

Supplementary material

Heparin encapsulated metered-dose topical “Nano-spray gel” liposomal formulation ensures rapid on-site management of frost-bite injury by inflammatory cytokines scavenging

Kalpesh Vaghasiya¹, Ankur Sharma¹, Kushal Kumar², Eupa Ray¹, Suneera Adlakha¹, Om Prakash Katare³, Sunil Kumar Hota² and Rahul K Verma^{1#}

¹Institute of Nano Science and Technology (INST), Phase X, Sector 64, Mohali, Punjab 160062, INDIA

²Defence Institute of High Altitude Research, Defence Research and Development Organisation, C/o 56 APO, Leh-Ladakh, Jammu and Kashmir 901205, INDIA

³University Institute of Pharmaceutical Sciences, Panjab University, Sector-14, Chandigarh, INDIA

Chemicals and reagents

Heparin sodium salt low-molecular weight heparin (from porcine intestinal mucosa; Hp, ~200 U/mg, m.wt 4000), Cholesterol (CH), Carbopol 934, Oleic acid and Azure-II was purchased from Himedia laboratories (Mumbai, India). Mouse fibroblast cell line (L929) was obtained from National Centre for Cell Sciences (NCCS), Pune, India. Ibuprofen sodium, L- α -phosphatidylcholine (PC), Propylene glycol, Tris-Cl, and cell-culture medium were procured from Sigma Aldrich (St. Louis, USA). Aloe vera gel freeze-dried powder purchased from SV Agro-food (New Delhi, India). Themis Medicare (Mumbai, India) supplied Ketamine hydrochloride injection (50 mg/ml). Cytokine ELISA kits (IL-6, TNF- α , IL-4, IL-10) were procured from Kristhgen systems (Mumbai, India). Merck Limited (Mumbai, India) supplied chloroform and methanol. All other chemicals were analytical reagent grade. Deionized (Milli-Q Plus system, Millipore, USA) was used in all the experiments.

***In vitro* drug release studies**

In vitro release studies of Hp from HLp was carried out at 37 °C up to 24 h by suspending 10 mg of lyophilized HLp in release medium pH 7.4. Phosphate buffered saline containing 1% ascorbic acid and 0.05% sodium azide at pH conditions of 7.4 were used for this purpose. A 100 ml beaker was used at a stirring speed of 100 rpm for these studies. A dialysis membrane (Sigma, molecular weight cut-off >900. kDa, 25mm×16mm) was cut into equal pieces of about 6 cm×2.5cm and pre-treated as suggested by the manufacturer. Next, 1 mL of samples was withdrawn in duplicates at appropriate intervals and an equal volume of dissolution medium was added to maintain constant volume. Samples were filtered through Millipore 0.22 µm filter and filtrate was stored at −20°C till analysis by UV-Vis spectrophotometer. The portion of Hp released at a particular time can be considered as a cumulative frequency function. HLp showed sustained release behaviour up to 18 h. The release kinetics of Hp from HLp in phosphate buffer are shown in Figure 4.13. As evident from the Figure S1, in the initial 12 h, 45 % of Hp was released from the HLp.

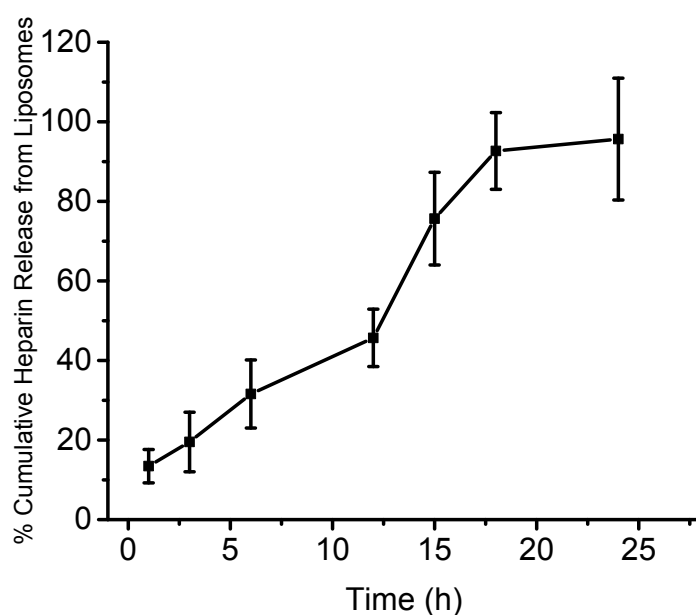


Figure S1. Release profile of Heparin from Heparin-loaded cationic liposome.

Table S1. Stability studies of HLp-NSG over a period of 28 days

	40 °C	25 °C	0 °C	-10 °C	-15 °C	-20 °C
Viscosity (cps)	5124± 741	7421± 532	8128± 814	9514± 629	Not measurable	Not measurable
Spreadability (sec)	27.54± 7.45	19.31± 6.78	15.21± 3.54	13.54± 6.31	No Spreadability	No Spreadability
pH	7.1± 0.4	6.3± 0.6	6.7± 0.45	6.6± 0.7	6.7± 0.6	6.8± 0.3
Entrapment efficiency (%)	93.14±16.51	96.78±11.25	98.31±13.14	95.17±17.54	93.14±13.54	91.25±15.21

Table S2. Stability studies of HLp over a period of 28 days

	40 °C	25 °C	0 °C	-10 °C	-15 °C	-20 °C
Size (nm)	229 ± 18	204 ± 12	Not measurable	Not measurable	Not measurable	Not measurable
pH	7.1± 0.4	6.3± 0.6	6.7± 0.45	6.6± 0.7	6.7± 0.6	6.8± 0.3
Entrapment efficiency (%)	92.67±12.56	96.98±13.28	82.55±17.84	77.26±13.66	72.65±14.28	72.96±16.54

Table S3. *In-vitro* Re-epithialization (Scratch Test)

	Control	Empty Liposomes	Heparin Liposomes (20U/ml)	Heparin Liposomes (200U/ml)
12 h	8.82±3.98	5.06±3.67	51.61±8.32	6.54±2.368
24 h	8.63±4.56	5.126±2.65	42.61±8.26	4.68±71.9
48 h	19.12±6.35	15.62±4.58	95.16±6.65	22.14±6.34

Table S4. *In-vivo* Re-epithialization

	Control	SSD	BLp-NSG	HLp	HLp-NSG
Day 2	24.71±5.45	52.85±8.54	31.64±6.15	63.05±6.15	76.78±6.48
Day 10	33.76±3.54	80.35±8.65	52.83±5.66	73.99±7.78	93.80±7.89
Day 14	40.86±5.11	87.14±6.53	61.03±7.58	78.91±10.24	99.76±8.99