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Characterization of reconstructed human skin using Photoacoustic Spectroscopy

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Abstract. Recent progress in skin culture techniques has led to the development of systems in which the reconstructed human skin obtained exhibits morphologic characteristics similar to those observed *in vivo*. Reconstructed human skin may be the best substrate for pharmacological tests of topically applied drugs; besides, it can be employed in the treatment of burns wounds and chronic skin ulcers. However, this newly developed material must be validated by comparison with human skin, in order to show that reconstructed skin presents characteristics similar to those of human skin. This was accomplished in the present work, through photoacoustic spectroscopy (PAS) measurements. Results show similarity between reconstructed skin and *ex-vivo* human skin, validating possible therapeutic and cosmetic treatments to be developed using the reconstructed human skin analyzed in this work.

1 Introduction

Recent progress in skin culture techniques has led to the development of culture systems in which the reconstructed epidermis obtained exhibits morphologic characteristics similar to those observed *in vivo* [1,2]. Reconstructed human skin may be the best substrate for pharmacological tests of topically applied drugs [3]; in addition, it can be employed in the treatment of burn wounds and chronic skin ulcers. In 2004, Puzzi et al. [1] have successfully reproduced human skin at the Skin Cell Culture Laboratory (LCCP) of the Medical Sciences School (FCM), UNICAMP. The human skin samples produced at the LCCP, reconstructed from isolated cells of patients, were multilayered and presented functional keratinocytes and melanocytes correctly disposed in epidermis, similarly to *in vivo* human skin, allowing the realization of autologous grafts in patients with scarce donor sites [2]. However, this newly developed material must be validated by comparison with human skin.

In photoacoustic spectroscopy (PAS) measurements, the signal is proportional to the heat generated in the sample after absorption of modulated light. PAS allows the depth analysis of

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opaque samples: the heat generated in the sample propagates over a distance determined by the modulation frequency of the light. Multilayered, the human skin can be characterized using this technique [4].

PAS presents advantages over conventional spectroscopic techniques: a) as it is directly based on the absorption of incident light, it allows the study of highly light-scattering samples; b) PAS is a non-destructive technique; c) it requires low or none sample preparation; d) it allows measurements in opaque and non-opaque samples. These characteristics make the PA technique suitable for the required characterization of human skin samples.

The goal of this work was to characterize, through PAS, reconstructed human skin samples developed for transplantation at the LCCP/FCM/UNICAMP, in order to evaluate the correspondence between this laboratory-grown skin and *ex-vivo* human skin.

2 Methodology

Samples

Comparison was performed between human skin samples (obtained from abdominoplasty) and reconstructed (cultured) human skin samples developed at the LCCP. To obtain reconstructed human skin samples, on the human dermis reconstructed *in vitro*, on a Petri dish (Corning), mixed epidermal cells were seeded (ratio between melanocytes and keratinocytes 1:4) with approximately $5 \cdot 10^6$ cells (10^6 melanocytes and $4 \cdot 10^6$ keratinocytes). The system was immersed in skin culture medium composed of 3 parts of IMDM (Iscove's Modified Dulbecco's Medium - GIBCO cat.12200-036) + 1 part of keratinocyte culture medium (Defined Keratinocyte Medium - GIBCO cat.10785-012), 10% of fetal bovine serum (GIBCO cat.10437-028) and supplemented with Ca^{++} 1.5 mM [1]. The culture medium was changed three times a week. Human skin reconstructed *in vitro*, containing dermis and epidermis, was obtained in 7 days (starting with the human dermis previously reconstructed *in vitro*).

PAS measurements

The experimental setup utilized was described elsewhere [4,5]. Skin samples (1 cm^2) were positioned inside the PA cell, receiving light from a 1,000W Xenon lamp mechanically modulated at 17 Hz (whole epidermis spectra) and 70 Hz (stratum corneum spectra). The use of different modulation frequencies allows the separation of contributions from different layers in the same sample; this is a feature of the PAS described by the RG model [6]. Spectral analysis was performed between 270 and 400 nm (UVA and UVB).

3 Results and discussion

Figures 1 and 2 show the PA spectra obtained for human skin and reconstructed human skin samples. These absorption spectra reveal that the obtained spectra for the reconstructed human skin under analysis were similar to those of the human skin, showing high absorption around 280 nm for stratum corneum and a large absorption band in the UVB range (290–320 nm) for the epidermal layers below stratum corneum. Previous work [4] shows that skin normally presents high UVB absorption in the stratum corneum layer; UVB radiation is highly absorbed by epidermal constituents (mainly melanin), which avoids its penetration in the dermal layer, while UVA radiation is more likely to reach internal layers, being absorbed by melanocytes [7].

Considering the whole epidermis, both samples show critical wavelength (50% of the maximum absorption level) at about 350 nm. This result is connected to an essential function of the human skin: to offer protection against ultraviolet (UV) radiation, avoiding or minimizing its penetration in deeper tissues. Such (deep) penetration could lead to deleterious effects, because

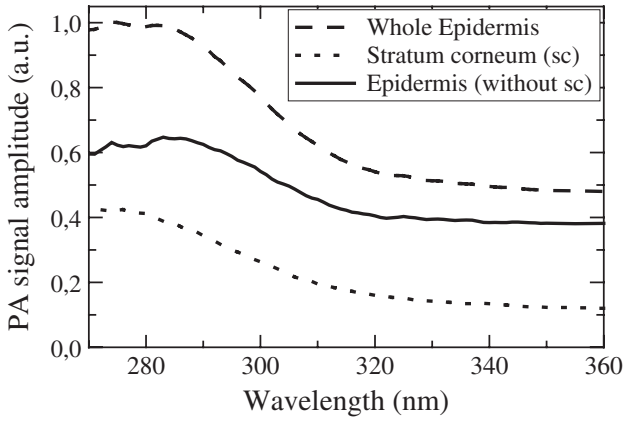

Fig. 1. PA spectra of human abdomen skin samples (N = 3): stratum corneum isolated (70 Hz), whole epidermis (including stratum corneum, 17 Hz) and epidermis without stratum corneum (difference spectrum).

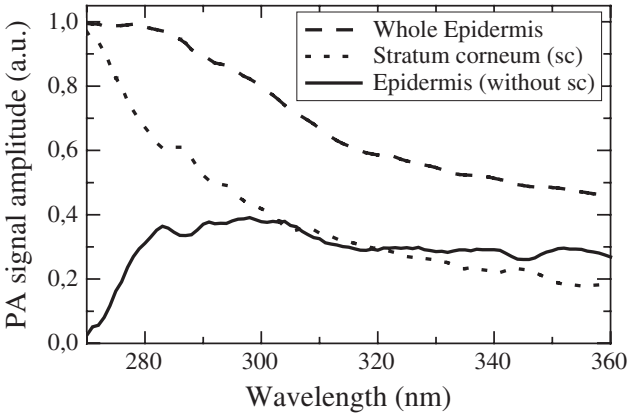

Fig. 2. PA spectra of reconstructed skin samples (N = 3): stratum corneum isolated (70 Hz), whole epidermis (including stratum corneum, 17 Hz) and epidermis without stratum corneum (difference spectrum).

UV radiation tends to increase the number of active melanocytes, producing erithema (sunburn), solar melanosis, telangectasis, actinic keratosis and even cancer lesions, as a result of continued exposition [8]. Actually, the pigment melanin, a proteic polymer present in epidermis, acts as an endogenous filter against UV radiation, and skin pigmentation depends mainly on the amount of melanin.

It can be observed that the absorption spectrum for the stratum corneum shows a higher absorption band in the UVB region for reconstructed human skin than for the human skin. This is what produces the distinction between the difference spectra of these samples. The absorption spectrum of the stratum corneum is mainly related to the presence of the protein keratin, while the absorption spectrum of the epidermis is related to the presence of the pigment melanin. Actually, the whole epidermis spectrum observed in Figs. 1 and 2 shows a high correlation with the absorption spectrum of melanin, plus some other radiation absorber. Melanin presents higher absorption from about 290 nm to 400 nm [9], as in the epidermis spectrum at Fig.2. It is known that keratin has an absorption peak at about 280 nm [10], similar to that observed at Fig. 2 for stratum corneum, but in Fig. 1 the stratum corneum spectrum is similar to that of the whole epidermis, with minimum differences. To explain that, we must observe that the spectra for both samples were obtained at the same thermal diffusion lengths. Therefore, the

changes in the PA spectra of the reconstructed human skin, when compared to that of human skin samples, can be attributed to the difference in the thickness of these samples.

The better discrimination of keratin at Fig. 2 and the similarity of epidermis and stratum corneum spectra at Fig. 1 must be related to the fact that the stratum corneum layer at the reconstructed human skin is thicker than the same layer in the human skin sample. In this way, the 70 Hz spectrum at Fig. 1 also has a contribution from the epidermis. In fact, this must be expected because the human skin is continuously changing its stratum corneum as a result of the interaction skin-ambient, but the reconstructed human skin must not change its stratum corneum at the same rate that *in vivo* human skin.

Despite this possible difference in layer thickness, the analysis of the PA spectra presented here lead us to conclude that the reconstructed human skin has optical properties and layer distribution similar to that of the human skin, with the absorption spectra indicating the presence of melanin and keratin in both samples.

These results support the potential utilization of the reconstructed human skin developed at the LCCP/FCM/UNICAMP: a) in pharmacological tests, substituting *in vivo* measurements and evaluations performed using artificially developed substrates [5]; b) in cellular therapies and autologous grafts, with no rejection risk.

4 Conclusion

PAS was successfully employed in the characterization of recently developed *in vitro* reconstructed human skin and comparison between reconstructed skin and human skin samples. The results reported in the present work can represent a significant issue for clinical translation, validating possible therapeutic and cosmetic treatments to be developed using the reconstructed human skin analyzed in this work. Future utilization of this reconstructed human skin as a substrate for pharmacological tests of topically applied drugs can eliminate the risks of *in vivo* human experimentation in this research field.

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