

Supplementary Method 2 (Fixation, embedding, and sectioning for LM microscopy analysis)

The samples were fixed in formaldehyde: acetic acid: alcohol 96% solution in the ratio of 10%: 5%: 5% v/v at room temperature for 48 h. After washing the tissue segments with deionized distilled water, they were dehydrated in a graded series of 50%, 60%, 70%, 80%, 90%, 100%, and 100% ethanol for 30 min each. Then, the ethanol was replaced by a graded series of ethanol: xylene solutions with ratios of 75%:25%, 50%:50%, 25%:75%, and 0.0%:100% at room temperature for 1 h each. The fixed tissues were soaked in paraffin wax (melting point 53–58 °C (ASTM D87): CAS No. 8002-74-2; Sigma-Aldrich, USA) at a temperature of 60 °C for 16 hr. Both dehydration and waxing processes were performed using an automatic tissue processor (DS-2080/H, Did Sabz Co., Tehran, Iran). A heated paraffin embedding station (Paraffin Dispenser Plus, DS 4LM, Did Sabz Co., Tehran, Iran) was used for paraffin embedding. The 8- μ m-thick sections were cut with a rotary microtome (DS 8402, Did Sabz Co., Tehran, Iran). The sections were floated in a water bath at 40 °C to be mounted onto New Silane II-coated microscope slides and heated at 50 °C for 10 seconds. The sections were deparaffinized in xylene and rehydrated in a series of 100%, 90%, 70%, and 50% ethanol and running tap water (5 min each). The dewaxed sections were stained with methylene blue 1.5% (w/v) in 96% ethanol for 10 seconds.