## Supplementary:

# Proteolytic Nanoparticles Replace a Surgical Blade by Controllably Remodeling the Oral Connective Tissue

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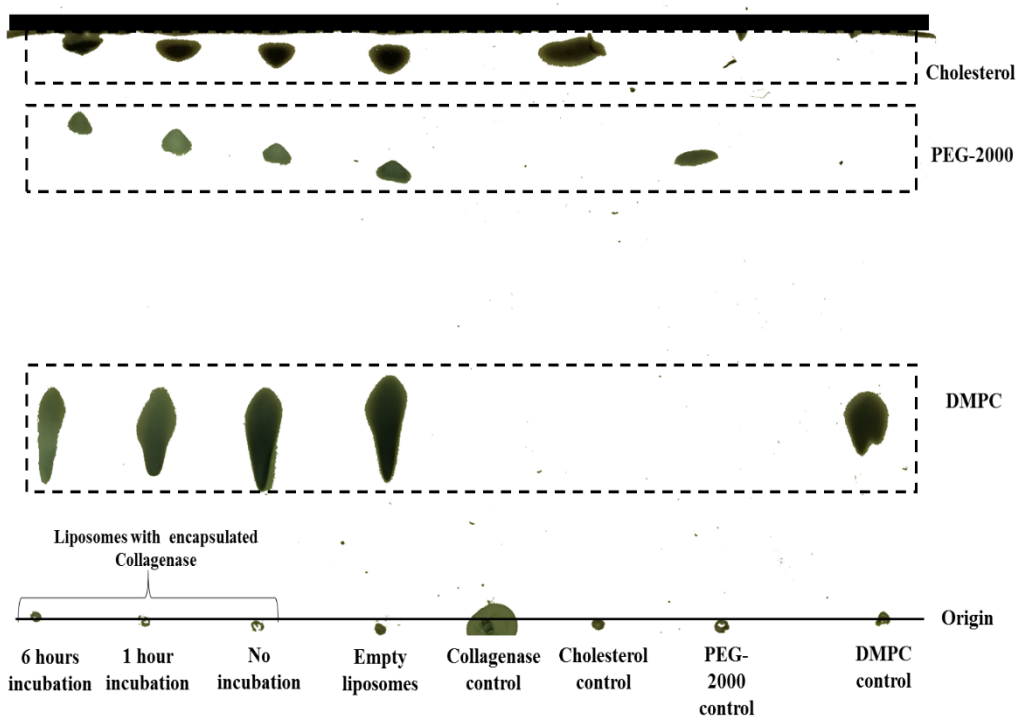
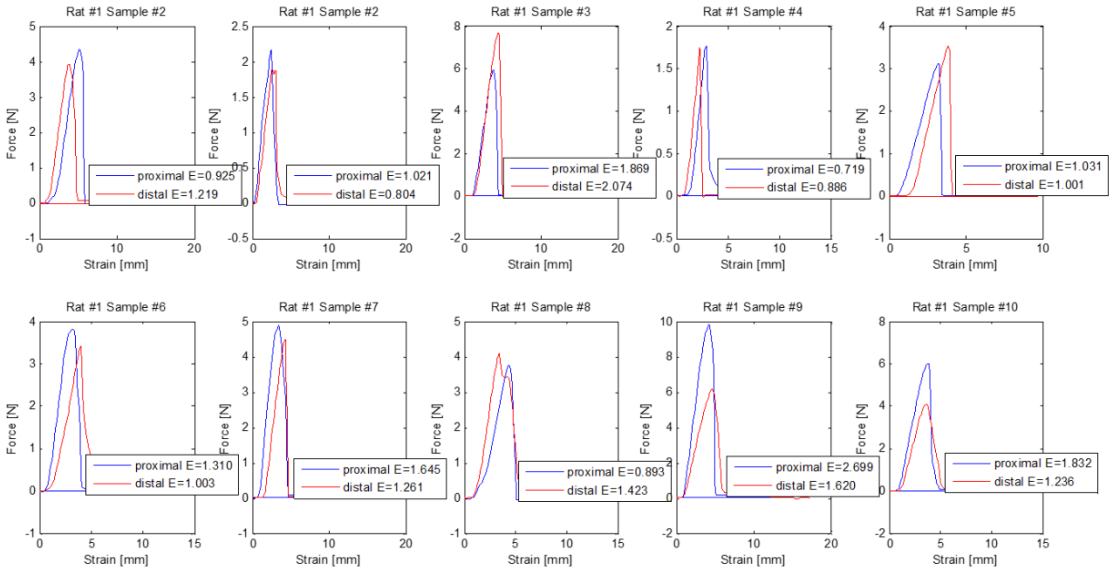
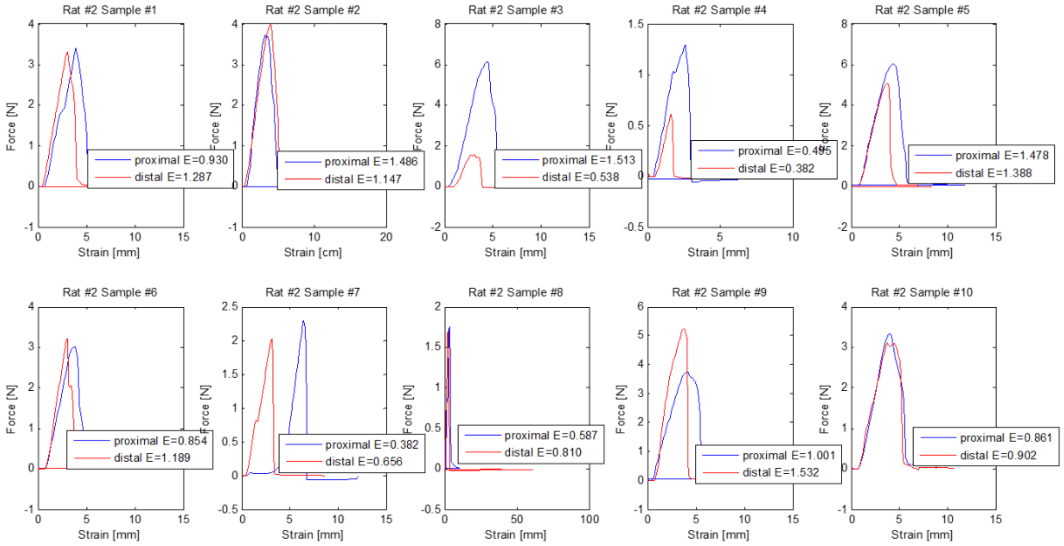



**Fig. S1. Collagenase doesn't affect the liposome's structure.** Liposomes were synthesized and split into two groups: empty liposomes and collagenase encapsulated. Liposomes were dissolved in a Tris-buffer with $Ca^{+2}$ ions. All groups were tested in a TLC assay, versus all 4 of their components separately: collagenase, cholesterol, PEG-2000 and DMPC. The liposome's components show a similar displacement to their respective control test, and the same results can be seen between both test groups. We conclude that the enzyme has no effect on the liposome's structure.

**A**


Rat #1 Sample #2
proximal E=0.925
distal E=1.219
Rat #1 Sample #2
proximal E=1.021
distal E=0.804
Rat #1 Sample #3
proximal E=1.869
distal E=2.074
Rat #1 Sample #4
proximal E=0.719
distal E=0.886
Rat #1 Sample #5
proximal E=1.031
distal E=1.001
Rat #1 Sample #6
proximal E=1.310
distal E=1.003
Rat #1 Sample #7
proximal E=1.645
distal E=1.261
Rat #1 Sample #8
proximal E=0.893
distal E=1.423
Rat #1 Sample #9
proximal E=2.699
distal E=1.620
Rat #1 Sample #10
proximal E=1.832
distal E=1.236
Force [N]
Strain [mm]

**B**


Rat #2 Sample #1
proximal E=0.930
distal E=1.287
Rat #2 Sample #2
proximal E=1.486
distal E=1.147
Rat #2 Sample #3
proximal E=1.513
distal E=0.538
Rat #2 Sample #4
proximal E=0.495
distal E=0.382
Rat #2 Sample #5
proximal E=1.478
distal E=1.388
Rat #2 Sample #6
proximal E=0.854
distal E=1.189
Rat #2 Sample #7
proximal E=0.382
distal E=0.656
Rat #2 Sample #8
proximal E=0.587
distal E=0.810
Rat #2 Sample #9
proximal E=1.001
distal E=1.532
Rat #2 Sample #10
proximal E=0.861
distal E=0.902
Force [N]
Strain [mm]
Strain [cm]

**C**

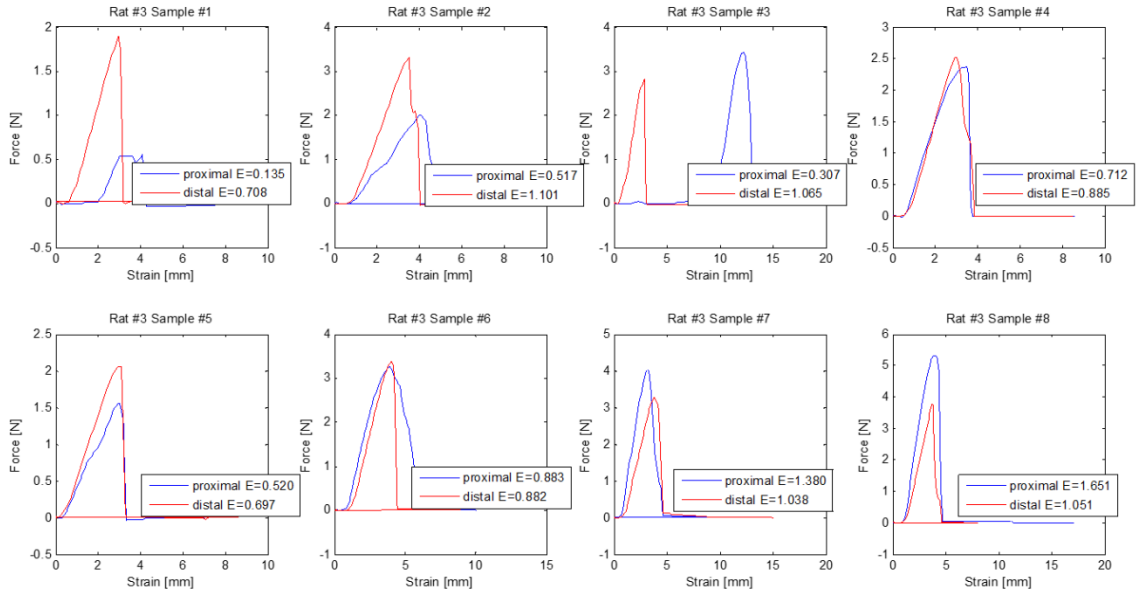



**Fig. S2. More than 300 Collagen fibers were stressed during the ex-vivo experiments.** Each fiber served as is its own control by exposing half the fiber to collagenase and the other half to an aqueous buffer. Each fiber was cut to: proximal and distal part. The two parts were stressed using the sensitive Instron machine and exhibited the same mechanical characters (A, B, C). This allowed us to treat one half of the fiber as control while the other half was treated as test during this research.

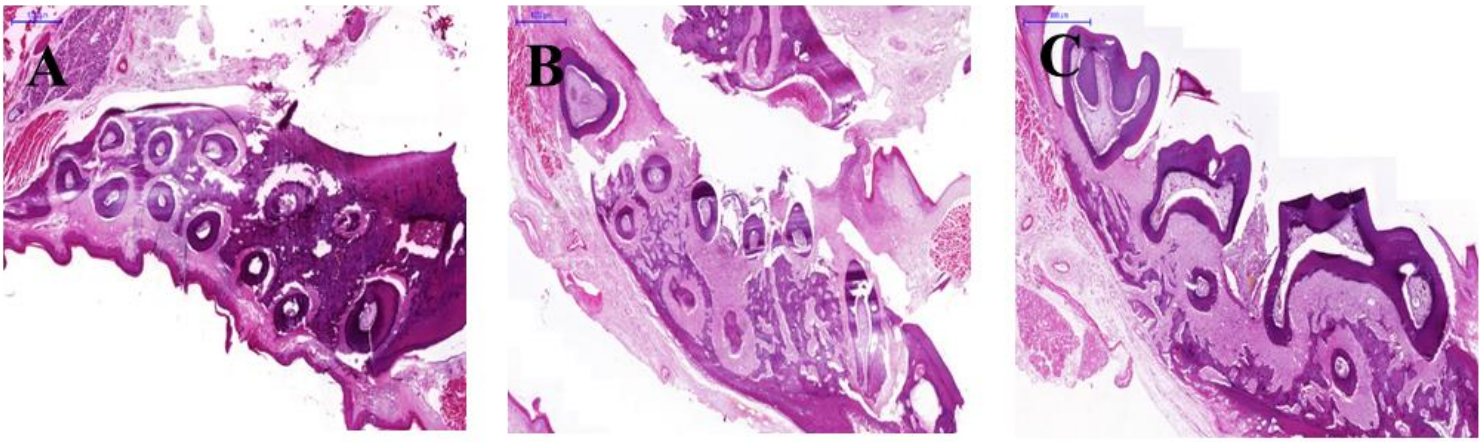



**Fig. S3. Inflammation assessment**. Axial histology cuts were performed in order to evaluate the damage caused by the different treatments: (A) braces, (B) traditional surgery plus braces, or, (C) nano-surgery plus braces. Among all cuts the same mild inflammation, typical for orthodontic treatment was observed.

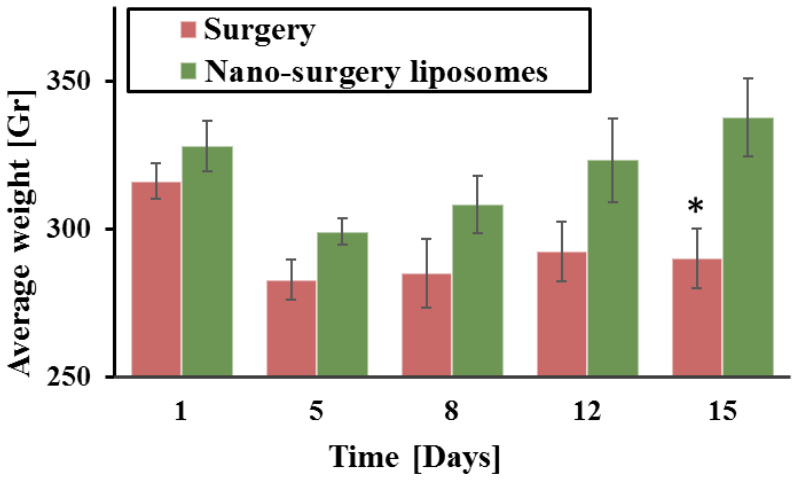



**Fig. S4. Animals weight fluctuations among the Nano surgery liposomes group and surgery groups throughout the experiment were measured.** The rats average weight in the surgery group decreased by 8.2%, while the average weight of the rats in the nano-surgery group decreased by only 2.7%. The wellbeing of the rats was monitored by weighing them every 3-4 days and following any behavioral changes. The weight decrease in both groups is attributed and is expected in orthodontic treatments, over the first 5 days. No unusual behavioral changes were observed.

A statistical analysis of day 15, that included Leven's Test for variances and a two-sample t-test assuming equal variances, concluded that the difference between the decrease in weight of the rats in both groups is significant.

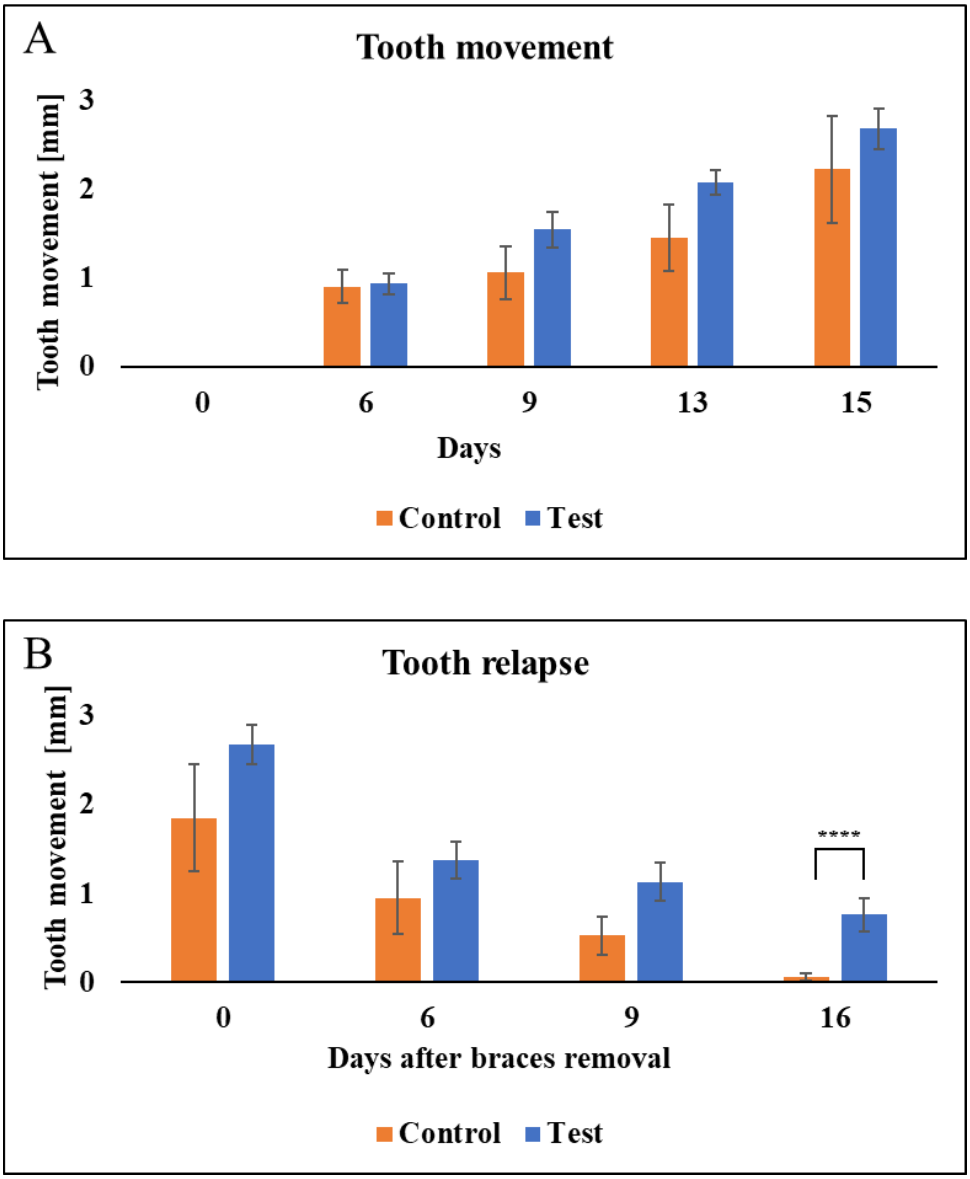



**Fig. S5. Enzymatic nano-surgery reduces tooth relapse.** 15 days after completing the tooth movement (A) we removed the braces and we measured tooth relapse disposition in both groups (B). We found that the nano-surgery treated group did not complete their tooth relapse, while the control group completed their tooth relapse within 16 days. We attribute this elongated tooth relapse to the rapid regeneration of the collagen fibers and bone (Fig. 3H.); ****, indicates two tail t-test with equal variances P-Value <0.0001.

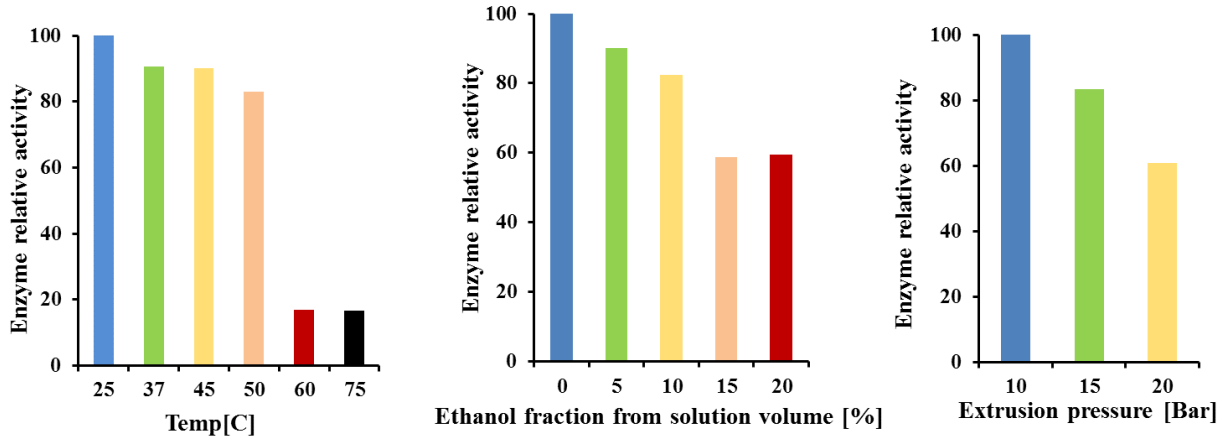



**Fig. S6. Collagenase durability test assists in collagenase encapsulation characterization.** Activity of the enzyme was determined according to the fluorescence change incline with respect to time. Optimize temp= 37C, ethanol fraction=10% and extrusion pressure= 10 bar were determined for optimal encapsulation.

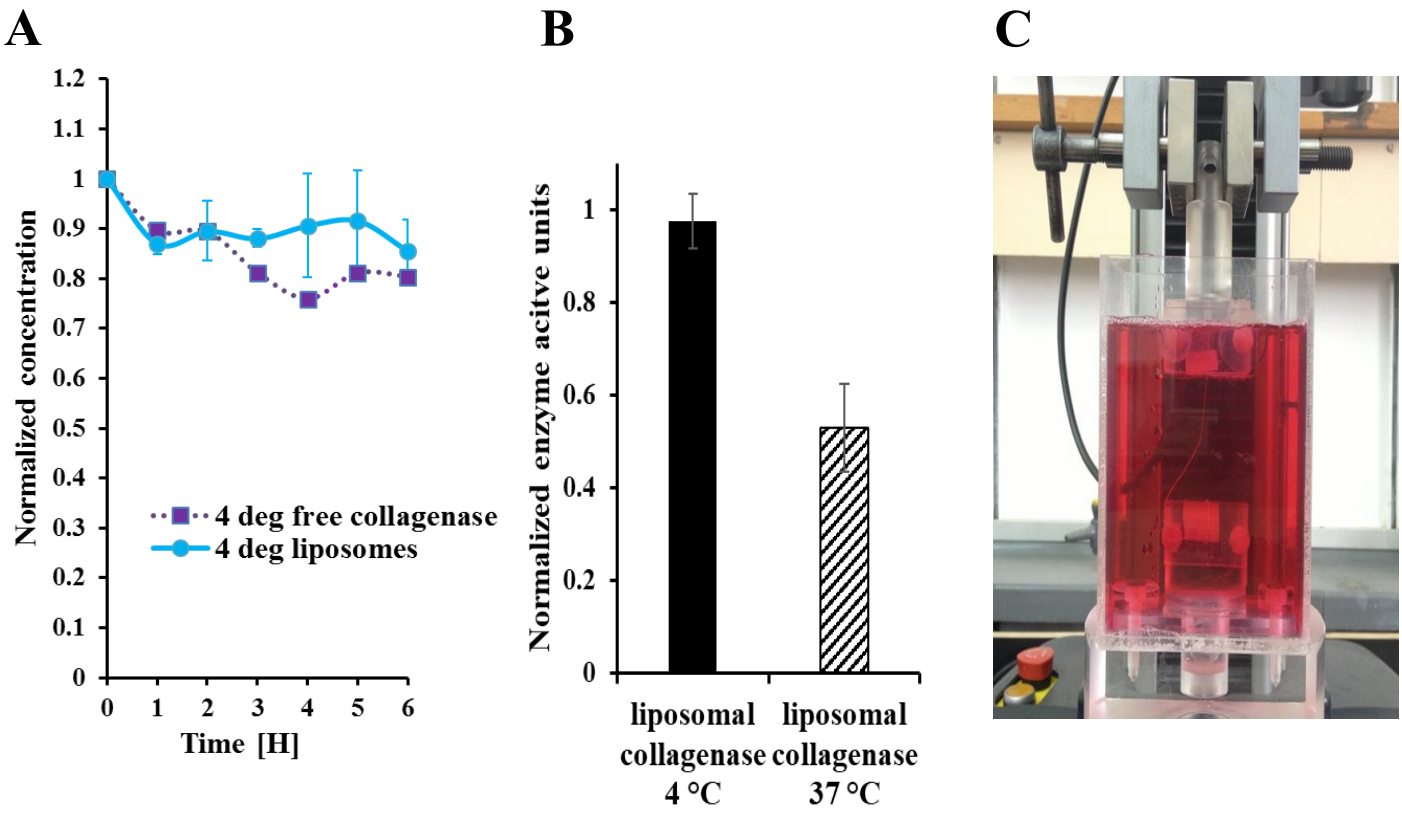



**Fig. S7 Collagenase activity profile.** (A) Aactivity of free collagenase and collagenase encapsulated liposomes was measured every hour for 6 hours while incubating at 4˚C. The activity of the liposomal collagenase was also tested 24 hours after the incubation beginning (B). This activity was normalized to the initial time measurement which remained similar in both test groups.

**(C) Measuring the fiber normalized strength**. To test the fibers normalized strength before and after the treatment, the collagen fibers were stressed using a LLOYD LF-Plus Materials Testing Machine, in physiological buffer containing: DMEM, 10% FCS, 1% PS,1% L-Glu, 1%. Collagen fiber stressing movie using this system is available on: https://youtu.be/jNtSw1hBpSM

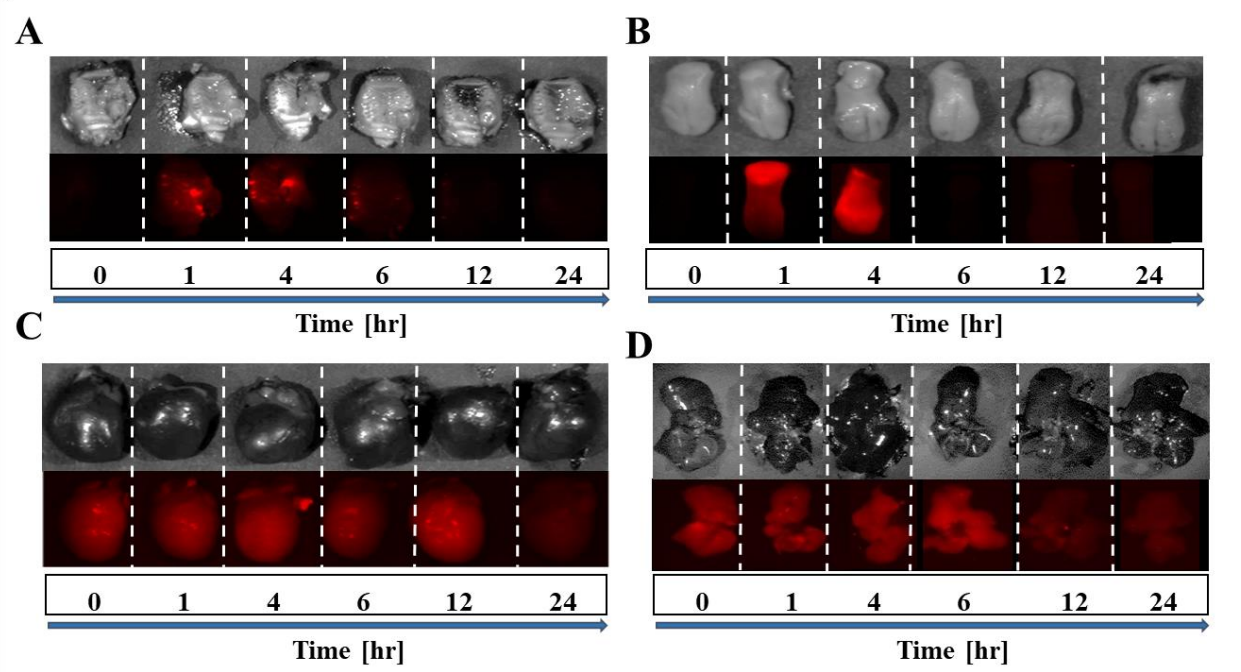



**Fig. S9. Biodistribution of the enzyme over time**. Florescent (ICG) liposomes were placed in the sulcus and the florescent signal was recorded over time in the gingival tissue (A), tongue (B), heart (C) and liver (D).