear model, and in a solitary human sebaceous gland, isolated from one biopsy.⁶ However, undamaged glands were seen in repeat biopsies at 7 days, and quantitative assessment of the effect of laser therapy on sebum excretion was not done.

Our study has a number of limitations: firstly, we examined the effect of a specific short pulse duration NA-PDL. Many different nonablative laser and light devices are currently used for photorejuvenation or the treatment of acne, and many produce similar long-term clinical and histological effects.¹ Nevertheless, our results cannot necessarily be extrapolated until similar studies have been performed with these other devices. Secondly, our study is relatively small in scale, so that some effects of treatment may have been missed. This is particularly the case in assessment of microbial colonization, which varies between individuals by several orders of magnitude. Thirdly, we have made no attempt at this stage to correlate our findings with clinical outcomes. Fourthly, biopsies were taken from an area not normally involved in acne, and importantly we have assumed that changes in cytokine expression following laser treatment of buttock skin reflect the changes seen at other sites. Site-specific differences in clinical response to laser therapies do occur but, to the best of our knowledge, there are no published data to indicate that cytokine responses following laser irradiation of the skin vary between different skin sites. Other studies examining ultrastructural or molecular changes following nonablative laser have also made this assumption.^{20,22} Finally, we have assumed that cytokine expression in the biopsy specimen obtained after 24 h would not be significantly affected by the 3-h biopsy which was 15 cm distant.

In summary, we have shown firstly, that TGF- β is an early marker of photothermal damage following NA-PDL therapy and probably triggers the early inflammatory infiltrate seen after treatment of normal skin. TGF- β could prove a useful experimental molecular signal of nonablative laser interaction with tissue, because it increases very much earlier than standard research endpoints such as collagen production or clinical

scar improvement. Secondly, the upregulation of TGF- β , a key trigger for neocollagenesis, provides, for the first time, an early molecular link between nonablative laser therapy and dermal remodelling. Finally, the concept that nonablative laser therapy modulates acne by damaging sebaceous glands or *P. acnes* may be an oversimplification. The induction of a highly potent immunosuppressive molecule by this laser provides a possible mechanism for nonablative laser therapy in acne, a disease initiated, influenced and maintained by inflammatory cells and cytokines.

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