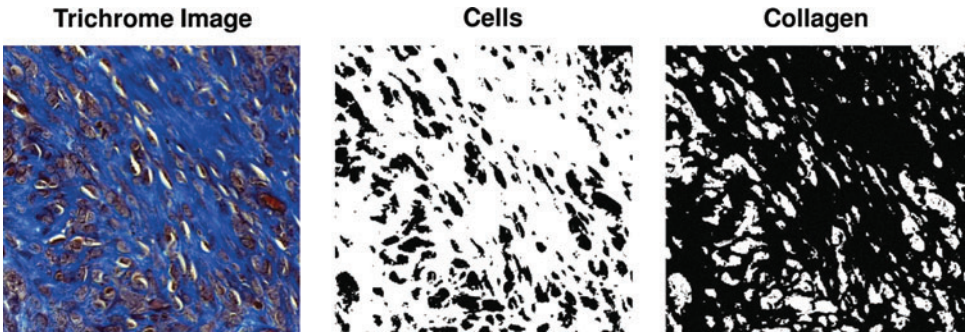
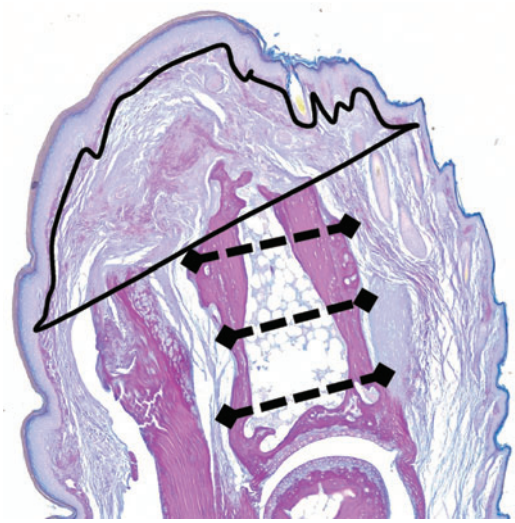
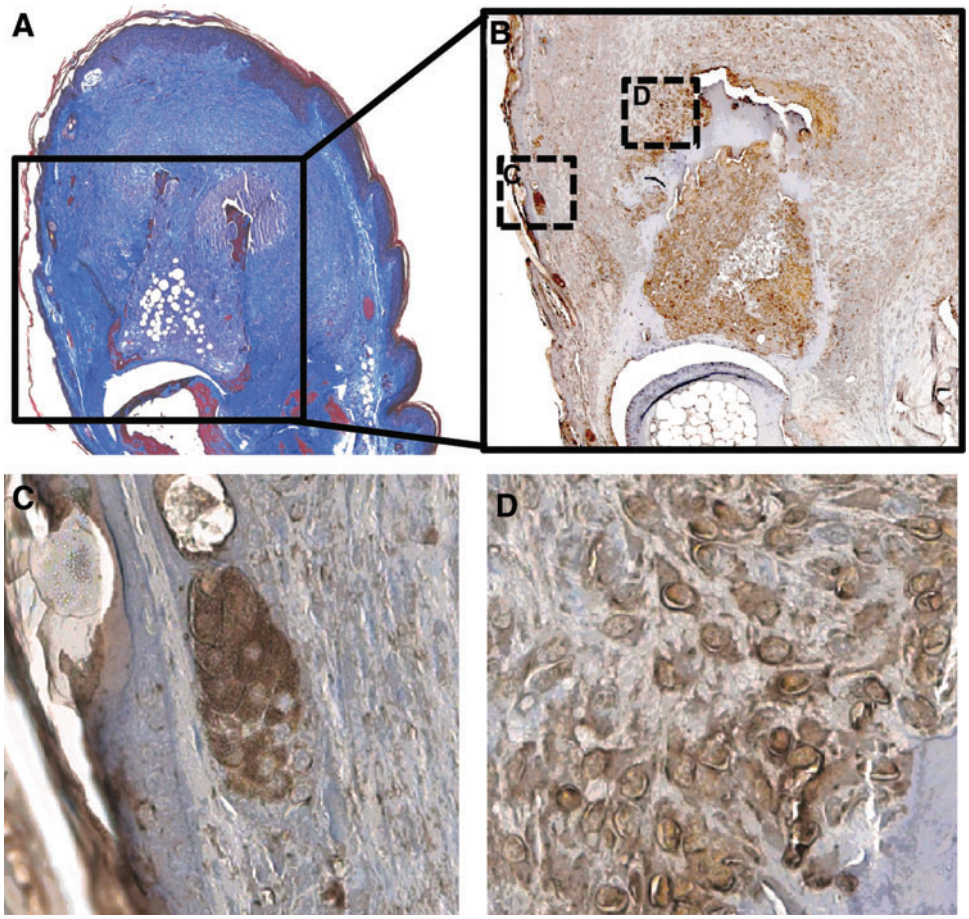
Supplementary Data



SUPPLEMENTARY FIG. S1. Examples of Trichrome nuclei-to-collagen ratio. After mid-second phalanx digit amputation and treatment with either extracellular matrix degradation products or corresponding control treatment, animals were euthanized at day 14 postamputation, and amputated digits were harvested, fixed in 10% neutral buffered formalin, decalcified, and sectioned for histologic analysis. Sections were stained with Masson's Trichrome stain to evaluate the ratio of cell to connective tissue at the site of amputation. Three images of the Trichrome-stained sections were randomly taken at a 32× magnification on an inverted microscope using AxioVision Rel 4.8 software. The relative cellularity and connective tissue in each image were calculated using a custom MATLAB script (version 7.11.0.584 R2010b; MathWorks). The MATLAB script is available upon request. Nuclei (purple) and connective tissue (blue) were isolated by hue histogram filtration and subsequent thresholding. Cellular density for each image was calculated as the ratio of the number of brown pixels to the number of blue pixels. Brown staining for nuclei and blue staining for collagen were isolated by hue histogram filtration. Cellular density was calculated as the ratio of the number brown pixels to the number of blue pixels.



SUPPLEMENTARY FIG. S2. Examples of Trichrome quantification of area. Analysis of soft-tissue area was found using ImageJ 1.44p (National Institutes of Health). Blue soft tissue distal to the site of amputation was manually outlined, and the area of the complex object was analyzed (in pixels). Three width measurements (in pixels) of the second phalanx were also taken at distal, mid, and proximal locations on the bone. Tissue growth was found as the area of blue soft tissue, as well as area of blue soft tissue normalized to the measured bone widths. There was no qualitative difference in the soft-tissue measurements distal to the site of amputation between un-normalized and normalized digits.



SUPPLEMENTARY FIG. S3. Example of the Sox2 staining. **(A)** Immunolabeling of amputated digits for Sox2 showed that Sox2+ cells were found predominantly either within or around the amputated P2 bone of the digit **(B)**. In each section, the hair follicles of the overlying skin were used as positive control for Sox2 immunoreactivity, since Sox2+ dermal stem cells are known to reside within the hair follicles **(C)**. Sox2 expression within the cells near the bone was predominantly cellular **(D)**.