SUPPLEMENTARY INFORMATION

DNA hydrogels for bone regeneration

Dimitra Athanasiadou[1], Nadeen Meshry[1], Naara G. Monteiro[2], Ana C. Ervolino-Silva[2], Ryan Lee Chan[3], Christopher A. McCulloch[1], Roberta Okamoto[2], Karina M. M. Carneiro[1,3]

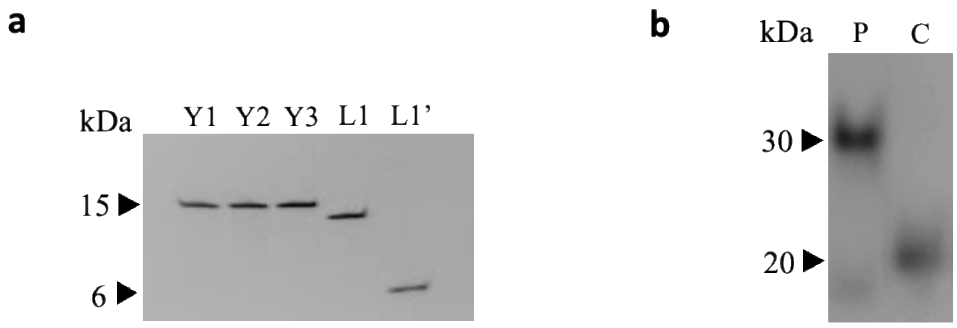
[1]Faculty of Dentistry, University of Toronto, Toronto, Canada

[2]Department of Basic Sciences, São Paulo State University - UNESP School of Dentistry at Araçatuba, Araçatuba, SP, Brazil

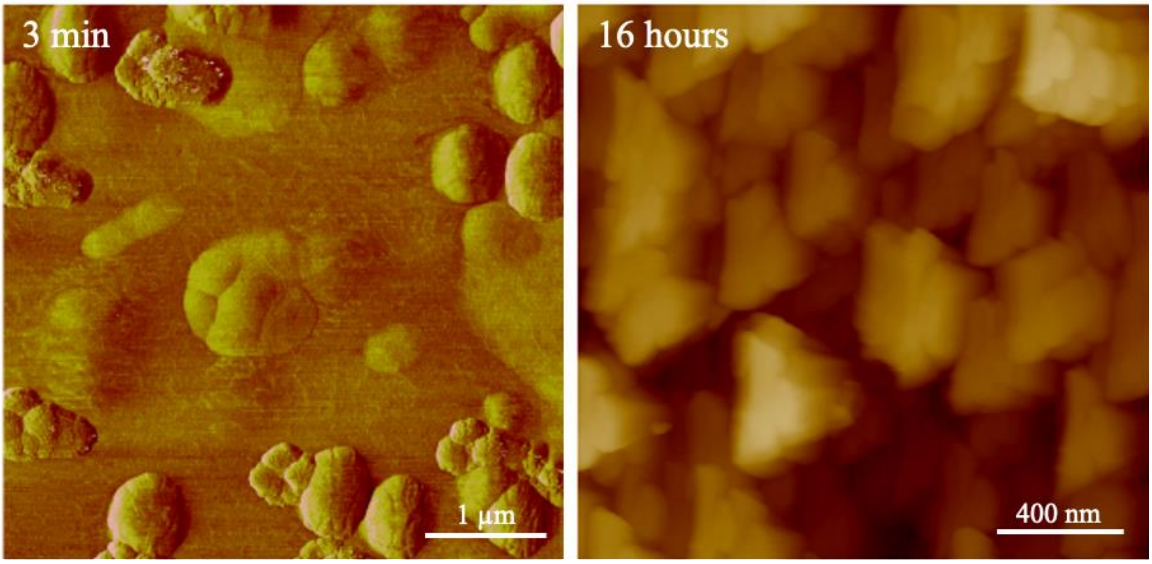
[3]Institute of Biomedical Engineering, University of Toronto, Toronto, Canada

Supplementary Table 1: Complementary strands for DNA hydrogel synthesis.

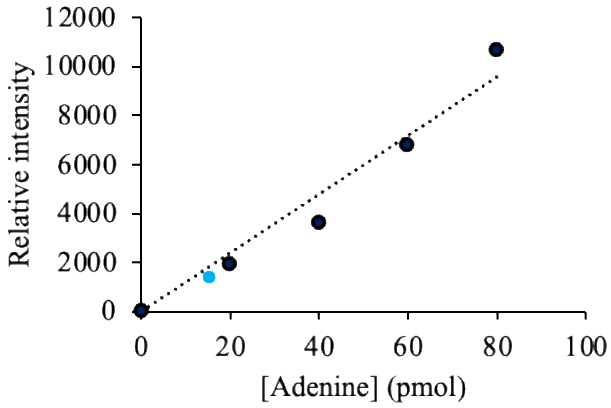
Strand	Sequence
Y1	5’-CGA TTG ACT CTC CAC GCT GTC CTA ACC ATG ACC GTC GAA GCG ATT GAC TCT C-3’
Y2	5’-CGA TTG ACT CTC CTT CGA CGG TCA TGT ACT AGA TCA GAG GCG ATT GAC TCT C-3’
Y3	5’-CGA TTG ACT CTC CCT CTG ATC TAG TAG TTA GGA CAG CGT GCG ATT GAC TCT C-3’
L1	5’-GAG AGT CAA TCG CGT CGT AGC AGT GTC AGG TAA GAG AGT CAA TC-3’
L1’	5’-TTA CCT GAC ACT GCT ACG ACG-3’



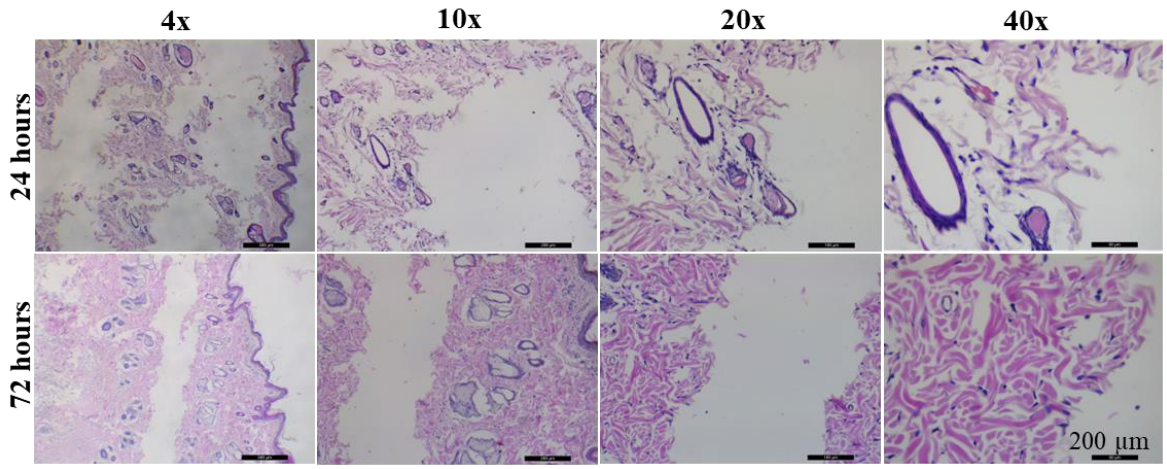
Supplementary Fig. S1: a Denaturing and **b** native PAGE experiments characterizing the DNA constructs that make the DNA hydrogel: three (Y1, Y2 and Y3) and two (L and L1’) DNA strands assemble into prepolymer (P) and crosslinker (C) DNA constructs, respectively.



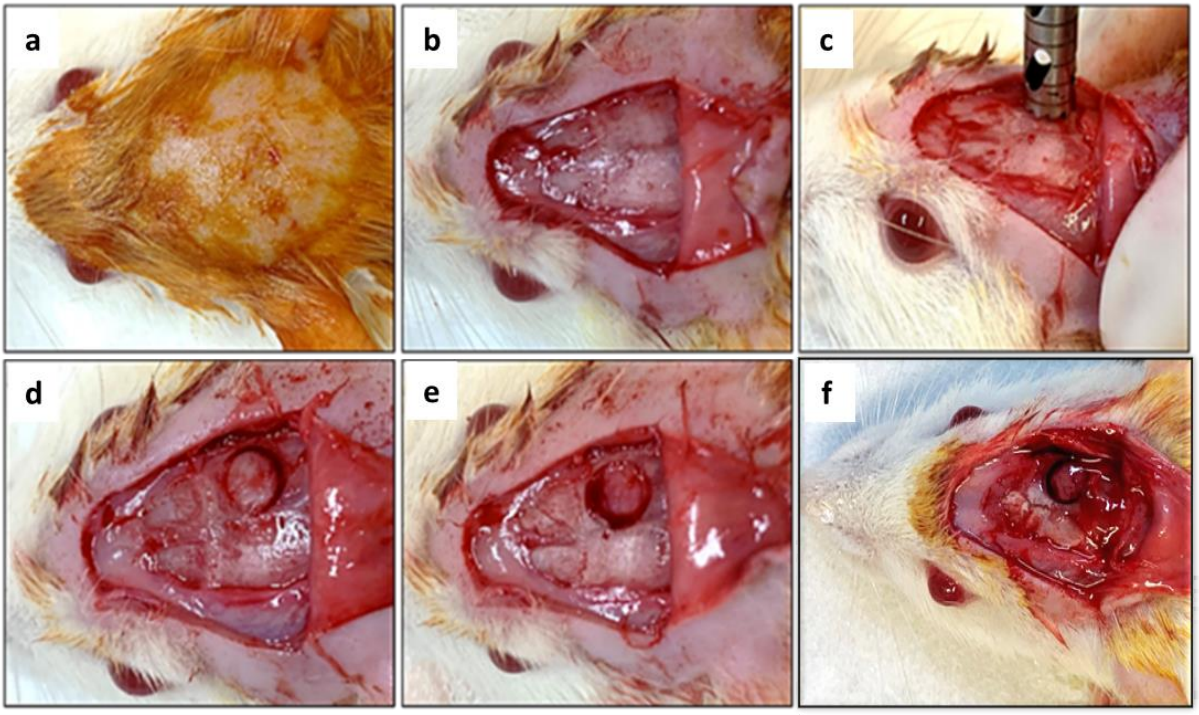
Supplementary Fig. S2: Calcium phosphate formation of DNA hydrogel after 3 minutes (left) and 16 hours (right) of mineralization.



Supplementary Fig. S3. Adenine quantification in degraded DNA hydrogel after 7-day incubation in cell culture medium containing 10% FBS (blue) in comparison to a standard curve (black).

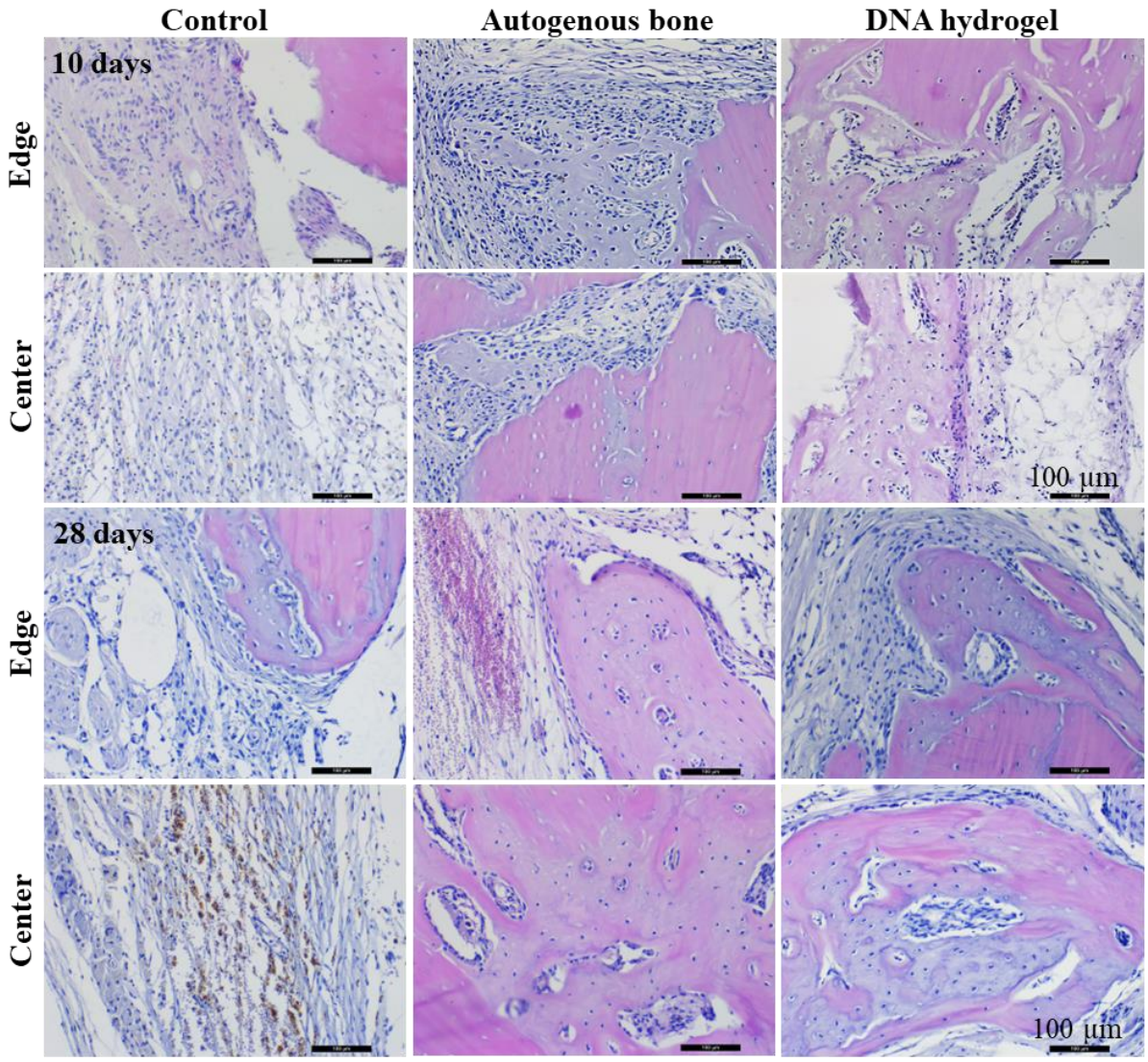


Supplementary Fig. S4. Inflammation experiment by implantation in the dorsal subcutaneous tissue of rats after 24- and 72-hours post-surgery.

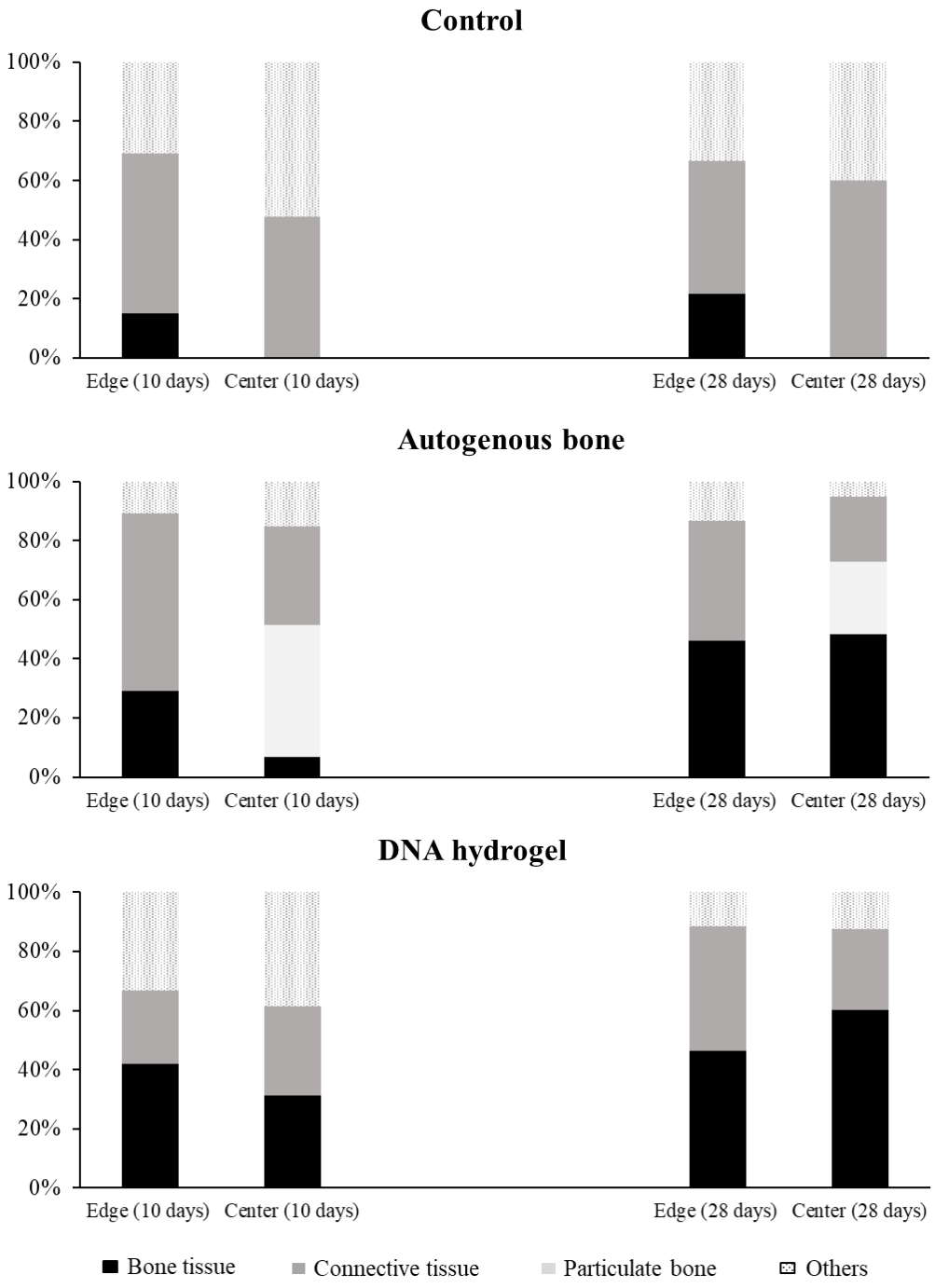


Supplementary Fig. S5. Surgical procedure for creating a critical defect in calvaria. a Trichotomy and antisepsis of the operative area. **b** U-shaped incision. **c** 5 mm diameter inner drill

bit positioning for defect creation. **d** Osteotomy in right parietal bone. **e** Parietal bone remotion from inside the defect. **f** DNA hydrogel.



Supplementary Fig. S6. Histological analysis of the studied groups from the edge and the center of the rat calvarial defect at 10- and 28-days post-surgery.



Supplementary Fig. S7. Quantitative analysis of the formed tissue among the studied groups.

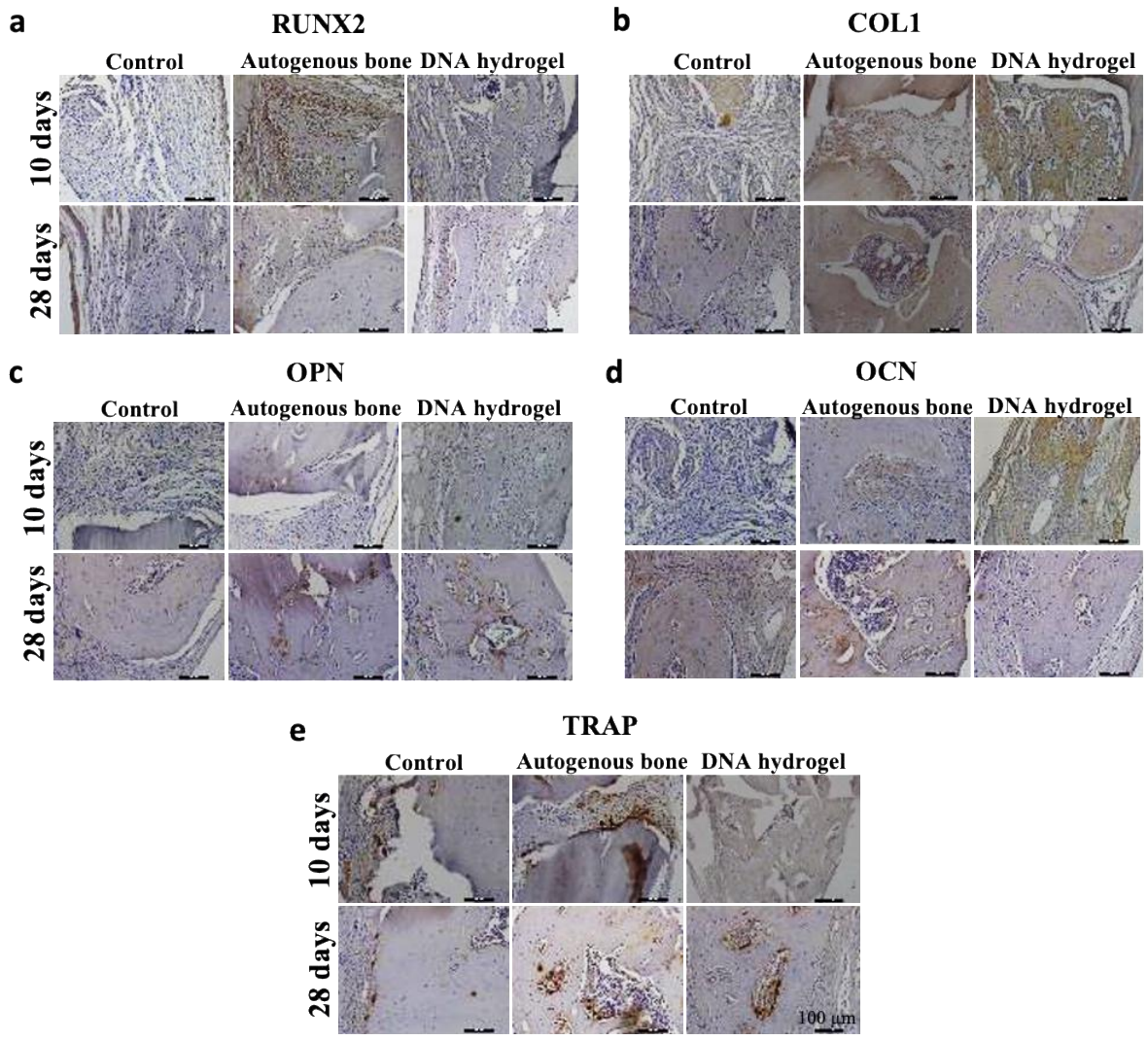


Fig. S8: Immunolabeling analysis during DNA hydrogel calvaria healing. Immunohistochemical detection of osteogenic differentiation markers **a** Runt-related transcription factor 2 (RUNX2), **b** Collagen type I (Col), **c** Osteopontin (OPN), **d** Osteocalcin (OCN) and **e** tartrate-resistant acid phosphatase (TRAP) among the studied groups at 10- and 28-days post-surgery.