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MDPI

Supplementary Materials

Regenerative Activities of ROS-Modulating Trace Metals in Subcutaneously Implanted Biodegradable Cryogel

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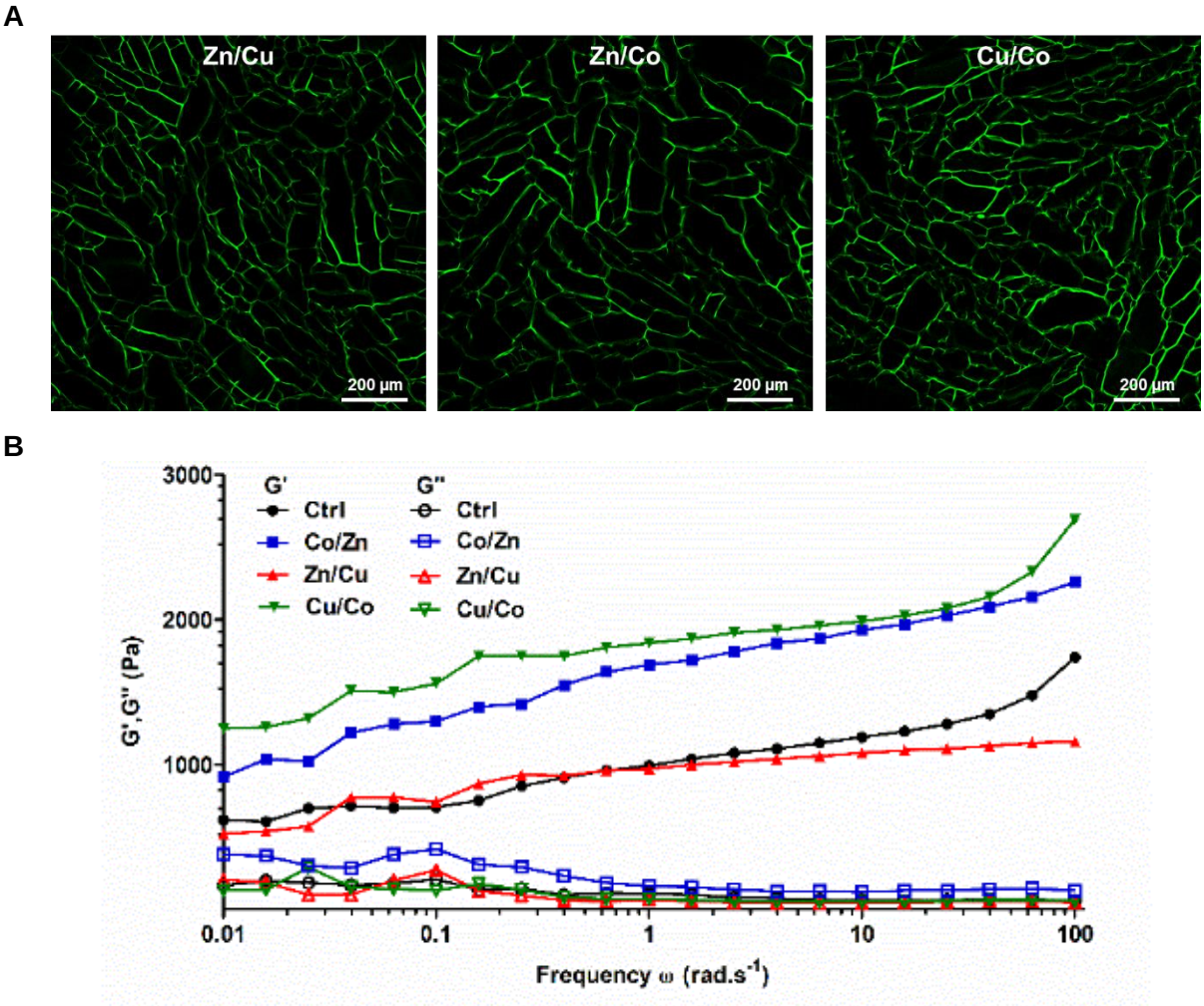


Figure S1. A. LSCM images of dual TM-doped cryogel sheets (top surface) visualized by autofluorescence upon argon laser excitation (488 nm). B. Frequency sweep test (strain deformation δ = 1%) data for the cryogels. TM-doped cryogels (1 mM for each metal) were analyzed.

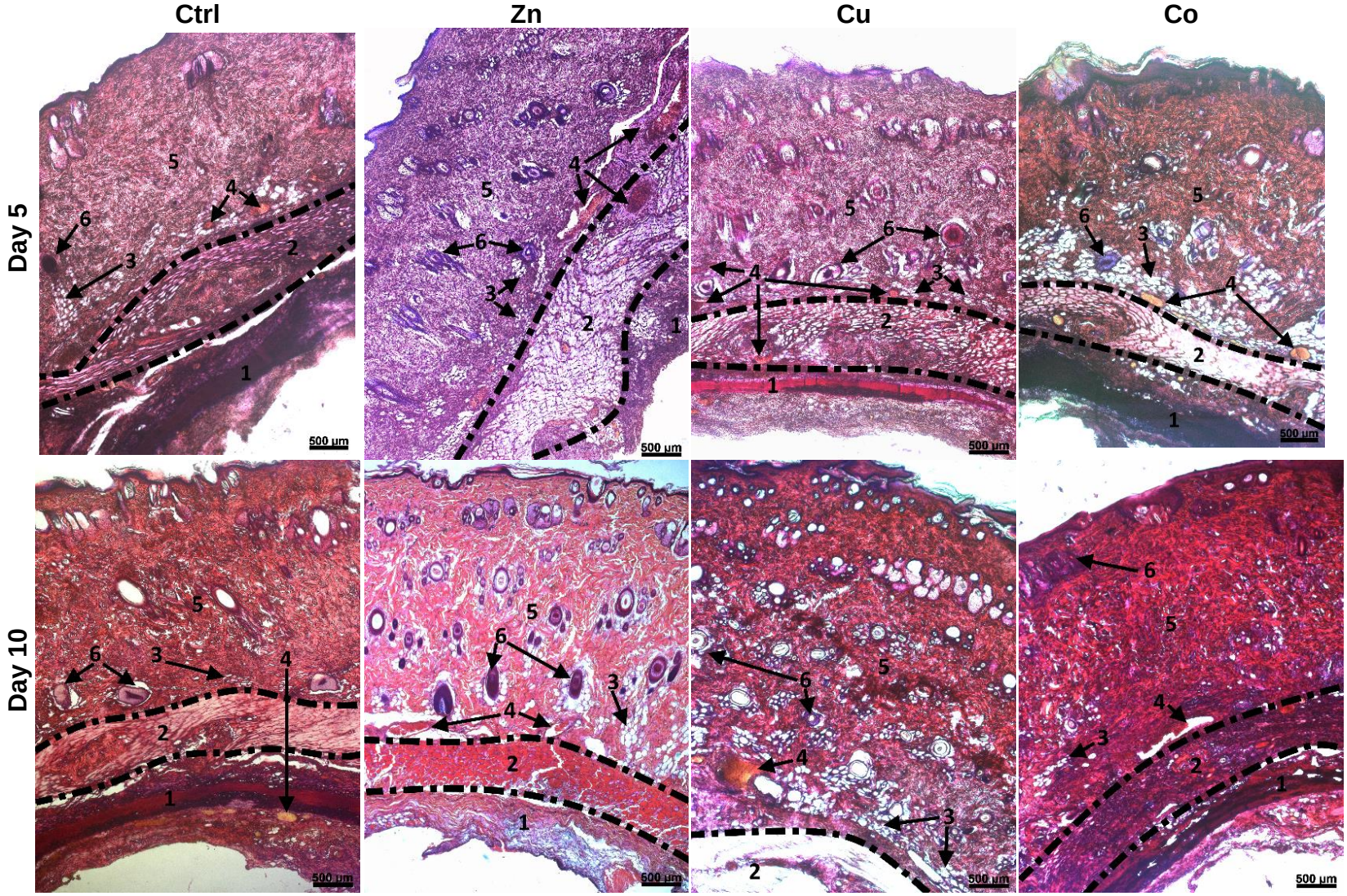


Figure S2. Representative bright-field microscopy images of H&E-stained cross-sections of skin explants contacted with subcutaneously implanted TM-doped cryogels.

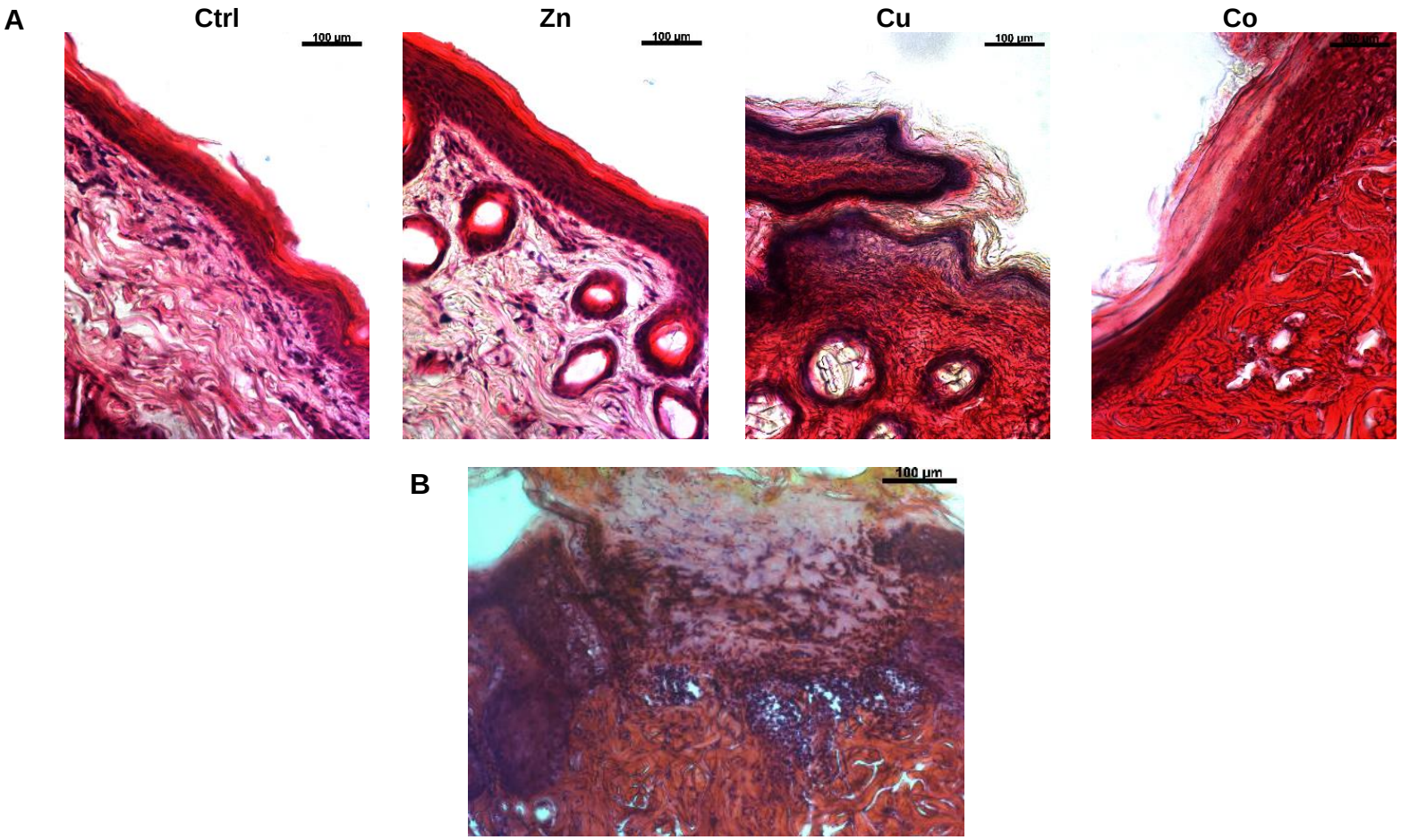


Figure S3. Visualization of epidermal layer of treated skin according to H&E staining. **A**. Representative bright-field microscopy images for non-doped and TM-doped cryogels. **B**. Localized area with dermatitis manifestations caused by Co-doped cryogel.

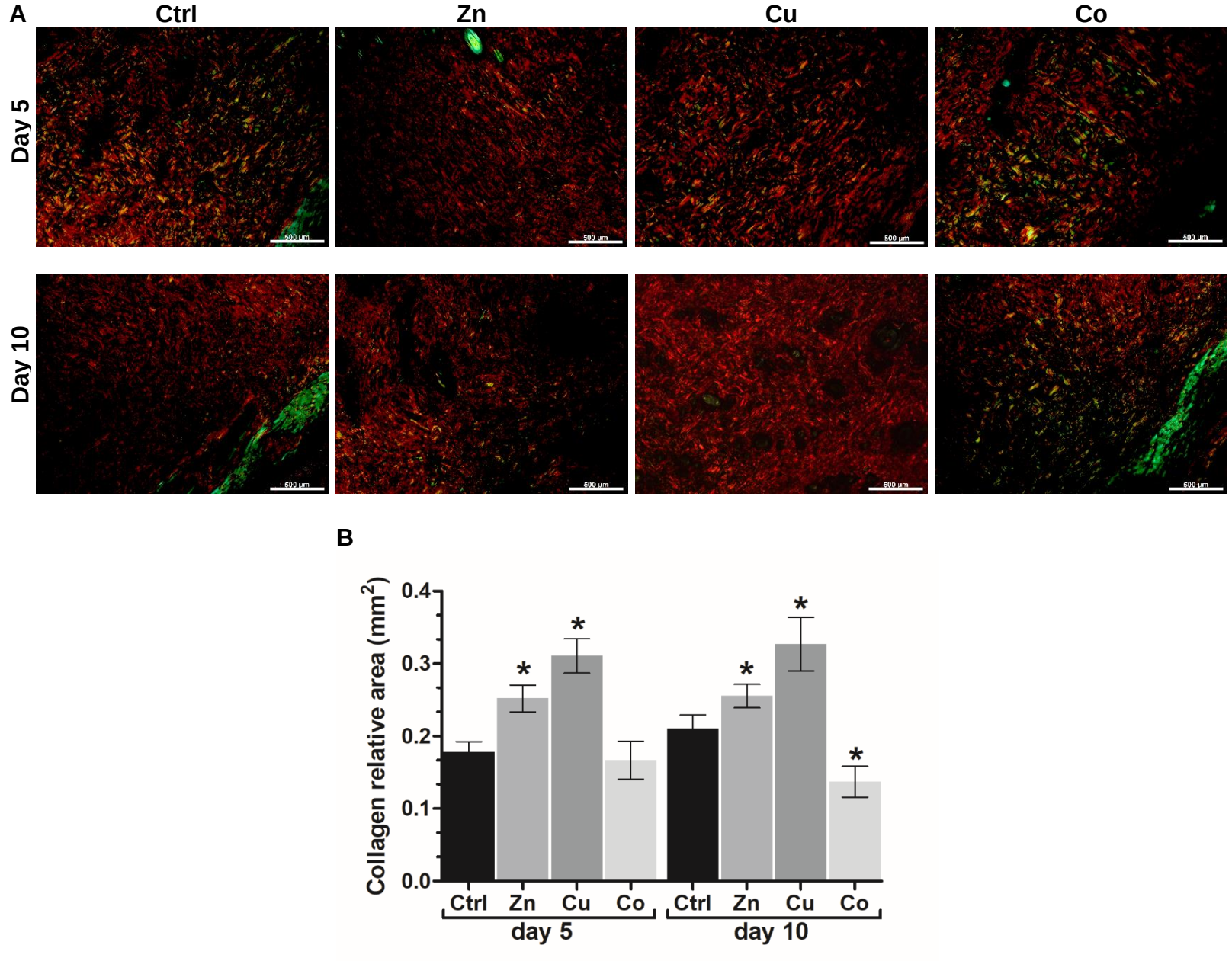


Figure S4. A. Representative polarization microscopy images of Picrosirius red-stained cross-sections of skin explants (dermal area) contacted with subcutaneously implanted TM-doped cryogels. B. Relative area of mature collagen per field of view (mean ± SD, $*p < 0.05$). Red-yellow and green structures, respectively, correspond to mature and immature collagens [41].

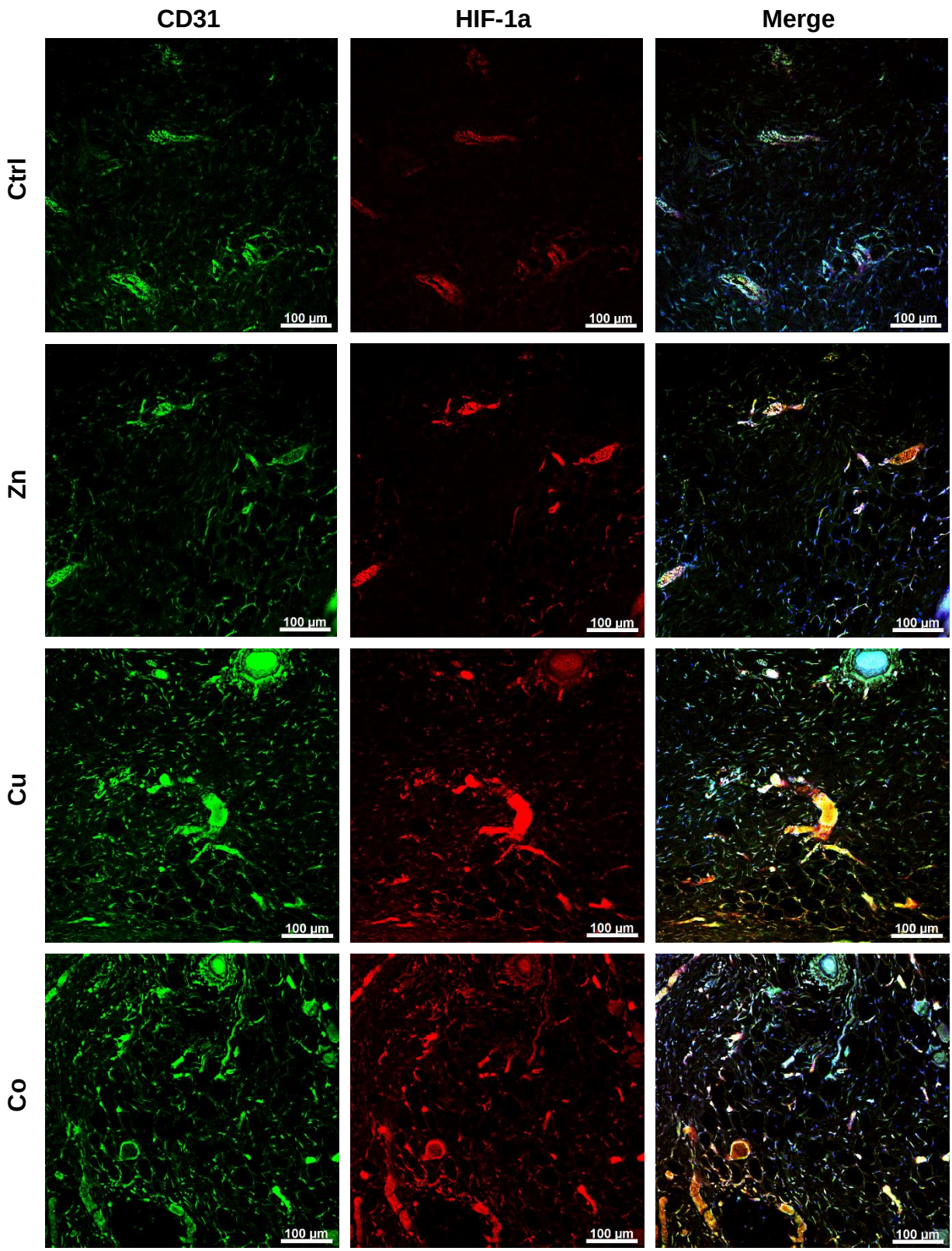
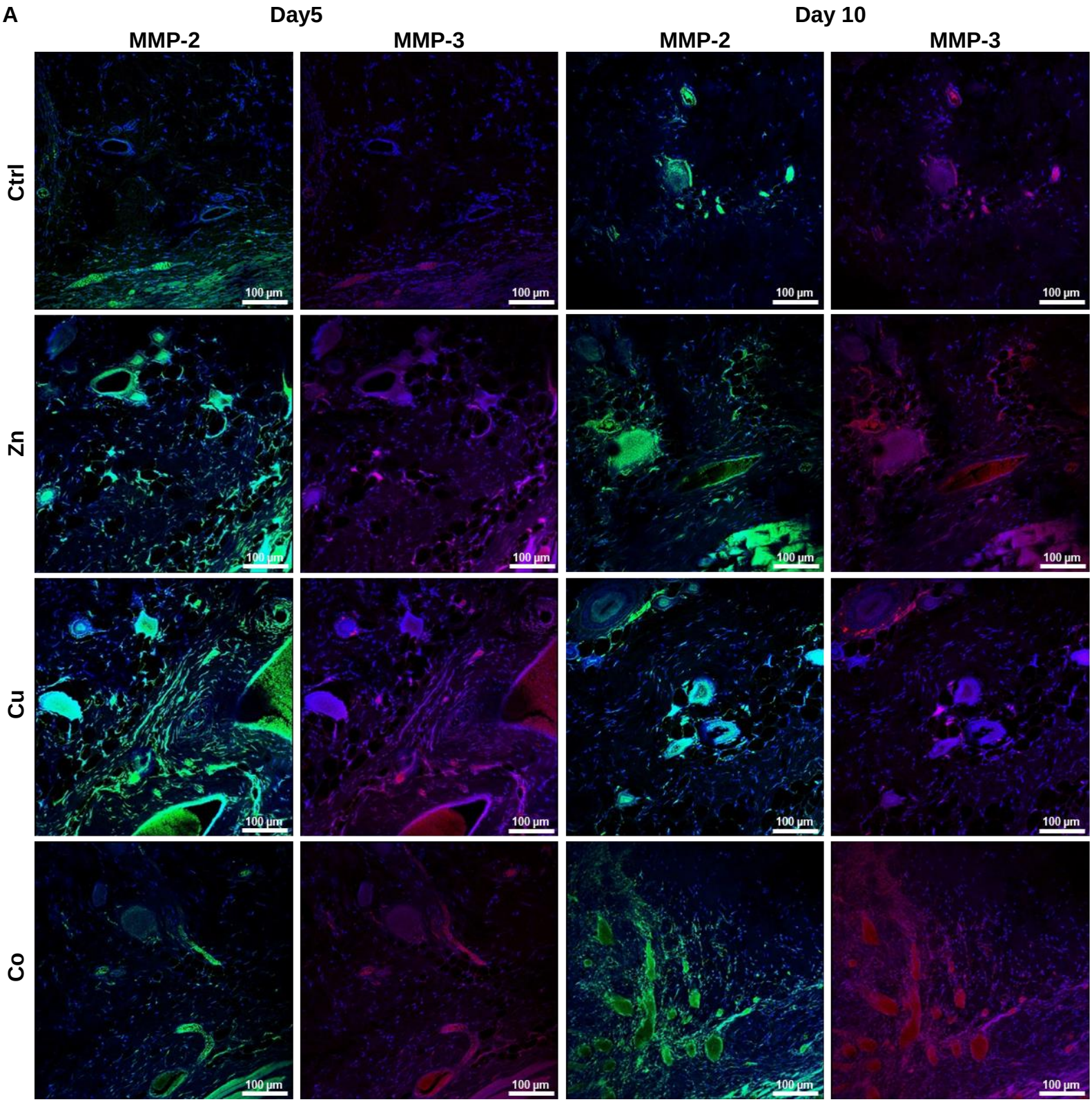


Figure S5. Immunofluorescent analysis of cross-sections of skin explants contacted with subcutaneously implanted TM-doped cryogels at day 5 (Zn and Cu) and day 10 (Ctrl and Co). Simultaneous CD31 CruzFluor™ 488 (green), HIF-1α Alexafluor 647 (red) and DAPI staining was performed.


A
Day5
Day 10
MMP-2
MMP-3
MMP-2
MMP-3
Ctrl
Zn
Cu
Co
100 µm

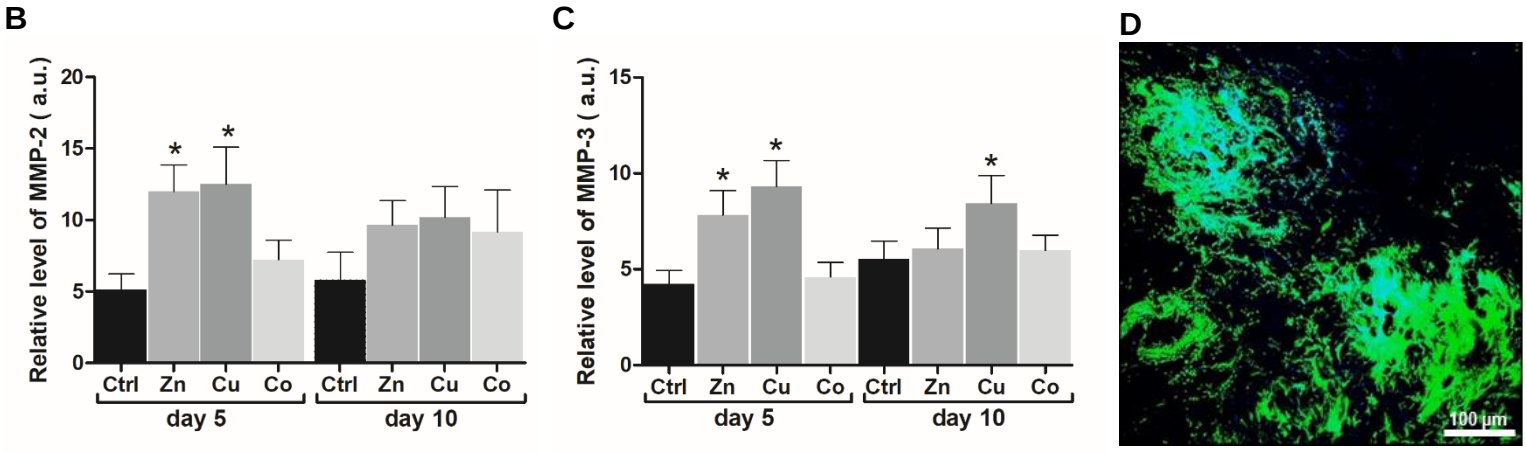


Figure S6. A. Immunofluorescent analysis of cross-sections of skin explants contacted with subcutaneously implanted TM-doped cryogels (MMP-2 AlexaFluor 488 (green) and MMP-3 Alexafluor 647 (red)). B, C – Relative MMP levels in the dermis per field of view (mean ± SD, *$p < 0.05$). D. The area with localized giant cells (MMP-2, Co-doped cryogel).

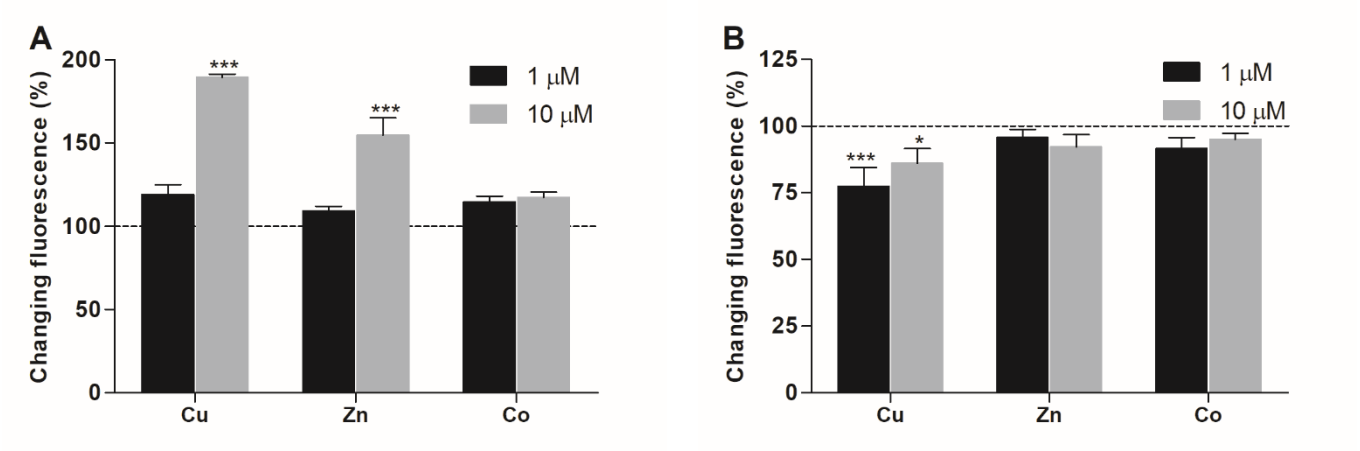


Figure S7. Effect of dissolved metals on relative levels of (A) ROS and (B) reduced glutathione in 3T3 fibroblasts according to DCFDA ($\lambda_{ex}/\lambda_{em}$ = 490/526) and monochlorobimane ($\lambda_{ex}/\lambda_{em}$ = 380/480) fluorescence, respectively. The cells were exposed to metal compounds for 1 h in HBSS. The values were presented as a relative fluorescence signal against the control cells with 100% fluorescence (mean ± SD, $n = 3$, *$p < 0.05$, ***$p < 0.001$).