

# A STUDY ON THE STRUCTURAL INTEGRITY OF HEALTHY AND DISEASED NAILS: THE RETENTION OF DISULPHIDE BONDS

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Onychomycosis is the most common disorder of the nail plate. *Trichophyton rubrum*, the organism most abundant during onychomycotic infections, is known to embed within the nail, produce keratinolytic proteinases<sup>1</sup> and change the visual characteristics of the local tissue, but there is not, at present, a clear understanding of how the infection impacts the nail integrity. The hard, yet slightly elastic structure of the nail plate is believed to be at least in part a consequence of extensive keratin disulphide links (-SS-). Breakage of disulphide bonds, consequently generating -SH groups, often result in a reduction in barrier function<sup>2</sup>. The aim of this work was to characterize the -SS- bonds in healthy and onychomycotic nails and investigate the link between -SS- bonds and nail barrier integrity.

Raman infrared spectroscopy was used to characterise the -SS- and -SH bonds on the dorsal and ventral sides of 4 healthy fingernail clippings, 4 healthy toenail clippings and 4 diseased toenail clippings. Tris(2-carboxyethyl)phosphine (TCEP) was used as a positive control and nail barrier integrity was determined using rhodamine B and water as markers. The Raman spectra (Fig.1) showed that healthy nails have a high content of -SS- bonds, in agreement with previously published work<sup>3</sup>. Upon treatment with TCEP, nail -SS- bonds are reduced and -SH groups are formed as shown by the presence of the -SH band in the raman spectrum. Interestingly, the Raman spectra of diseased nails showed that the -SS- content remains high and comparable to that of healthy nails and no -SH groups are evident. Rhodamine B and water ingress (measured as nail swelling) into the nail were significantly lower in nails with a high proportion of -SS- bonds (rhodamine penetration:  $0.16 \pm 0.07 \mu\text{g}/\text{mg}$  and nail swelling:  $15.21 \pm 1.54\%$  weight increase) than in nails containing a proportion of -SH (rhodamine penetration:  $0.39 \pm 0.03 \mu\text{g}/\text{mg}$  and nail swelling:  $277.02 \pm 29.75\%$  weight increase).

The data presented herein suggest that the human nail plate barrier properties are at least in part a function of -SS- bond integrity and that these bonds are retained in onychomycotic nails.

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