



SUPRAMOLECULAR ORGANISATION OF COLLAGEN FIBRILS IN HUMAN TISSUES

**C. MÉRIGOUX¹, D. DURAND¹, J. DOUCET¹, M. EUGÈNE²
AND O. DIAT³**

1 LURE, ORSAY (FRANCE),

2 LABORATOIRE RMN, HÔPITAL SAINT-LOUIS, PARIS (FRANCE),

3 ESRF, EXPERIMENTS DIVISION

The very rich SAXS pattern of human dermis reveals a well defined supramolecular organisation of collagen molecules into fibrils and bundles. The quality of dermis used for skin graft can be checked by this technique.

The structure of skin is not well characterised despite the essential protection role played by this tissue against physical, chemical and microbiological aggression. An improvement in our knowledge of the whole structure is crucial for medical applications (graft, substitution, etc.).

Skin is composed of three different layers; epidermis, dermis and hypodermis (Figure 1). The epidermis serves mainly as a chemical barrier (to water permeation for instance) while the dermis provides most of the toughness of the skin and the hypodermis acts as a thermal and mechanical insulator.

The epidermis is mainly made up of cells (keratinocytes) which during their migration from the dermis to the stratum corneum (upper layer of the epidermis) release lipids which aggregate into bilayers in the intercellular matrix of the stratum corneum. These bilayers have been widely studied by electron microscopy and X-ray diffraction. The lower layer of skin, the hypodermis, contributes to the fatty reserves of the body. As far as the dermis is concerned, fibroblasts are the main cells present in a matrix mostly comprising a protein called collagen and also a small amount of proteoglycans and elastin.

The molecular structure of collagen is a triple helix of about 3000 Å length (Figure 2). The tips which are called telopeptides are partially responsible for the connection between molecules. Consequently, molecules are gathered into fibrils and their arrangement is such that two adjacent molecules are parallel and shifted by 652 Å. In the dermis, these fibrils are also parallel and arranged in bundles which are more or less randomly organised in the tissue.

The classical analytical technique of studying skin-type tissues is transmission electron microscopy which provides structural information from 150 Å to several microns resolution. However, samples undergo several treatments before observation (fixation, staining, dehydration, inclusion) and these are known to modify structural parameters in the whole tissue and at

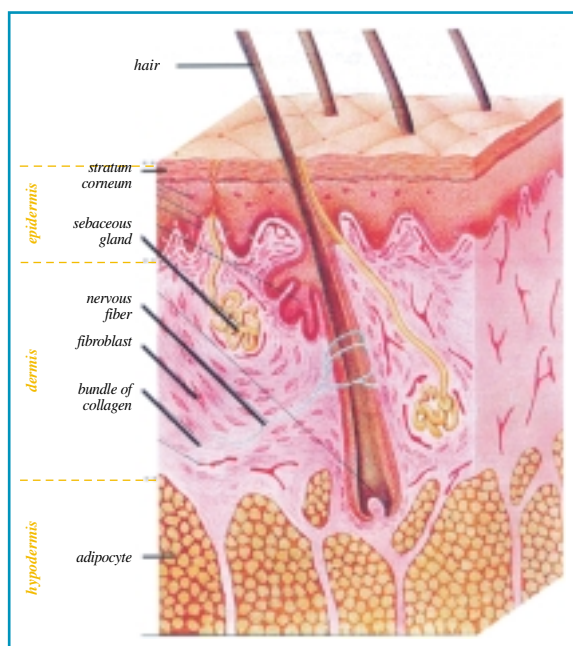
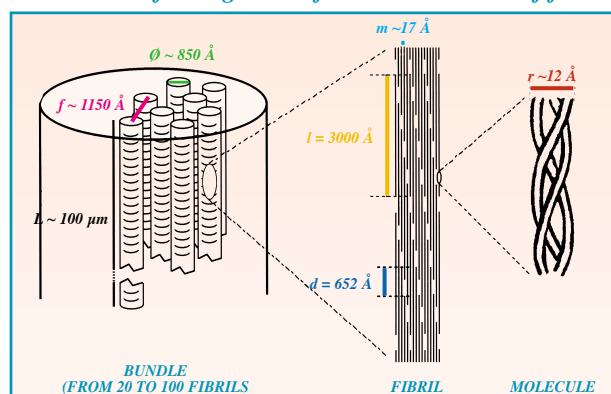


Fig. 1: Schematic representation of human skin (from M. Bago et al, Transplantation, 41, 316 (1986)).

Fig. 2: Supramolecular organisation of collagen into fibrils and bundles of fibrils.



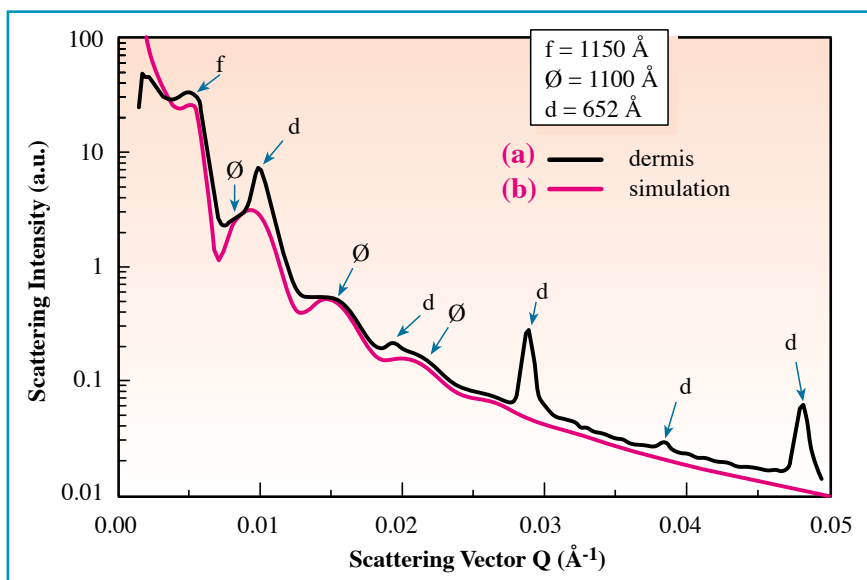


Fig. 3: Scattering pattern for human dermis recorded on ID2 (a) compared to a simulated pattern (b) without taking into account the longitudinal periodicity 652 Å (peaks d). f: peak due to the lateral packing of fibrils. Ø: scattering features due to the cylindrical shape of the fibrils. d: peaks due to the intrafibrillar longitudinal period 652 Å.

each measurement scale [Fullwood]. A complementary technique is therefore needed which does not require chemical modification of the tissue and allows appropriate measurements to be made before the tissue is irreversibly damaged. Such a technique is provided by Small-Angle X-ray Scattering (SAXS), which gives structural information on the packing of the collagen fibrils into bundles on a scale ranging from 10 to 1000 nm. Experiments have been undertaken on fresh untreated human dermis on beam line ID2 at the ESRF. Samples, 1 mm in thickness, were exposed to a high brilliance, highly collimated X-ray beam and diffraction patterns recorded using a multi-wire gas-filled area detector placed at 10 m from the sample, allowing the recording of scattering signals below the mrad angular range. A typical scattering curve (a) is shown in Figure 3 after circular averaging and detector corrections. The scattering intensity is plotted versus the scattering vector Q , which is proportional to the scattering angle θ , $Q = 4\pi/\lambda \cdot \sin(\theta/2)$ with $\lambda = 1 \text{ \AA}$. The peaks labelled «d» are well known and correspond to the five first orders of the periodical arrangement of the collagen molecules in fibrils (652 Å period). The envelope of the curve (a) is the signature of the scattering from the fibrils themselves; it corresponds to the product of the form factor (oscillations ϕ from which we can

extract a diameter of the fibrils, 1100 Å) and the structure factor (correlation peak «f» from which the average distance 1150 Å is determined). The scattering feature «f» had never been observed up to now and the excellent quality of the data has permitted to interpret for the first time the whole scattering pattern in terms of fibril geometry and interfibrillar packing. From a model of these fibrils, packed with a short range position order in a gel matrix, the simulated scattering envelope (b) of the scattering curve can be calculated; it is in good agreement with the experimental curve, indicating the correctness of the model.

Similar SAXS experiments carried out on frozen dermis lead to an improved model of the fibrils structure. They consist in a proteoglycan coating surrounding a collagen core of diameter of 850 Å. This type of analysis gives accurate information on the state of the dermis (at the supramolecular scale) and more especially on the quality and nature of the fibrils and how these vary under chemical and physical treatment of the skin tissue (reconstructed tissues, cryoconcentration, etc.). ■

Reference

N.J. Fullwood, K.M. Meek, «A synchrotron X-ray study of the changes occurring in the corneal stroma during processing for electron microscopy», (1993), *J. Microscopy*, 169, 53-60.