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Ex Vivo Modeling of Multidomain Peptide Hydrogels with Intact Dental Pulp

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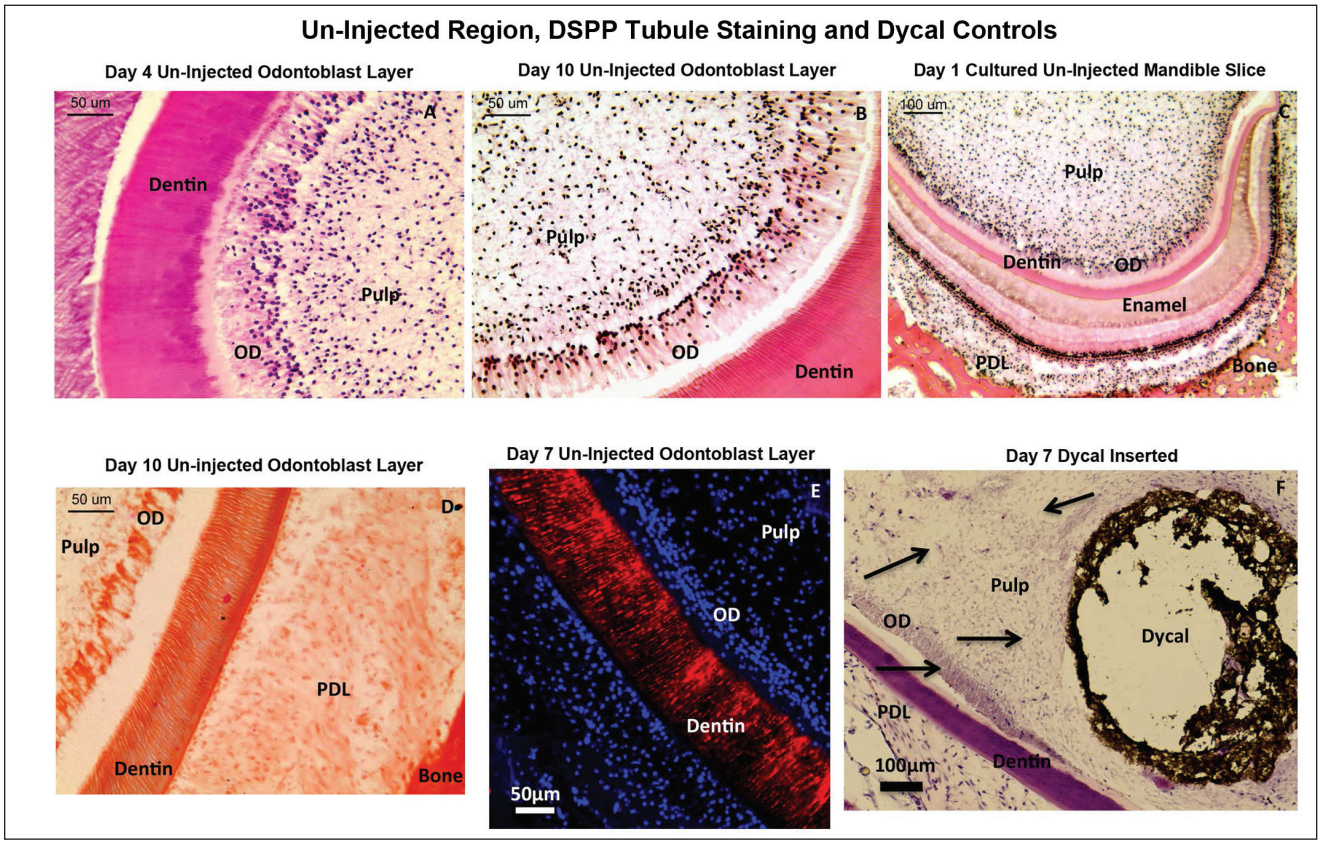
Appendix



Appendix Figure 1. Uninjected region, dentin sialophosphoprotein tubule staining and Dycal controls. Hematoxylin and eosin images of uninjected regions of the odontoblast (OD) space from days 4 (**A**) and 10 (**B**) post-injection. Normal pulp tissue architecture is seen. (**C**) A hematoxylin and eosin image of an uninjected mandible section after 24 h in culture. Normal pulp architecture is seen and is comparable to uninjected regions shown in panels A and B. PDL, periodontal ligament. (**D**) An image of a noninjected region of the OD layer stained with alizarin red at day 10 post-injection. This figure shows brighter red staining of the OD layer, dentin, and bone than surrounding soft tissues. (**E**) A representative dentin sialophosphoprotein immunostain of an uninjected region; extensive positivity in the dentinal tubules is seen. (**F**) A representative image of a mandible slice with Dycal inserted into the pulp and cultured for 7 d. In contrast to injected multidomain peptide scaffolds, a loss of both pulp core and OD cell nuclei adjacent to the inserted Dycal suggests a deleterious effect on pulp tissues. There is also evidence of emerging gaps in the matrix of the pulp, indicating a loss of vitality. These regions are indicated by arrows. In contrast, regions of the pulp farther from the inserted Dycal exhibited a normal histologic appearance and visible cell nuclei.

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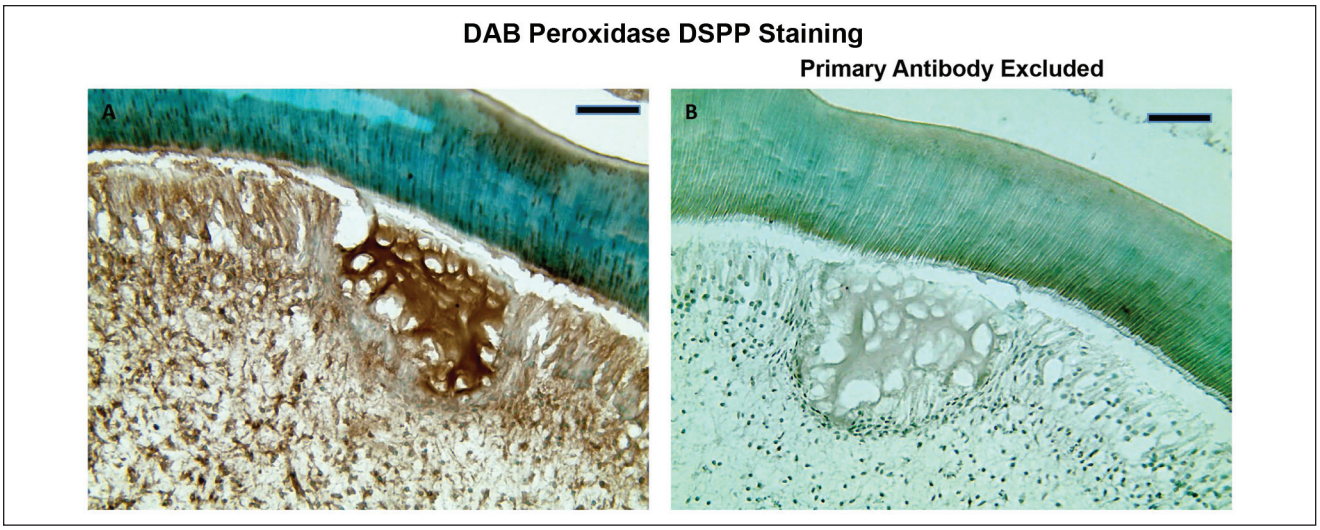
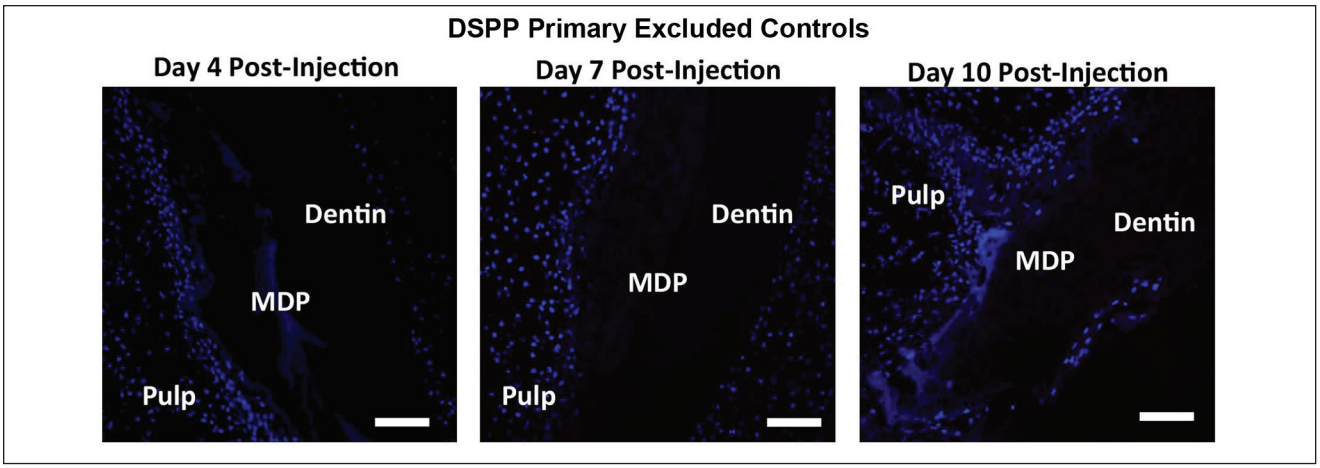
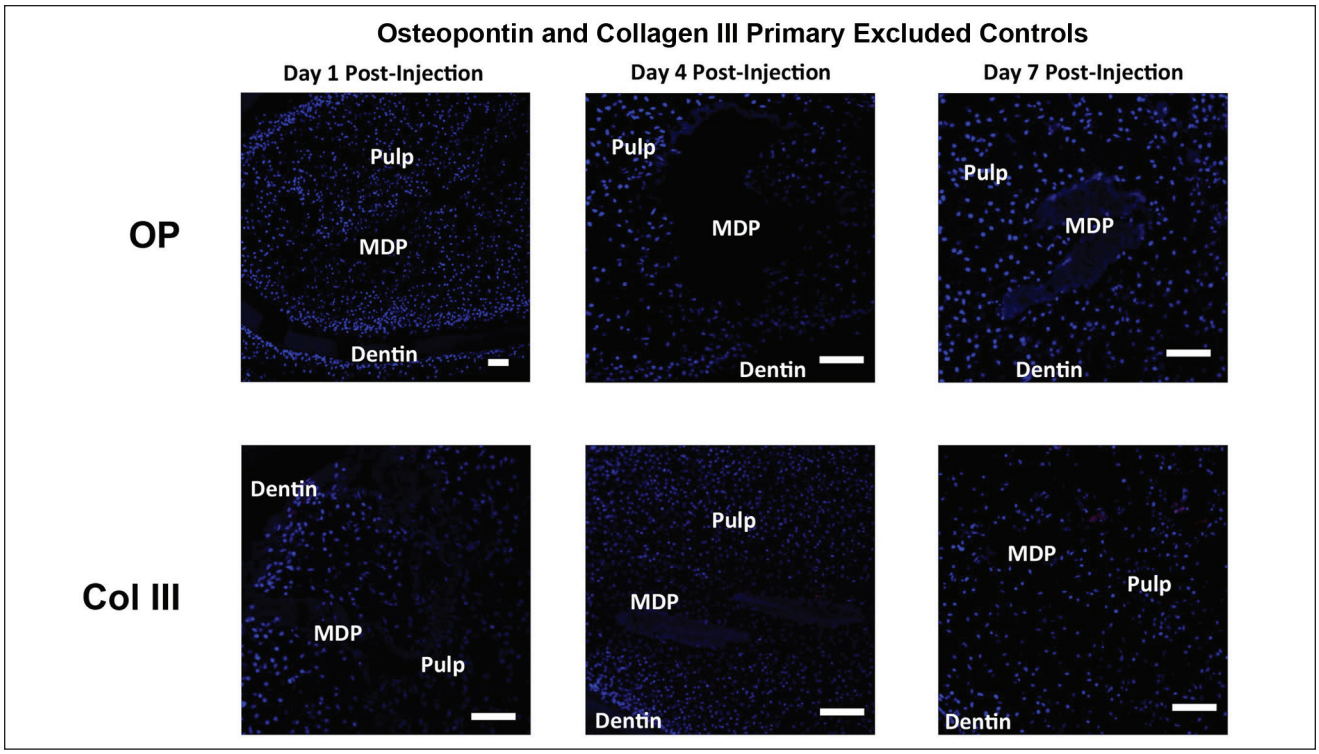


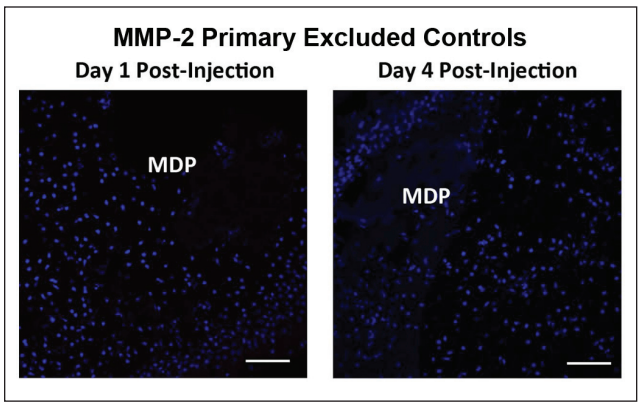
Appendix Figure 2. Diaminobenzidine (DAB) peroxidase dentin sialophosphoprotein (DSPP) staining. (**A**) DAB peroxidase immunostaining of DSPP expression at 7 d post-injection. These were performed to confirm the observed distribution of DSPP shown in Figure 4. Relatively high levels of DSPP expression are seen in the dentinal tubules, associated with odontoblasts and in the injected multidomain peptide hydrogel. (**B**) A primary excluded negative control is shown, with minimal staining. Green, methyl green counterstain; brown, DSPP expression. Scale bars = 50 μm.



Appendix Figure 3. Primary excluded negative controls for dentin sialophosphoprotein immunostaining at days 4, 7, and 10 post-injection. No red staining is visible in any of the negative controls. Blue, DAPI-stained nuclei. Scale bars = 50 μm. MDP, multidomain peptide.



Appendix Figure 4. Primary excluded negative controls for collagen III (Col III) and osteopontin (OP) immunostaining at days 1, 4, and 7 post-injection. No red staining is visible in any of the negative controls. Blue, DAPI-stained nuclei. Scale bars = 50 µm. MDP, multidomain peptide.



Appendix Figure 5. Primary excluded negative controls for matrix metalloproteinase 2 immunostaining at days 1 and 4 post-injection. No red staining is visible in any of the negative controls. Blue, DAPI-stained nuclei. Scale bars = 50 µm. MDP, multidomain peptide.