Healing status of burn wound healing: ATR-FTIR study

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***Abstract*—The purpose of this study is to use infrared spectroscopy (FTIR) for monitoring biological changes in burned skin. Wistar rats dorsum samples were compared to healthy group samples at 7, 14, 21 days after burn. Proteins changes of burn wounds were monitored by area under the curve (AUC) of bands at 1630 cm 1, 1543 cm 1 and 1743 cm 1. Kruskal-Wallis normality tests, unpaired t test with Welch's correction were used to evaluate the differences between AUC. These bands suggest association between collagen activity during wound healing stages. Our result indicates progressive recovery of 7,14 and 21 days tissues when compared with the healthy group.**

Keywords—ATR-FTIR, burned skin, wound healing

I INTRODUCTION

Advances have been made in the treatment and diagnostic of critical skin burned patients due to the increase in multidisciplinary collaboration[1]. The methods routinely used to evaluate the mechanisms of wound tissue repair include histological biopsy, which is the gold standard for diagnosing the healing status of burn wounds. For burn wound biopsy assessment, an experienced pathologist takes into account the scar appearance, blood supply, and feeling to the touch[2]. These classifications accuracy can be enhanced through a deeper understanding of the wound healing biomarkers. Therefore, in order to overcome these limitations optical techniques have been developed to determine the healing status as well as to provide valuable information about the tissue repair process in a simple and fast way[1,2] In this way, infrared spectroscopy(FTIR) is a powerful tool that can provide valuable insights about the properties of the biological materials Therefore, the current study aims to investigate the feasibility of FTIR spectroscopy as a biochemical indicator of the burn wound healing phases.

II. MATERIAL AND METHODS

A. Animal Experiment and Sample Preparation

After approval of Ethics Committee for Animal Research (CEUA IPEN 165/15), third degree burn, which reaches both epidermis and dermis, spots were generated on the back of Wistar rats by water vapor at 90◦C during 12 seconds. The tissue samples were collected after thermal injury: 7, 14 and 21 days of in order to assess the wound healing process and its stages. The severity of thermal damage was confirmed based on histopathology analysis All specimens were cryopreserved, and sections of 5 μm thickness were placed in MirrIR low-E coated slides (Kevley Technologies, Chesterland, OH) [3]

B. FTIR Spectroscopy

The spectra were recorded by FTIR (Thermo Nicolet 6700, Waltham, MA) using an Attenuated Total Reflectance (ATR) (Smart Orbit, Thermo scientific, Waltham, MA). Spectral resolution was 4 cm^{1} and each spectra was an average of 150 scans, in in the range between 4000 400 cm^{-1} Fingerprint region (900 1800 cm^{-1}) was separated for baseline correction and normalization by robust normal variate (RNV) [4]. Proteins changes of burn wounds were monitored by area under the curve (AUC) of bands at 1630 cm^{1}, 1543 cm^{1} and 1743 cm^{1}. Spectra data assessments were conducted on MATLAB R2015a (MathWorks,Natick, MA).

C. Statistical Analysis

The AUC results were statistically analyzed using the software GraphPad Prism For bands at 1630 cm^{1} and 1543 cm^{1} the normality were validated using Kruskal Wallis and evaluated with unpaired t test with Welch's correction. In the case of band at 1743 cm^{1}, the statistical analysis was examined using the Mann-Whitney non-parametric test. All AUC findings were estimated with significance level set at $p < 0.005$. Table I shows the experimental design.

TABLE I. EXPERIMENTAL DESIGN

Group	*Animal*	*Tissue samples*	*Spectra obtained*
Control	5	25	60
Day 7 post burn	10	25	65
Day 14 post burn	10	25	60
Day 21 post burn	10	25	70

III. RESULT AND DISCUSSION

The infrared spectra can be affected by environmental factors such as water or carbon dioxide [6]. In particular, the water vapor spectrum in amide bands regions (1600 1800 cm^{-1}) [6,7]. With the aim of reducing the effects of the background peaks associated with water vapor and carbon dioxide, we minimize the interval between the background measurement and purge the FTIR equipment with dry air associated with desiccants. Furthermore, according to Diem *et al*, remaining variations associated to rot vibrational transitions of the water molecule can be validated with a principal component analysis on the entire data set [7] Based on this chemometric quality procedure, Figure 1 evaluate the entire spectral dataset

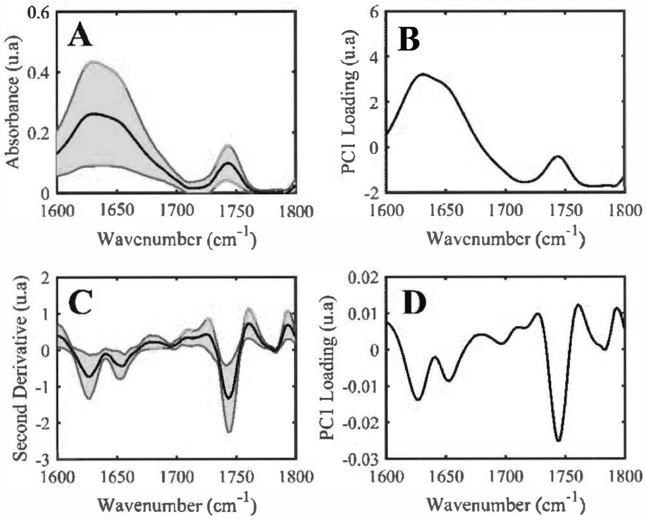
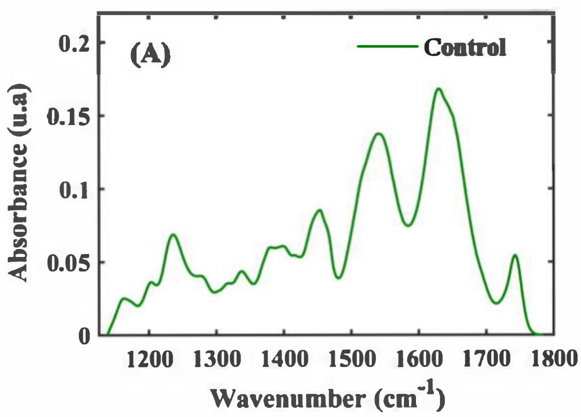


Fig. 1. Entire spectral dataset over the wavenumber interval 1600-1800 cm^{-1}: (A) average and standard deviation of spectral dataset; (B) First principal component of spectral dataset ; (C) average and standard deviation of spectra second derivative; (D) First principal component of spectral second derivative.

The comparison between first component of spectra and second derivative spectra shown in the Figure 1 demonstrated that there is no significant detrimental outcome related to water vapor on the spectra quality over the range 1600-1800 cm^{-1}.

Figure 2 depicts the average spectra of healthy and post-burn injuries over a range from 1000-1800 cm^{-1}. As shown in Figure 2, the peak positions between the spectra does not evidence differences in the appearance and disappearance of infrared bands.

An assessment of the potential effects on the overall biochemical status caused by thermal injury were estimated using the AUC Figure 3 depicts the AUC bands comparison of the healthy skin to post burn wounds



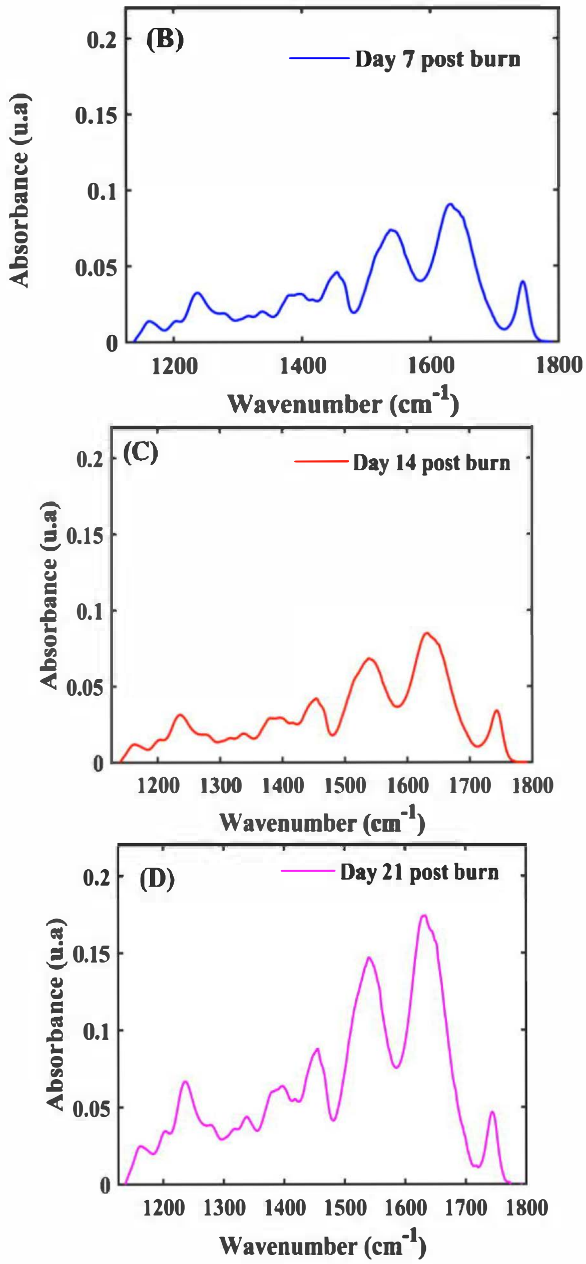


Fig. 2. Averages spectra over the wavenumber interval 1000-1800 cm^{-1}. (A) Healthy group; (B) to (D) post-burn wounds

The Fig.3 shows AUC results which were progressively higher according to the wound healing day after the thermal injury. It is possible to notice that significant statistical difference between the groups control to 21 day post burn at Fig. 3(A). When comparing the AUC values obtained at 1630 cm^{-1} and 1743 cm^{-1}, the significant differences were observed These bands may be related with an increase in the relative amount of protein tissue which suggest association between collagen activity during wound healing stages [2] The molecular and physiological bases of wound healing could lead to proliferation of fibroblasts and deposition of proteins in the extracellular matrix [5]. The high difference of these molecular bands during the healing process could be the spectral hallmarks of the tissue repair mechanisms.

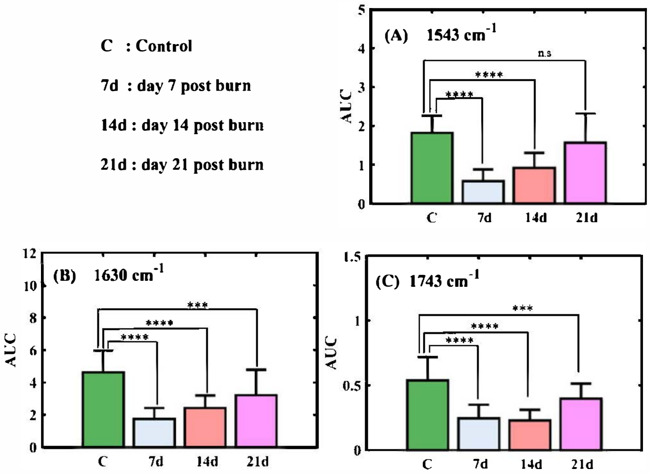


Fig 3 Intergroup (A) to (C) comparison of burn wounds to healthy skin A column indicates the mean values, and a bar represents the standard deviation of mean values. The *n.s* indicates that no statistical difference was observed and the asterisks represent the **statistical significance (* $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$) obtained with the pairwise evaluation. For (A) to (B), the** statistical analysis applied is *t*-Student test For (C), the statistical analysis employed is Mann-Whitney.

The healing response occurs when skin integrity is compromised by thermal injury. Consequently, the regenerative process could be potentially hampered by infection. Also, recent studies have shown the importance of low-cost vibrational spectroscopic methods for evaluating spectral markers that are label-free in comparison to the current diagnostic measures [8]. For this reason monitoring is a critical step.

IV. CONCLUSION

In this work, we demonstrate the potential of FTIR spectroscopy as a tool for increasing the biochemical reports of burn wounds status. Our findings indicate that the burn wounds contain biochemical activity related to wound healing stages. Therefore, FTIR spectroscopy results could be a first step to further improve pathologist examination of burn outcomes and contributing to future perspectives in the treatment of wound burns.

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REFERENCES

[1] G. M.G. Jeschke, M.E. van Baar, M.A. Choundhry et al, **"Burn injury".** Nat Rev Dis Primers. 2020, vol. 6(1), pp.1-25.

[2] M.O ,Santos, *et al*, "Multimodal evaluation of ultra-short laser pulses **treatment for skin burn injuries". Biomed Opt Express. 2017, vol. 8**(3), pp. 1575-1588.

[3] M. J. Baker, *et al*, **"Using Fourier transform IR spectroscopy to analyze biological materials". Nat Protoc, 2014, vol. 9(8), pp.** 1771-91.

[4] **E.E, Storey, A.S, Helmy, "Optimized preprocessing and machine** learning for quantitative Raman spect**roscopy in biology" J Raman** Spectrosc. 2019; vol.50, pp.958–968.

[5] **N. X. Landn, D. Li, and M. Sthle, "Transition from inflammation to proliferation: a critical step during wound healing", Cellular and** Molecular Life Sciences, 2016, vol. 73, pp. 38613885.

[6] E.R , Stuart, *et al* , **"The selective enhancement and subsequent** subtraction of atmospheric water vapour contributions from Fourier **Transform infrared spectra of proteins" Spectroc Acta Part A 1996,** vol. 52(1), pp. 1347-1356.

[7] M. Die**m, "Comments on recent reports on infrared spectral detection of disease markers in blood components", J Biophotonics. 2018, vol.** 11(7).

[8] K.V, Hackshaw, *et al*, **"Vibrational Spectroscopy for Identification of Metabolites in Biologic Samples" Molecule**s 2020, vol 25(20), pp.4725.