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Supplementary Material

Integrating 3D-Printed Hydrogels and Polymeric Nanofibers in a Double-Layered Transdermal Patch for the Treatment of Rheumatoid Arthritis

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1. Preparation and preservation of skin samples

The preparation of the skin samples followed a precise methodology to ensure the integrity of the tissues for subsequent analysis. Firstly, skin samples from all animal groups were collected post-euthanasia under aseptic conditions. The collected samples were carefully removed from the ankle joint areas to include both affected and surrounding tissues. Secondly, the samples were immediately immersed in 10% neutral buffered formalin for fixation and to ensure the preservation of the tissue structure and prevent degradation. Thirdly, the samples were dehydrated through an ascending ethanol series, cleared in xylene, and embedded in paraffin wax blocks for sectioning and histological examination.

2. H&E staining procedure

To investigate the histological alterations in the tissue samples, H&E staining was done. Tissue slices (4 μm thick) were cut from paraffin-embedded blocks and placed on glass slides for deparaffinization and rehydration. After being deparaffinized in xylene, the sections were rehydrated using distilled water and a declining ethanol series. To stain the nuclei, the slides were then submerged in haematoxylin for five minutes. The excess haematoxylin was removed using running water. For distinction, the portions were immersed in 1% acid alcohol and then washed with water one more time. The cytoplasm and other cell components were counterstained with eosin for two minutes. The stained sections were dehydrated using a sequence of increasing ethanol concentrations, cleaned in xylene, and then mounted with a coverslip using a resinous mounting media for dehydration and mounting. Stained sections were viewed under a light microscope for microscopic evaluation in order to detect pathological characteristics such tissue damage, inflammation, and cell infiltration.