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Assessment of Fish Skin Grafts in the Burn Wound Healing through Synchrotron FT-IR Microspectroscopy

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Abstract

The application of fish skin grafts in the management of burn injuries has garnered significant interest recently due to their distinctive properties. This research focuses on the healing process of wounds in a rat model following third-degree burns. During the healing phase, the rat skin exhibits various structural and molecular alterations, particularly concerning proteins and lipids. In this study, synchrotron radiation Fourier-transform infrared microspectroscopy (SR-FTIRM) was employed to examine the dermal region of rat skin post third-degree burns. The experiment involved three groups of rats: one group received treatment with white fish skin, another with carpio fish skin, and a control group with untreated wounds. The analysis of collagen fiber orientation, determined by the ratio of amide I to amide II (integrated intensities: 1600-1710 cm^{-1}/1492-1598 cm^{-1}), revealed that the group treated with white fish skin exhibited the most organized arrangement of fibers. Gaining insights into such structural characteristics may significantly improve our comprehension of wound healing mechanisms and tissue regeneration.

Key words: Fish skin graft; Burned wound healing; Collagen; Synchrotron radiation FT-IR microspectroscopy

1. Introduction

Skin burns represent a prevalent category of injuries, with their severity contingent upon the depth of the burn and the extent of tissue damage involved. A third-degree burn is characterized by its penetration through all layers of the skin, potentially affecting the underlying tissues as well. Such burns typically necessitate medical treatment and may require surgical interventions, including skin grafting [1]. The appropriate and timely application of dressings to burn wounds is crucial for ensuring optimal recovery and enhancing patient outcomes. The selection of a wound dressing is influenced by various factors, including the nature of the wound, its stage of healing, and the site of the injury. Conventional wound dressing materials, such as gauze, synthetic or natural bandages, cotton wool, and lint, continue to be employed in wound management. These materials are readily available, economical, and facilitate wound drainage. Nevertheless, they often adhere to the wound as they dry, leading to discomfort upon removal. Furthermore, the necessity for frequent dressing changes can incur significant costs and may elevate the risk of tissue damage. Consequently, contemporary wound dressings that maintain a moist environment conducive to healing have largely supplanted these traditional methods [2].

In the initial phases of wound healing, collagen type III experiences degradation, while collagen type I levels rise as the healing process advances towards scar formation and tissue remodeling, thereby improving the tensile strength of the scar tissue [3]. This process is vital for ensuring that the tissue appears "normal" and contributes to the restoration of physiological function. Given its significant role in these essential processes, collagen has been utilized as an adjunctive treatment for burn wounds to facilitate healing. Its low immunogenicity, biocompatibility, and capacity to attract macrophages and fibroblasts render collagen-based biomaterials ideal for wound dressing applications.

Fish skin serves as a valuable source of collagen. Biomedical researchers have increasingly focused on fish skin due to its superior physicochemical properties, low immunogenicity, biodegradability, high porosity, excellent biocompatibility, ease of processing, natural affinity for both synthetic and natural substances, and minimal religious restrictions [4-6].

FT-IR microspectroscopy is extensively employed in various fundamental and applied biomedical research fields due to its remarkable sensitivity in revealing details about molecular composition, structure, and interactions. The effectiveness of FT-IR microspectroscopy is significantly improved when employing synchrotron infrared radiation sources, which offer exceptional brilliance, thereby enhancing spatial resolution to the diffraction limit. This study investigates the healing of third-degree burn wounds in rat skin treated with the skins of Caspian white fish (Caspian kutum) and Caspian common carp (Cyprinus carpio), two prevalent fish species available in local markets. The research utilizes synchrotron radiation FT-IR

microspectroscopy (SR-FTIRM) with a particular emphasis on detecting changes in the biochemical composition of tissues as well as conformational alterations in proteins and lipids.

2. Materials and methods

Fresh specimens of Caspian common carp (Cyprinus carpio) and Caspian white fish (Caspian kutum) were acquired from the fish market in Babol, Mazandaran, Iran. All chemicals utilized in this study were of analytical grade and did not require further purification. In this investigation, six male Wistar rats, weighing between 200 and 215 grams, were sourced from the Animal House of Babol University of Medical Sciences. The animal experiments received approval from the Animal Experiment Ethics Committee at the same institution. The rats were housed in cages under controlled environmental conditions, including a 12-hour light/dark cycle, a temperature of 25°C, and humidity levels maintained at 50–60%. On the experimental day, the rats were anesthetized using a combination of Ketamine and Xylozin (2:1 ratio). Subsequently, the fur on their backs was shaved using an electric clipper and disinfected with 70% alcohol. Deep burn wounds, classified as third-degree burns, were created by applying a 2 cm^2 probe heated to 100°C to the rats' backs for 30 seconds. The rats were then randomly assigned to three groups: group 1 served as the control with no treatment, group 2 received sterile strips made from white fish skin, and group 3 was treated with sterile strips derived from carp skin. Each group was maintained in separate, clean cages. Wound dressings were replaced every three days with freshly prepared sterilized fish skins. On the 10^{th} day, the rats were euthanized through ether inhalation. The wound area was excised, immediately frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. Serial sections of rat skin tissue from each group were cut to a thickness of 5 μm at -20°C using a Microm HM 525 cryostat (Thermo Scientific, Germany) and were placed on slides for SR-FTIRM measurements.

2.1. SR-FTIRM measurements

The SR-FTIRM measurements on rat skin tissues affected by third-degree burns were performed at the SMIS infrared beamline located at the French National Synchrotron Facility (SOLEIL) in France. The spectra were acquired utilizing a Bruker INVENIO R spectrometer in conjunction with a Bruker Hyperion II ILIM infrared microscope, operating in transmission mode. For this investigation, the microscope was fitted with a 36× objective (0.5 NA) and a corresponding condenser, along with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector, an automated sample stage, and Bruker Opus software for data collection. The spectra were gathered using a top aperture size of 10 × 10 μm^2 and a bottom aperture size of 20 × 20 μm^2. Two hyperspectral maps were generated for each specimen, focusing on areas approximately 40 μm × 40 μm. The spectral data were recorded with a resolution of 4 cm^{-1},

incorporating 64 co-added scans for each spectrum, within the mid-infrared range of 900 to 4000 cm^{-1}. Prior to analysis, all data underwent atmospheric water correction using OPUS 8.9 (Bruker Optik GmbH, Ettlingen, Germany).

3. Results and discussion

The spectroscopic data were acquired through SR-FTIRM. Figure 1 illustrates the normalized and averaged FTIR spectra of the dermis from burned rat skin that was treated with white fish skin, as well as that treated with carpio fish skin, alongside the dermis of control rat skin on the tenth day following a third-degree burn injury. The normalized average spectra for the control sample are depicted for two selected regions in Fig. 1. It is evident that the intensity and spectral characteristics of these two regions differ, indicating that the microenvironment of the burned wound is more complex and heterogeneous in the control group. The spectral data indicate that the bands linked to proteins, specifically amide I (~1658 cm^{-1}) and amide II (~1547 cm^{-1}), exhibited greater absorbance in the treated groups relative to the control one. Additionally, absorption bands observed at approximately ~1454 cm^{-1}, ~1400 cm^{-1}, ~1340 cm^{-1}, and ~1238 cm^{-1} are associated with the wagging and deformation of CH_2 and CH_3 groups, as well as C–N stretching in collagen [7]. The absorption band observed at approximately 1340 cm^{-1} is associated with the CH_2 wagging vibration of the proline side chains found in collagen. This specific vibration at around 1340 cm^{-1}, which serves as an indicator of collagen integrity [8], confirmed that the collagen structure was preserved during the entire treatment process.

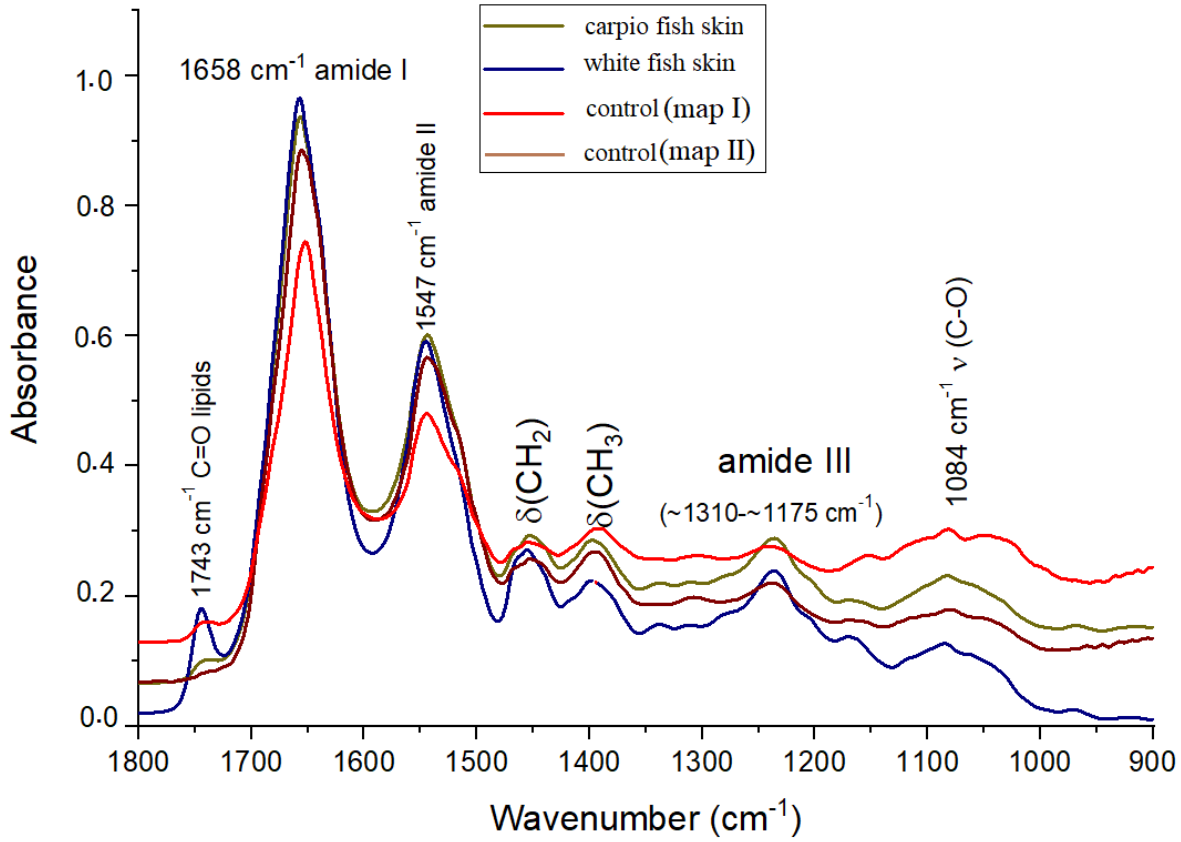

Figure 1. The normalized average spectra obtained from the skin tissues at 1800-900 cm^{-1}.

The absorption peaks observed at 1033 cm^{-1}, 1059 cm^{-1}, and 1084 cm^{-1} are attributed to the C–OH stretching vibrations associated with the carbohydrate components linked to the protein [9]. The alignment of collagen fibers was assessed by evaluating the ratio of amide I to amide II (integrated intensities: 1600-1710 cm^{-1}/1492-1598 cm^{-1}). Higher values in this ratio suggest a disordered configuration of collagen fibers with various orientations [10]. In the control group within the map I region (control I), a disorganized arrangement of collagen fibers was noted, while the group treated with white fish skin exhibited the most organized fiber structure, as illustrated in Fig.2. Prior research has indicated that collagen fibers are arranged in a way that allows for the continuous mobility of each fiber, which enhances the resilience of collagen against significant stretching; the strength of the skin is dependent on the superstructure of the collagen fibers [11].

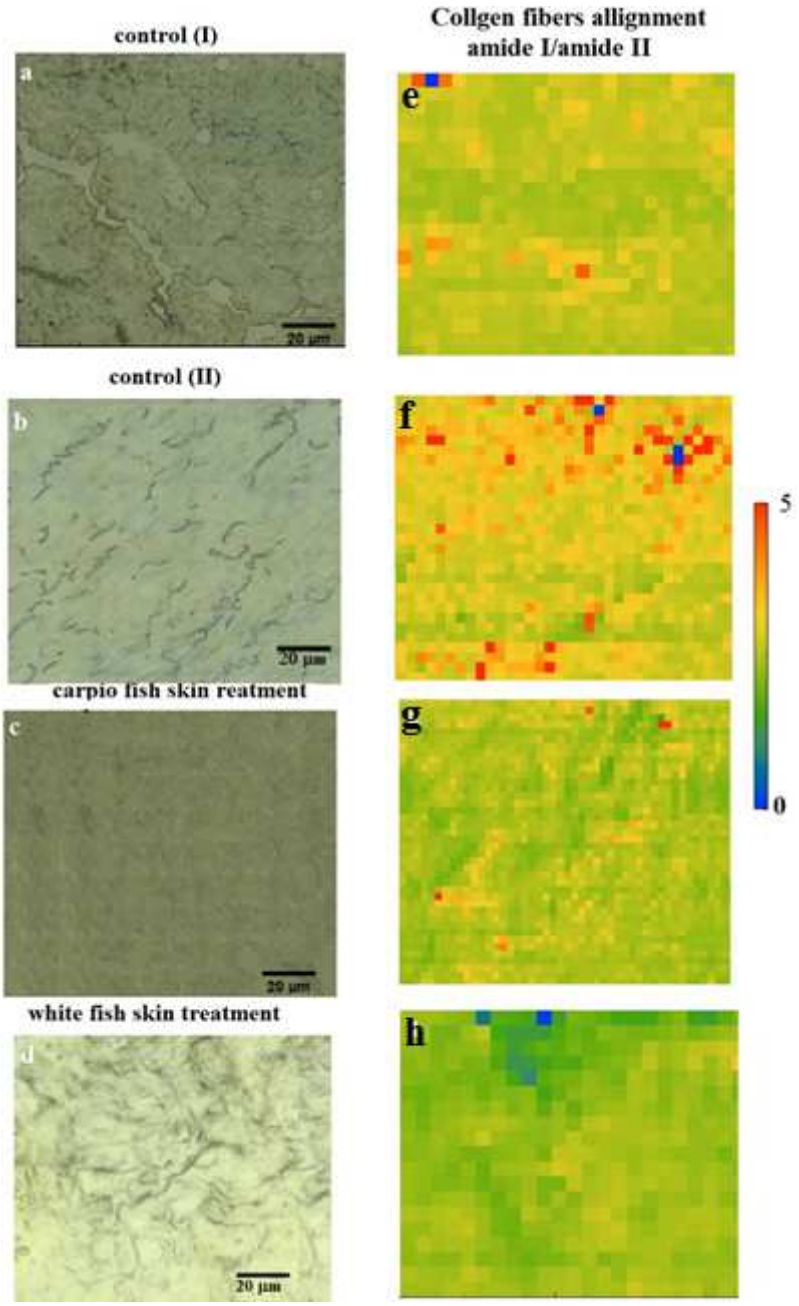

Figure 2. SR-FTIR mappings (scale bar: 20 µm) in dermis region of the (a,b) control group, I carpio fish skin treatment group, and (d) white fish skin treatment group, their corresponding collagen fibers aligned (amide I/amide II) (e-h). Red and blue colors represent the strong and weak absorption of the infrared beam, respectively.

4. Conclusions

Fish skin grafts present a hopeful opportunity for pioneering approaches in wound care. The SR-FTIRM results indicated that lipids and collagen are major biochemical components altered during wound healing. Our study demonstrated that the application of white fish skin graft on burn wounds results in a higher content of lipids and more ordered collagen compared to carpio fish skin graft. Lipids are vital in the wound healing process as they influence inflammation, angiogenesis, proliferation, and tissue repair [64,36]. Therefore, the high lipid content found in rat skin may elucidate some of the beneficial characteristics of fish skin grafts in promoting wound healing. The spectral characteristic at approximately 1454 cm^{-1} specific to the δ (CH_2) signal and the peak at around 1400 cm^{-1} specific to the δ (CH_3) signal indicates superior fibrillar organization in white fish skin treatment.

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