Biochemical characterization of skin burn wound healing using ATR-FTIR

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***Abstract*— Efficient biochemical characterization of skin burn healing stages can improve clinical routine to adjust patients treatment. The golden standard for diagnosing skin burning stages is the histological biopsy. This practice is often expensive and technically challenging. There have been advances in the treatment, and diagnostic of the critical skin burned patients due to the increase of multidisciplinary collaboration. The contributions from different fields of biomedical engineering motivate to develop a better procedure for clinical applications. Considering the difficulty of monitoring wound healing the Fourier Transform Infrared coupled with an Attenuated Total Reflectance (ATR-FTIR) accessory is an analytical technique that can provide information regarding spectral biomarkers in biological materials. This study aimed to evaluate the classification feasibility provided by ATR-FTIR technique in the burned skin to follow the regenerative process in vivo. 40 skin burned samples from the Wistar rats dorsum at 3,7, 14, 21 days after burn were compared with the corresponded healthy group samples, by registering their infrared absorption spectra in FTIR Thermo Nicolet 6700 coupled to a diamond crystal ATR. The spectra were separated in the region 900 to 1800 cm^{-1} for further chemometric calculations. The second derivative of spectra was applied for discrimination, which results demonstrated differences from control and burns wounded groups, as well as among, burn wounded groups, using Amide I (1628 cm^{-1}) and Amide II (1514 cm^{-1}) bands. Amide I and Amide II bands are two significant bands of the infrared protein spectrum. The Amide I band is mainly associated with the C=O stretching vibration (70-80%) and is directly related to the backbone conformation. The Amide II band results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%). This band is conformationally sensitive. These bands suggest proteins activity changing associate to inflammatory and maturation stages when it is compared with the healthy group. The statistical difference with amide I occur in proliferation and maturation stages. These findings indicate that ATR-FTIR is suitable to detect the burn wound healing stages and in the future can be an auxiliary instrument for clinical routine. This study was supported by CEPID/FAPESP 05/51689-2, CAPES/PROCAD 88881.**

Keywords*— *ATR-FTIR; burned skin; wound healing

I. INTRODUCTION

Burns still represent a significant public health concern which incidence is estimated 11 million injuries per year [1]. The treatment of burns substantially changes depending on the outcome of the initial assessment, then the evaluation of deep-degree burns is a critical decision point [2], [3]. The current gold standard is histological skin biopsy for visual inspection by the histopathologist. This wound classification requires expensive clinical procedure and time-consuming. Misinterpretation of burn wound could induce undesirable surgical procedures. In this sense, diagnostic tools to improve the performance of the burn depth evaluation will support the histopathology analysis. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR), would enhance the biochemical report for the clinician. It produces a valuable report on the biochemistry changes of a tissue sample. Accurately and quickly status of the wound healing will provide a significant supplement to burn wound therapy [4]–[6].

II. MATERIAL AND METHODS

A. Animal Model

The third-degree skin burn experiment was carried out after approval of Ethics Committee for Animal Research from Nuclear and Energy Research Institute (CEUA IPEN 165/15). The skin burns tissues, in the dorsum of Wistar Rats, were acquired by exposure to a source of water vapor at 90°C with an 8 mm-diameter for 12 seconds. For burn wound healing investigation with histopathologic support, we conduct four stages (3, 7, 14 and 21 days) to collect ATR-FTIR spectra from wounds. Once all skin samples are properly for cryopreservation, they were a bath in isopentane and storage at bottle of liquid nitrogen.

B. ATR-FTIR spectroscopy

FTIR system (model 6700, Nicolet Instruments, USA), equipped with an ATR (Attenuated Total Reflectance) was performed to collect spectra. All spectra 150 scans were averaged with a sampling interval of 4 cm^{-1}, wavenumbers ranging from 4000 to 400 cm^{-1}. During the ATR-FTIR measurements, tissues were pressed onto a diamond crystal (refraction index of 2.41) and irradiated at 45°C incidence angle.

C. Chemometric analyses

Spectral preprocessing was performed using MatlabR2015a(MathWorks, EUA) software. The fingerprint region between 900 to 1800 cm^{-1} was separated and spectra corrected with vector normalization. In order to access sub-bands changes, second derivative spectra were computed. Afterward, all spectra were submitted to Savitzky-Golay filter with a polynomial of second order in an eleven points window. For classification of the spectral

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patterns, amides regions were elected to achieve significant insight into the proteins modifications at different wound healing stages. The amplitude of the second derivative was interpreted using Student *t*-test pairwise to consider if the chosen wavenumbers were statistically different.

III. Results and Discussion

During the wound healing stages, ATR-FTIR spectra from burn wounds have been obtained to recognize biochemical changes. Fig. 1 shows the spectral range corresponding to amide I (1628 cm^{-1}) and amide II (1545 cm^{-1})[7]

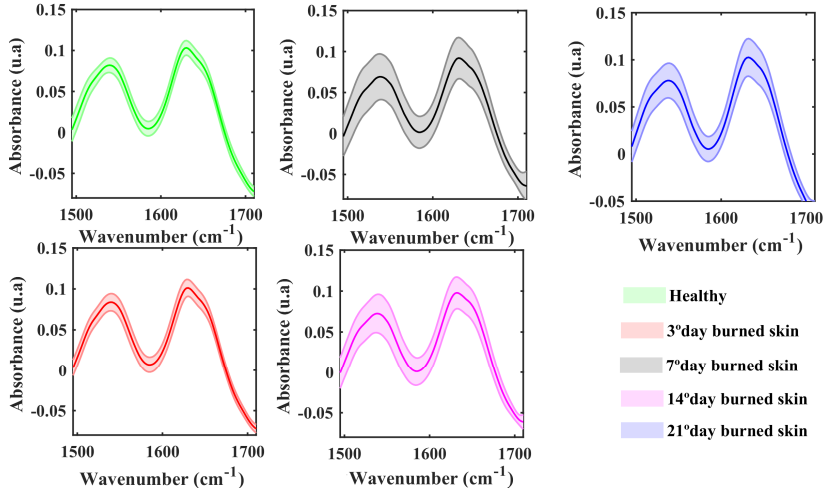

Fig. 1. Average ATR-FTIR spectra from Wistar tissues. The protein bands, Amide I and Amide II, regions of the normalized spectra and shaded region represent the standard deviation

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As exposed in Fig. 1, each averaged spectra had no significant variation among them. The ATR-FTIR alteration is not conveniently detected in the initial absorbance spectrum. Therefore, we employed the second derivative to monitor the behavior of amides bands as shown in Fig. 2.

Fig. 2 presents the average second derivative spectra from the burn wound tissues. From the spectra obtained, the comparatives absorbance differences indicate spectral change in the protein behavior of these tissues. The amide I profile is associated from the stretching vibration of the peptide carbonyl group and the amide II is related to bending vibrations of N- H bonds. Both of these bands concerns about the secondary structure of proteins, which can be valuable outcome because previous studies have demonstrate relation between enzymes protein digestion and molecular conformation of secondary structures [8-9].

The amplitude of the second derivative allowed the semi- quantitative evaluation by pairwise t-Student in Fig. 3. For the Amide I, our results exhibit a gradual increase in the statistical difference in Amide I when it is compared with healthy skin. The statistical difference only occurs after the 3day comparison with normal tissue. This behavior could be associated with the resolution of the inflammatory process, during which a new tissue will be develop. Due to these considerations, this absorption band modification seems as a spectral biomarker for collagen recovery stability in the dermis [10].

The Amide II is attributed to beta-turn protein conformation [11 - 12]. Their statistical difference could be observed particularly in the early inflammatory and the initial maturation phases when it is compared with healthy skin.

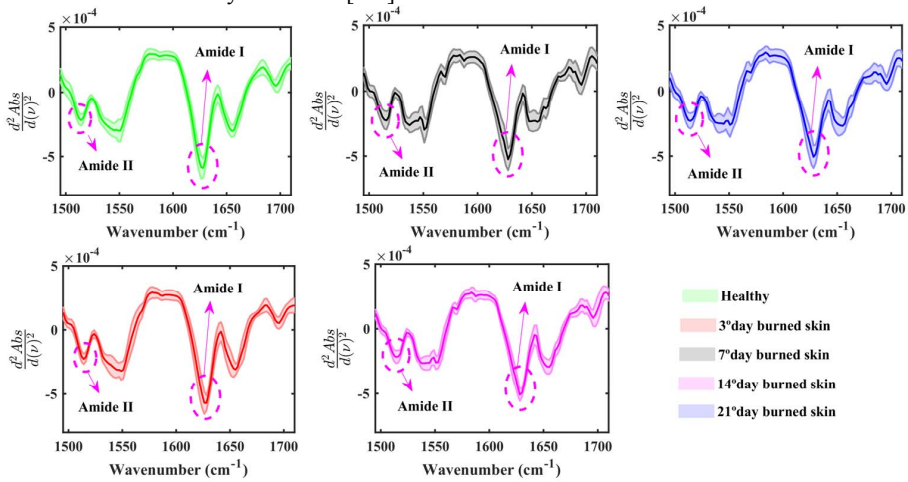

Fig. 2. Second derivative spectra calculated from Figure 1 spectra. The amide II and amide I (beta sheet) was highlighted with the pink ellipse and shaded region represent the standard deviation.

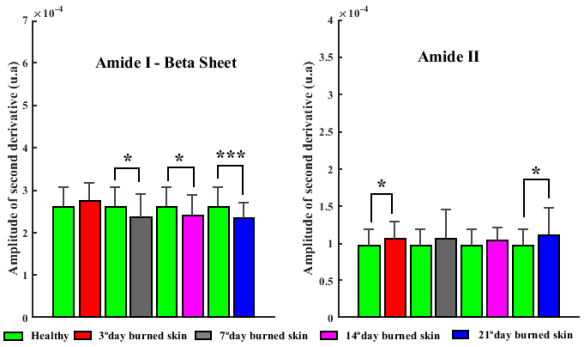

Fig. 3. The absence of asterisk indicate the no statistical difference. The asterisk presence represent the statistical difference (* p≤0.05, ** p≤0.01, *** p≤0.001) that comparison was evaluated with the pariwise comparison using *t* – Student test (p< 0.05).

The burned tissue experience acute denaturation of proteins [13]. In light of this, the collagen denaturation is directly related to the increase of temperature. This outcome results in a displacement of the triple helix and the enhance of disordered structures [14]. These proteins band changes seem to be associated with the collagen stability in the repair process. All of these spectral changes in burns wounds allow insights into the tissue repair mechanisms.

IV. Conclusions

The skin burn wound healing monitoring would be able to early report the physicians, and consequently, support the best decision to surgery and tissue sparing. This study demonstrates that amplitude of second derivative amide I and amide II are suitable to discriminate healthy skin with other healing stages. In fact, our findings revealed promising spectral markers in the burn wound healing and in the future can be a biochemical indicator to promote innovative therapeutic strategies for burns wounds.

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