Supplementary Materials

Histological Staining Methods

1. Hematoxylin and Eosin (H&E) Staining

Skin tissue samples were fixed, embedded in paraffin, and sectioned according to standard pathology protocols. Sections were deparaffinized by sequential immersion in clearing reagent I (20 min), clearing reagent II (20 min), absolute ethanol I (5 min), absolute ethanol II (5 min), and 75% ethanol (5 min), followed by rinsing in tap water. Frozen sections were re-warmed to room temperature and fixed in tissue fixative for 15 min before washing. Sections were then stained with hematoxylin for 3–5 min, rinsed, differentiated, blued, and counterstained with eosin for 5 min. After dehydration through graded ethanol and clearing in xylene, the slides were mounted with neutral resin and examined under a Nikon Eclipse E100 microscope. Nuclei appeared blue, and cytoplasm appeared red.

2. Masson's Trichrome Staining

Skin tissue sections (paraffin or frozen) were prepared following standard pathology protocols. Deparaffinization was performed as described for H&E staining. Sections were immersed in Masson's solution A overnight, rinsed, and then stained sequentially in a mixture of Masson's solutions B and C (1 min), differentiated, and washed. Sections were subsequently stained with solution D (6 min), solution E (1 min), and solution F (20–30 s), followed by differentiation in 1% acetic acid. After dehydration in absolute ethanol and clearing in xylene, the sections were mounted with neutral resin.

Under the microscope, collagen fibers appeared blue, while muscle fibers, fibrin, and erythrocytes appeared red.

3. Picrosirius Red (Sirius Red) Staining

Skin tissue sections were prepared as described above. After deparaffinization and rehydration, frozen sections were fixed for 15 min and rinsed in running water. Sections were immersed in Sirius Red solution for 8 min, followed by dehydration in absolute ethanol and clearing in xylene before mounting with neutral resin. Under light microscopy, collagen fibers appeared red against a yellow background. Under polarized light microscopy, type I collagen appeared as orange or bright red thick fibers, while type III collagen appeared as green thin fibers.