SUPPLEMENTAL FILE

Eugenol Nanocapsule for Enhanced Therapeutic Activity Against Periodontal Infections

Kannissery Pramod^{1,†}, M. R. Aji Alex², Manisha Singh³, Shweta Dang³, Shahid H. Ansari⁴, Javed Ali^{1,*}

¹ Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India.

² Centre for Biomedical Engineering, Indian Institute of Technology (IIT), Hauz Khas, New Delhi – 110 016, India.

³ Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector, Noida - 201307, India.

⁴ Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India.

[†] Present address: College of Pharmaceutical Sciences, Govt. Medical College, Trivandrum – 695011, India.

Corresponding author: * Javed Ali, E-mail address: javedaali@yahoo.com, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India. Tel.: +91 9811312247; Fax: +91 11 2605 9663.

Table S1 Grading system for semi-quantitative analysis of the interdental papilla

Observation	Grade
Continuity of epithelium	
Intact (continuity of the epithelium along the whole interdental papilla)	0
Partly absent (small or large disruptions of the epithelium)	1
Absent (complete absence of epithelium on the interdental papilla, but no exudates of inflammatory cells)	2
Absent + exudate (same as grade 2, but presence of an exudate)	3
Continuity of transseptal fibers	
Intact (no disruption of transseptal fibers)	0
Partly absent (some transseptal fibers still present)	1
Absent (complete disruption of transseptal fiber system)	2

Table S2 *In vitro* release profile of eugenol from optimized polycaprolactone nanocapsules

Time (h)	Mean CPDR* ± SD (%) (n=3)
1	29.73 ± 3.98
2	42.82 ± 6.17
4	63.06 ± 5.67
6	82.26 ± 5.08
8	85.11 ± 6.30
10	89.38 ± 6.21
12	88.96 ± 5.28
24	92.43 ± 4.12
48	97.36 ± 5.35
72	96.49 ± 3.87

*PCDR = percentage cumulative drug release

Figure S3 Eugenol release kinetics from the nanocapsules; (A) Zero order; (B) First order; (C) Higuchi model

Table S4 Kinetics of drug release from the optimized nanocapsules

Zero order		First order		Higuchi	
K_0 (h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H ($h^{-1/2}$)	r^2
0.6059	0.3607	0.0389	0.6917	7.2653	0.5555

Figure S5 Fitting of *in vitro* drug release data to quadratic and cubic models

Figure S6 Fitting of *in vitro* drug release data to the proposed Michaelis-menton like model using Graphpad Prism 5 software

Table S7 Cell viabilities of L-929 cell lines treated with different samples

Sample	Absorbance at 570 nm (MTT assay) (Mean $\pm$ SD, n=3)	Cell viability (% of control) (Mean $\pm$ SD, n=3)
Control	0.1147 $\pm$ 0.0172	-
Eugenol	0.1280 $\pm$ 0.0261	111.62 $\pm$ 22.72
Placebo NCs	0.1490 $\pm$ 0.0420	129.94 $\pm$ 36.63
Eugenol NCs	0.1333 $\pm$ 0.0196	116.31 $\pm$ 17.12

Table S8 Graded evaluation of the tissue disruption in the interdental papilla

Group Code	Group	†Epithelium Mean $\pm$ SD (n=5)	‡Transseptal fibers Mean $\pm$ SD (n=5)
A	No treatment (Model)	1.60 $\pm$ 0.55	0.80 $\pm$ 0.45
B	Eugenol	1.80 $\pm$ 0.45	0.40 $\pm$ 0.55
C	Minocycline (5 mg kg ⁻¹ day ⁻¹ orally)	0.20 $\pm$ 0.45	0.20 $\pm$ 0.45
D	Placebo nanocapsules	1.40 $\pm$ 0.55	0.60 $\pm$ 0.55
E	Eugenol loaded nanocapsules	0.20 $\pm$ 0.45	0.40 $\pm$ 0.55

(p<0.05 = Significant; p>0.05 = Not significant)

† p value (A & B >0.05; A & C <0.05; A & D >0.05; A & E <0.05; B & C <0.05; B & D >0.05; B & E <0.05; C & D <0.05; C & E >0.05; D & E <0.05)

‡ p value (p>0.05 between all groups)

Figure S9 Graded response for continuity of transseptal fibers in the interdental papilla