10 Understanding the Biological Effects of Ablative and Non-Ablative Laser Systems in the Skin as Key to Avoiding Complications

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10.1 Biological Effects of Laser Treatment on the Human Skin

Ablative CO_2 Laser

The CO_2 laser has been increasingly used in dermatological surgery in recent decades and has become the gold standard in the treatment of a large number of skin changes. The development of the fractionated application has led to further aesthetic dermatological indications for CO_2 laser and also offers the possibility of using it to rejuvenate light-aged skin and to revise scars. The ablative therapy of the skin by the fractionated CO_2 laser leads, depending on the amount of energy used, to an erosion of the epidermis and parts of the upper dermis. Controlled thermal treatment of the skin induces wound healing stimulation, which in turn leads to the remodeling of the corium and reepithelialization of the epidermis.

Fractional CO_2 laser systems have been successfully used to treat atrophic acne scars and in burn scars, skin rejuvenation, and laser-assisted drug delivery (LADD). The ablative CO_2 laser treatment can also reduce the risk of non-melanocytic skin cancer after UV exposure. The first clinical investigations dealt with whether laser treatment leads to molecular changes in the human skin. Here, there was evidence of the resulting regulation of the expression of heat shock proteins and molecules associated with the extracellular matrix and collagen network. In these studies, however, mostly nonstandardized patient biopsies were examined using different techniques. The biopsies were always performed at the same time. Time-dependent effects of laser treatment on human skin have therefore not been fully elucidated so far.

Therefore, in one of our investigations organotypic human 3D skin models were treated with a fractionated, nonsequential, ultra-pulsed CO_2 laser with different energy doses. In order to assess the morphological changes, histological examinations were subsequently performed at various points in time and microarray, and qRT-PCR analyses were performed to investigate the molecular effects of the laser treatment (Fig. 10.1). This showed that the fractionated CO_2 laser treatment in the skin models led to dose-dependent morphological changes and an almost complete restoration of the epidermis 5 days after irradiation. On day 5 after laser treatment with an absorbed dose of 100 mJ/cm^2, microarray analysis showed upregulation of genes associated with tissue remodeling and wound healing (e.g.,

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G. Kautz (ed.), *Energy for the Skin*, https://doi.org/10.1007/978-3-030-90680-1_10

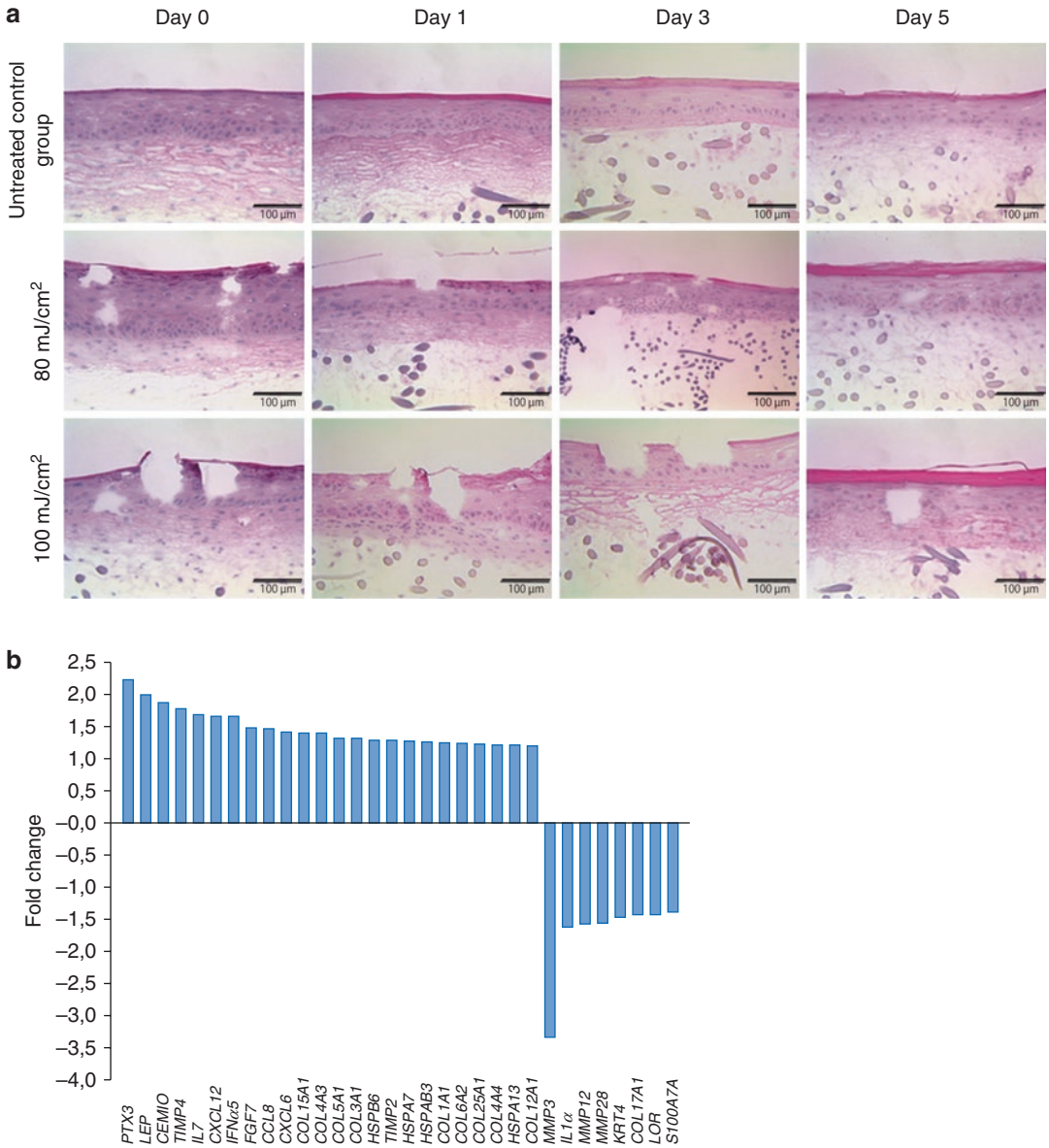

Fig. 10.1 (**a**, **b**) Molecular effects of CO_2-laser in a 3D skin model. (**a**) Histological sections of the laser-treated model show the wound healing process over a period of 5 days. (**b**) Chip-based gene expression analysis shows up- and downregulation of differentially expressed genes on day 5 after laser treatment with 100 mJ/cm^2

COL12A1 and FGF7) as well as proinflammatory genes involved in immune response (e.g., CXCL12 and CCL8) and proteins of the heat shock family (e.g., HSPB3). In addition, the regulation of mRNA expression of matrix metalloproteinases (e.g., MMP3) and epidermal differentiation markers (e.g., LOR) decreased. These data were confirmed by an independently performed RT-PCR analysis; the regulation of CXCL12 was demonstrated at the protein level. In summary, the results showed that ablative CO_2 laser treatment leads to morphological changes

and regulation of the expression of various genes associated with epidermal differentiation, inflammation, and dermal remodeling. Interestingly, these results were even seen when no inflammatory mononuclear cells were present.

Er:YAG Ablative Laser

Clinically, fractionated Er:YAG lasers are successfully used in indications similar to fractionated CO_2 laser-assisted drug delivery (LADD) which is currently the focus of particular attention. It can significantly strengthen established methods, such as photodynamic therapy, in the future, especially for the treatment of actinic keratoses (grade II and III). It has already been described that treatment with the fractionated Er:YAG laser exerts an effect on the dermal extracellular matrix, which also leads to the formation of new collagen. One theory says that this effect could be explained by the intensity of the treatment and the resulting micro wounds or thermal effects. However, the exact underlying molecular effects remain unclear.

Therefore, the organotypic human 3D skin models with microlens array and different parameters were investigated at the same cumulative energy doses. To assess the morphological changes, histological examinations (Fig. 10.2) as well as microarray and qRT-PCR analyses were performed at different points in time analogous to the previous examination with the fractionated CO_2 laser (Fig. 10.3). The 3D skin models were treated with different energy doses and settings recommended by the manufacturer. The following modes were selected: N10% (12 pulses, absorbed dose 5 J/cm^2 pulse length 300 µs); E10% (6 pulses, energy density 10 J/cm^{2}pulse length 300 µs); C10% (15 pulses, energy density 4 J/cm^{2}pulse length 100 µs); and W25% (15 pulses, energy density 4 J/cm^{2}pulse length 1000 µs). The cumulative energy dose of the laser treatment of the skin model was 60 J in all cases used.

This study showed that the fractionated Er:YAG laser treatment in the skin models resulted in dose-dependent morphological changes and an almost complete restoration of the epidermis 3 days after treatment. At the same time, there was a significant increase in mRNA expression of MMPs and their inhibitors (e.g., MMP1, MMP2, MMP3, TIMP1, and TIMP2) as well as chemokines (e.g., CXCL1, CXCL2, CXCL5, and CXCL6) and cytokines (e.g., IL-6, IL-8, and IL-24). In contrast, mRNA expression of epidermal differentiation markers such as keratin 4, filaggrin 1, filaggrin 2, and loricrin as well as antimicrobial peptides such as S100A7A, S100A9, and S100A12 was decreased. Interestingly, certain laser parameters (E10%; W25%) in these studies were associated with significantly higher gene regulation, while other parameters (N10%; C10%) did not show this effect. Pulse lengths of 1000 µs tended to lead to a stronger gene expression and showed a direct effect on the expression of collagen 1A2, 5A2, and 6A2. This can possibly be explained by the fact that this mode can also reach deeper dermal tissue layers and that the long pulse times also generate thermal effects that could play a role in the formation of collagen. Antimicrobial peptides such as S100A7 have antifibrotic effects and are significantly reduced in the tissue of keloids. The use of the Er:YAG laser seems to be rather unfavorable due to the described observations.

The study shows that time-dependent differences in gene expression exist, depending on the laser used and the settings used, which would allow the biological effect to be used more specifically or even predicted in the future.

Non-Ablative Er:Glass Laser

The Er:glass laser is used clinically, e.g., for scar treatment and for the treatment of stretch marks with the aim of achieving a deep dermal remodeling. Clinical improvements were also observed in melasma, female hair loss, and acne vulgaris. Studies of gene expression by a cDNA microarray 5 days after treatment of a 3D model showed increased regulation of epidermal differentiation markers such as loricrin and filaggrin 1 and 2

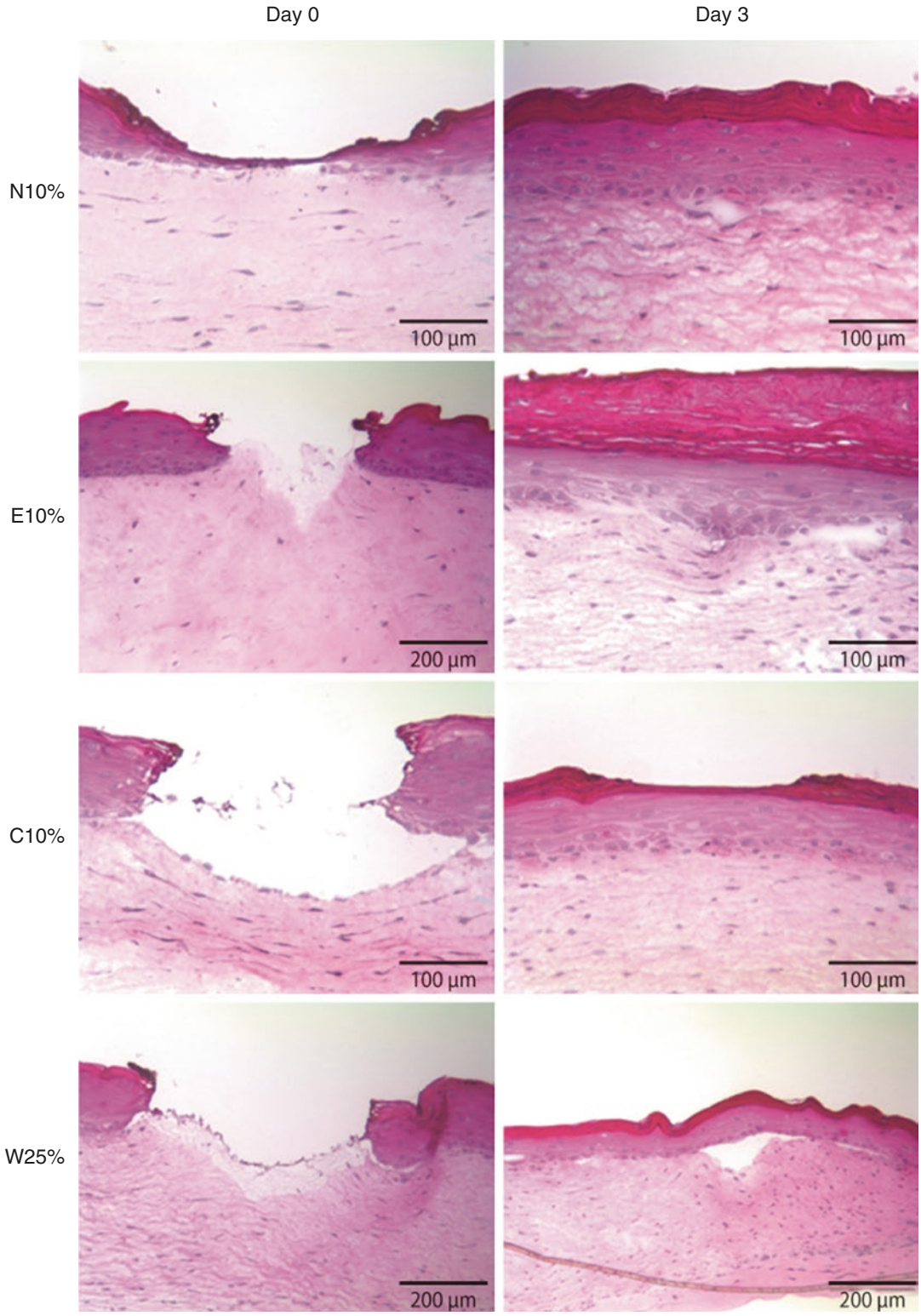

Fig. 10.2 Effects of the Er:YAG laser in a 3D skin model. Histological sections of the 3D model after treatment with different energy doses show the lesion immediately after treatment and wound healing on day 3. (From Aus Schmitt et al. 2017)

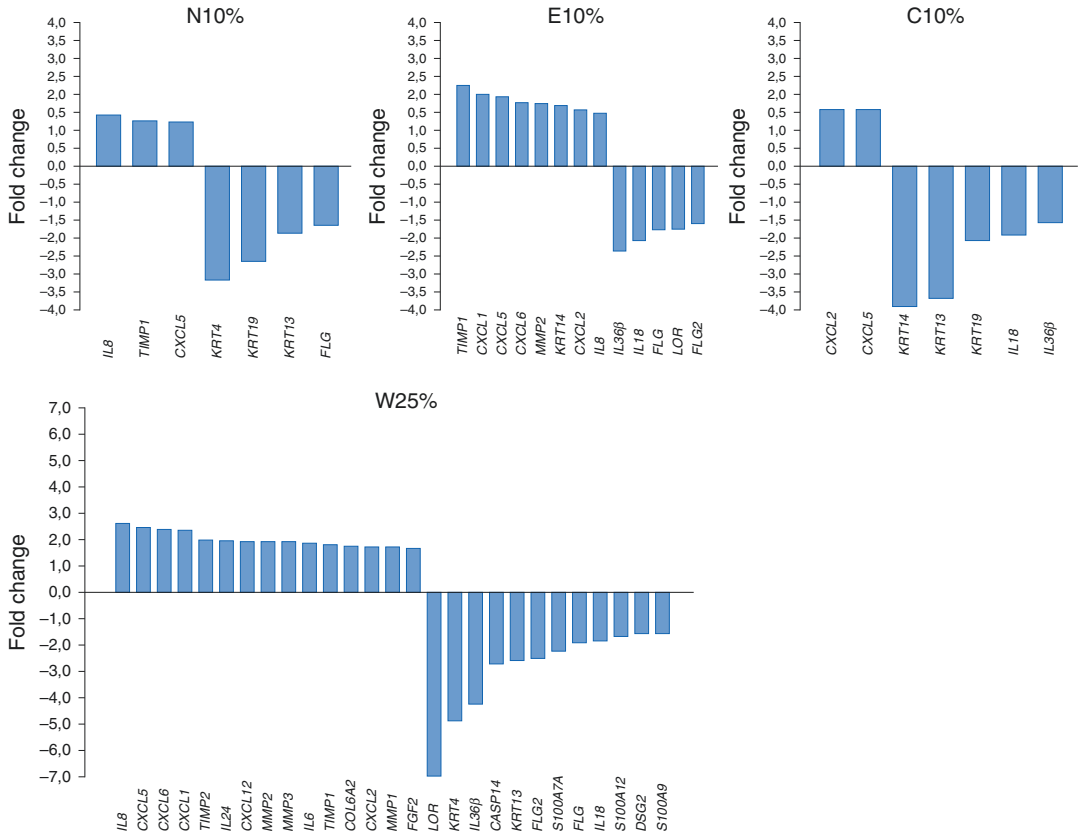

Fig. 10.3 Molecular effects of the Er:YAG laser in a 3D skin model. Chip-based gene expression analyses show the up- and downregulation of differentially expressed genes on day 3 after laser treatment with different parameter settings. (From Schmitt et al. 2017)

(Fig. 10.4). Proinflammatory chemokines such as CXCL1, CXCL2, CXCL5, and CXCL6 as well as interleukin IL-8 were predominantly decreased, as was caspase 14, which is involved in terminal differentiation of keratinocytes and thus in dermal remodeling.

It is known that chemokines (such as CXCLs) have chemotactic and activating functions on neutrophil granulocytes, which are particularly active in the acute inflammatory phase. The downregulation of chemokine expression as well as proinflammatory interleukins such as IL-6 and IL-8 shown in these investigations results in anti-inflammatory and differentiation-promoting effects for the Er:glass laser, which are important for the treatment of keloids or hypertrophic scars.

MMPs are also involved in the remodeling of the extracellular matrix, with this study showing an increased expression of MMP9 at mRNA and protein level. It is known that MMP9 can also be induced by compression therapy, which is beneficial in the treatment of keloids or hypertrophic scars. MMP3 is said to be responsible for fibroblast contraction and increased angiogenesis. Own investigations showed a decreased expression of MMP3 on mRNA and protein level. It follows from this that treatment with Er:glass lasers also has effects on the remodeling of the dermal extracellular matrix, which is mediated, among other things, by fine regulation of the expression of MMPs and chemokines.

Bottom Line

The molecular effect of fractionated nonsequential laser treatments on human skin has not yet been fully understood. Using a newly developed

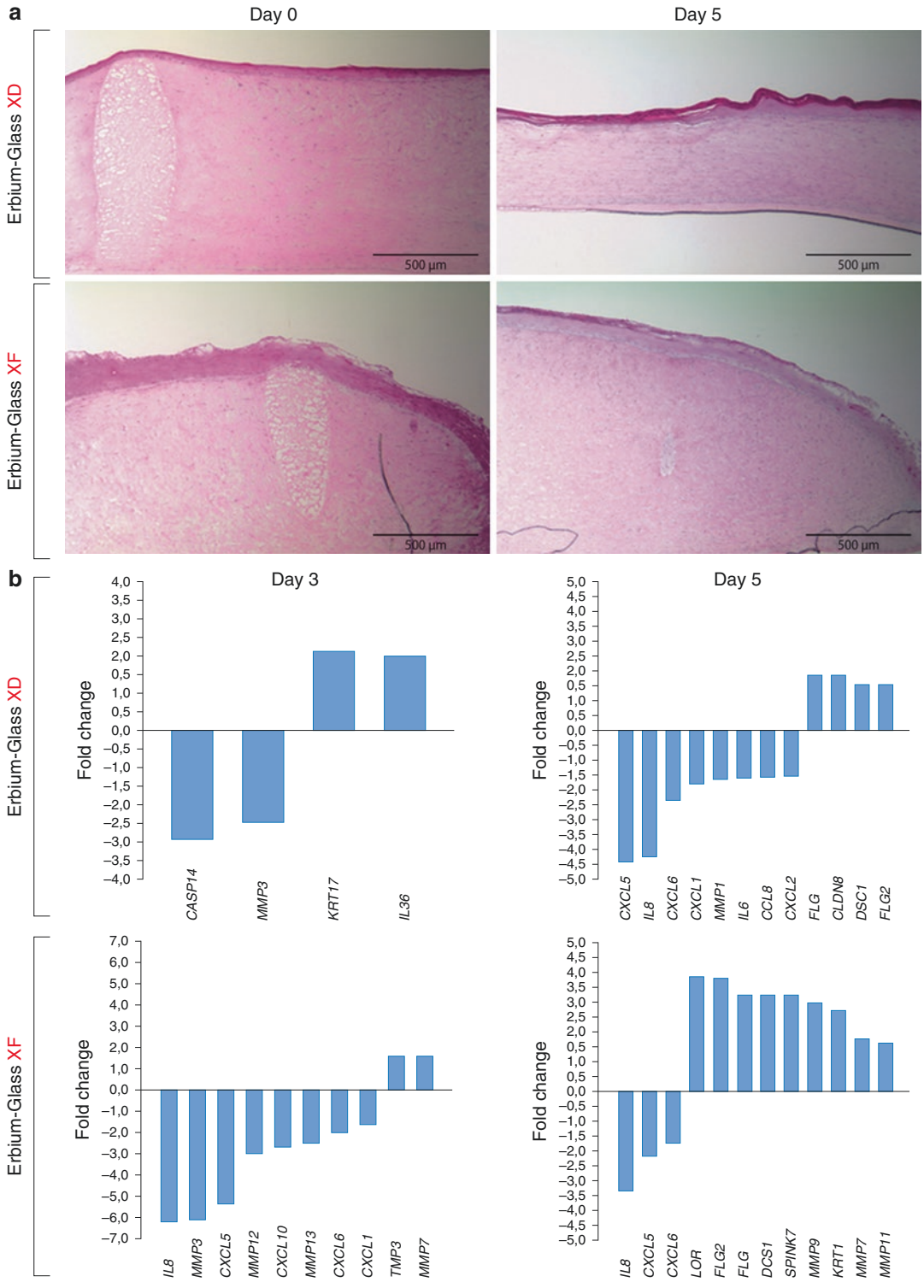

Fig. 10.4 (**a**, **b**) Molecular effects of the Er:glass laser with XD and XF lenses in a 3D skin model. (**a**) Histological sections of the laser-treated model show the wound healing process over a period of 5 days. (**b**) Chip-based gene expression analysis shows the up- and downregulation of differentially expressed genes on days 3 and 5 after laser treatment

standardized 3D skin model, the effects of different laser systems and their settings on both skin morphology and gene expression during wound healing could be investigated in more detail. In vitro studies have shown that CO_2 laser treatment of the 3D skin models led to histological changes and to the regulation of the expression of various genes associated with epidermal differentiation, inflammation, and dermal remodeling. In particular, the proinflammatory effect of the **ablative CO_2 laser systems seems** to be advantageous in the treatment of atrophic or burn scars. Investigations into the biological effect of the **ablative Er:YAG lasers** showed that these lasers induce different gene regulations through different modes, which reveal similar proinflammatory properties as the fractionated CO_2 lasers and are therefore also unsuitable for the treatment of keloids. In contrast to this the **Er:glass laser** with its anti-inflammatory and differentiation-promoting effect is better suited for the treatment of keloids or hypertrophic scars. Altogether, the studies on the standardized 3D skin model allow a better insight into the molecular effects of the different laser systems and thus a better understanding of the respective application possibilities. In the future, this could be used in a targeted manner to better predict treatment success.

Suggested Reading

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