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The role of topically applied L-ascorbic acid in ex-vivo examination of burn-injured human skin

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ABSTRACT

Wound treatment and healing is complex and is comprised of an elaborate set of processes including cellular, spectroscopic and biochemical ones as well as the "reaction" of local tissue to thermal injury. Vitamin C as L-ascorbic acid (LA) prevents injurious effects of oxidants because it reduces reactive oxygen species to stable molecules, it becomes oxidized to the short-lived ascorbyl radical. As a result, antioxidant treatment may contribute to minimizing injury in burn patients. The aim of this study is to assess changes in molecular structure of collagen extracted from human epidermis burn wound scab during incubation of the epidermis in L-ascorbic acid solution. The study will be performed using FTIR and FT Raman spectroscopies. During this research it was observed that the intensity of Raman peaks increased where healing was being modified by LA. The intensity of the amide III band at 1247 cm^{-1} relative to the intensity at 1326 cm^{-1} was used to test tissue repair degree at the incision site. FTIR spectra were recorded from frozen specimens of serum modified by LA; an analysis of shifts in the amide I band position was conducted. The appearance of a new band for frozen samples modified by LA was observed around 1149–1220 cm^{-1}. The above conclusions confirmed the creation of hydrogen bonds between N—H stretch and C═O. Samples being incubated in solutions of L-ascorbic acid demonstrated the absence of electrophoretic bands of albumin. Alterations in the surface of the skin incubated in L-ascorbic acid were investigated with the use of Scanning Electron Microscopy (SEM). A decrease in external symptoms of burn injury was noted in the damaged epidermis incubated in L-ascorbic acid.

1. Introduction

The application of diverse defensive means may counteract damage resulting from irradiation. β-Carotene, melanin, H_2O_2, riboflavin, methylene blue and thiourea have all been reported to modify the photochemical stability of collagen. Ascorbic acid is an example of one such means which counteracts the presence of free radicals. Being an antioxidant, L-ascorbic acid (LA, vitamin C) reacts with and deactivates biologically significant radicals and oxidants [1]. In general, vitamin C fortifies collagen biosynthesis (lack of vitamin C causes collagen malformation) and the synthesis of ceramides to form strong barrier lipids in the epidermis [2]. Darr et al. studied the capacity of locally applied vitamin C to stimulate dermal vitamin concentration and to decrease UV harm to porcine skin [3,4]. One study reported an increase of more than 200% in the collagen synthesis rate of Achilles ligament fibroblasts when vitamin C was coupled with the essential culture media [5].

Apart from functioning as an antioxidant and protecting against oxidative stress, vitamin C is a cofactor in numerous enzymatic responses, such as collagen synthesis, which is critical in wound-healing, and counteracting capillary bleeding. Locally applied vitamin C has also been proven to stimulate collagen production in human skin in vivo [6]. Since the skin functions as an organ that shields us from natural free-radical stretch, vitamin C seems to be of utmost importance. Vitamins C and E are more effective if applied locally to the skin, provided they are formulated accurately and in a sufficient concentration. Preparation of an efficient local delivery system is important since LA is an innately unstable molecule and is oxidized to dihydroascorbic acid in the presence of air. Numerous products include stable derivatives that the skin does not metabolize (for example, ascorbyl-6-palmitate or magnesium ascorbyl phosphate) and owing to this demonstrate little or no activity [7]. Created as by-products of cellular metabolism or acquired from natural sources, oxygen radicals and other activated oxygen species induce modifications of the amino acids of proteins which further affect functional shifts in structural or enzymatic proteins, and proteins are crucial targets for oxidative modifications [8]. Apart from altering

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amino acid side-chains, oxidation reactions may likewise facilitate fragmentation of polypeptide chains as well as intramolecular and intermolecular cross-linking of peptides and proteins. Therefore, the occurrence of carbonyl bands in proteins functions as a marker of reactive oxygen-mediated protein oxidation. On the basis of the introduction of carbonyl groups, protein oxidation is linked with maturing, oxidative anxiety and various illnesses, including premature aging ones. Protein aggregation is a typical component of several illnesses, yet the lack of homeostasis occurring between protein synthesis and protein degradation is by all accounts vital. Numerous investigations confirmed the occurrence of oxidative stress in rheumatoid arthritis patients, in Alzheimer's disease, in Parkinson's disease and cardiovascular disease [9]. In the early stage of burn disease markers of inflammation are elevated and oxidative stress markers are present in children's plasma [10].

Disturbances in the balance between reactive oxygen species (ROS) formation and antioxidants can damage cell components, including proteins, lipids, and DNA, and oxidative modification of any of these biomolecules, as well as a significant decrease in total antioxidant capacity, which protects the organism against ROS activity can lead to diverse functional changes and theoretically contribute to disease development [11].

The aim of this study is to assess the impact of a locally applied L-ascorbic acid solution on human burn wounds to investigate the structural changes of collagen using FTIR and FT Raman spectroscopies.

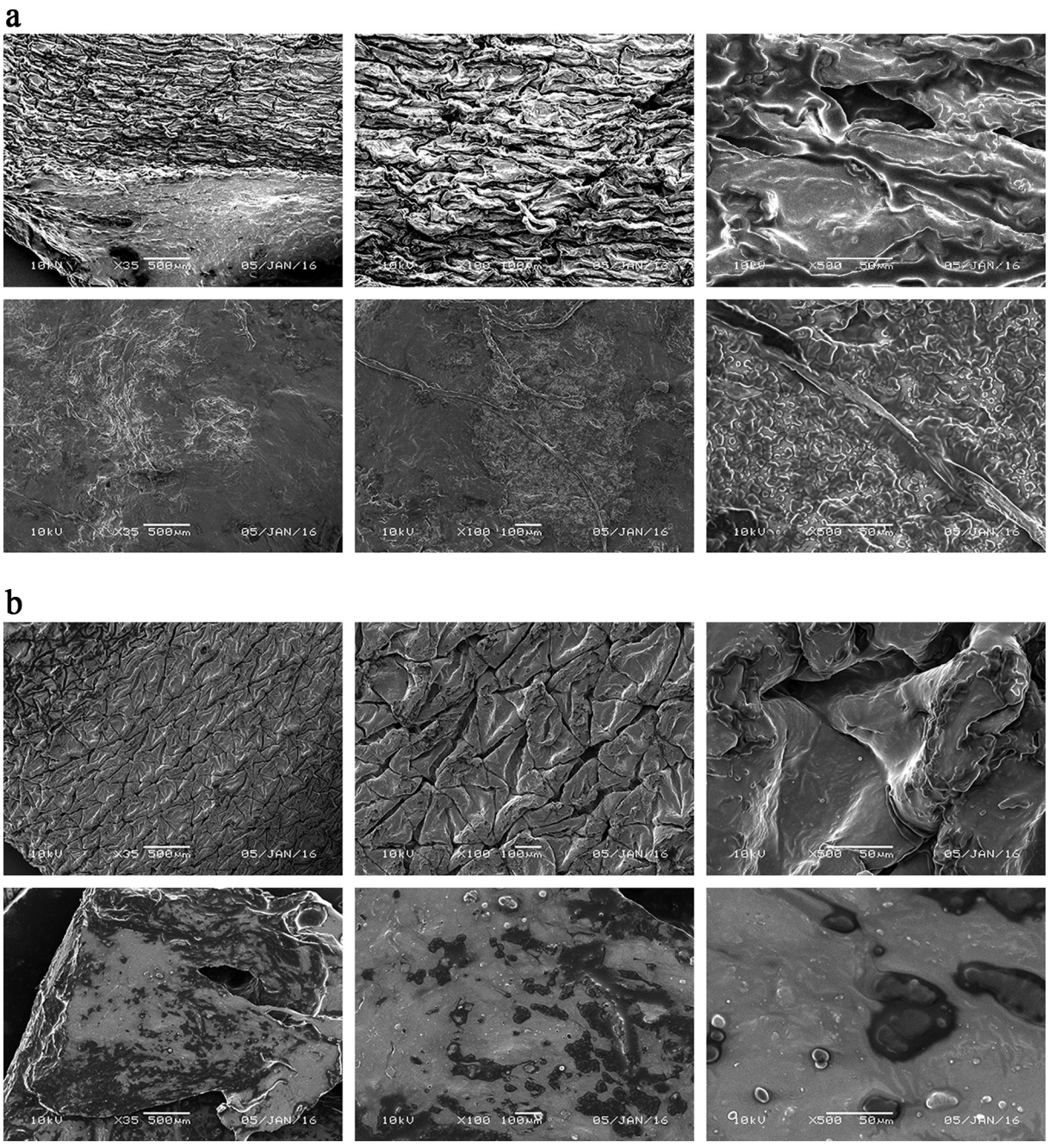

Fig. 1. a. Scanning electron microscopic images of the samples of human burn wound skin surface (1S and 2S) (×35; ×100; ×500). b. Scanning Electron Microscopy images of the samples of human burn wound skin surface and then incubated in L-ascorbic acid solution (1SLA and 2SLA) (×35; ×100; ×500). c. Scanning electron microscopic images of the samples of human burn wound skin surface (3S and 4S) (×35; ×100; ×500). d. Scanning Electron Microscopy images of the samples of human burn wound skin surface and then incubated in L-ascorbic acid solution (3SLA and 4SLA) (×35; ×100; ×500).