Supporting Information

Imiquimod-Loaded Nanosystem for Treatment Human Papillomavirus-Induced Lesions

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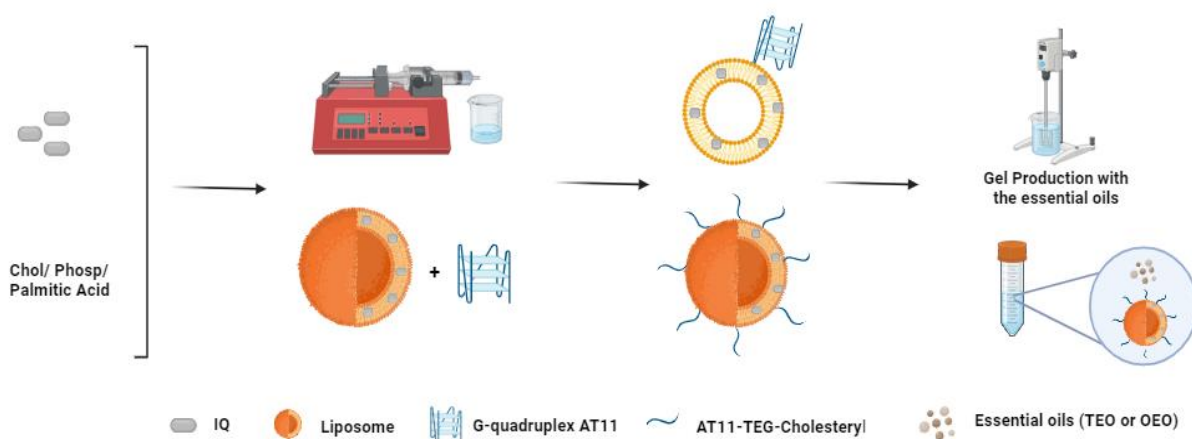*Corresponding author: carlacruz@fcsaude.ubi.pt

Figure S1: Synthesis of Lipo IQ AT11 and gel production with 0.25% of thyme or oregano essential oils and Lipo IQ AT11.
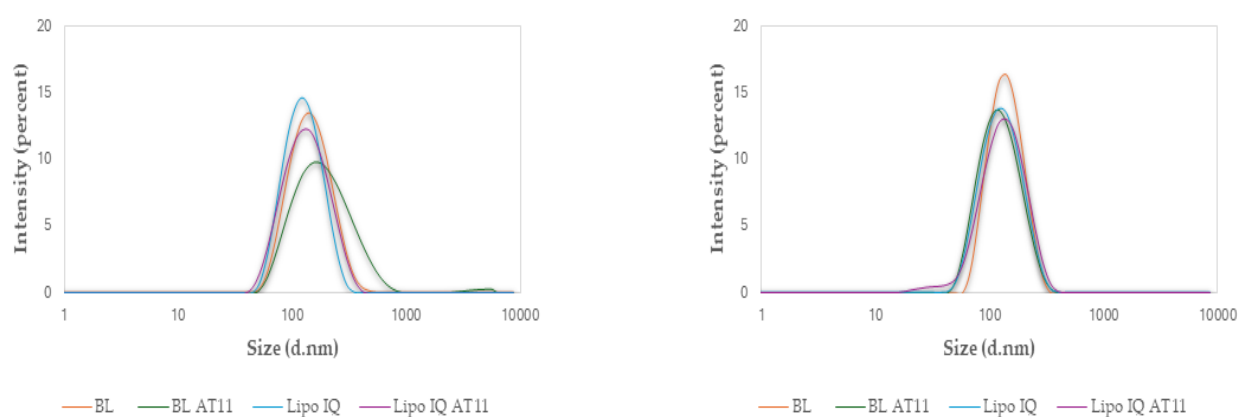

Figure S2 - Particle size distribution diagram for the liposomes BL, BL AT11, Lipo IQ and Lipo IQ AT11 on the left side, on the day they were prepared and on the right side, after 30 days.
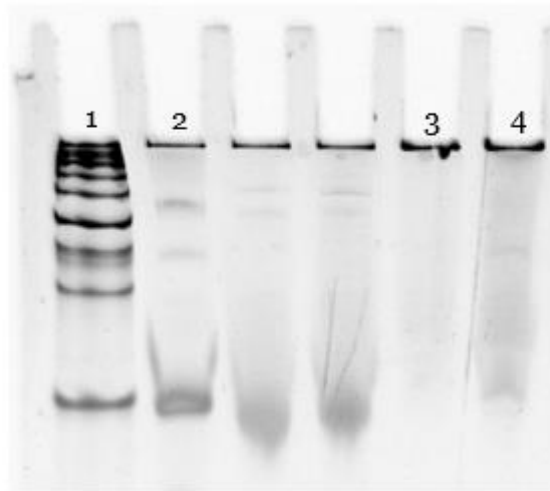
Figure S3 - Acrylamide gel of (1) 25bp Marker, (2) AT11-TEG-Cholesteryl, (3) Lipo IQ (1mg) + AT11-TEG-Cholesteryl (1 nmol), (4) Lipo IQ (1mg) + AT11-TEG-Cholesteryl (2 nmol).
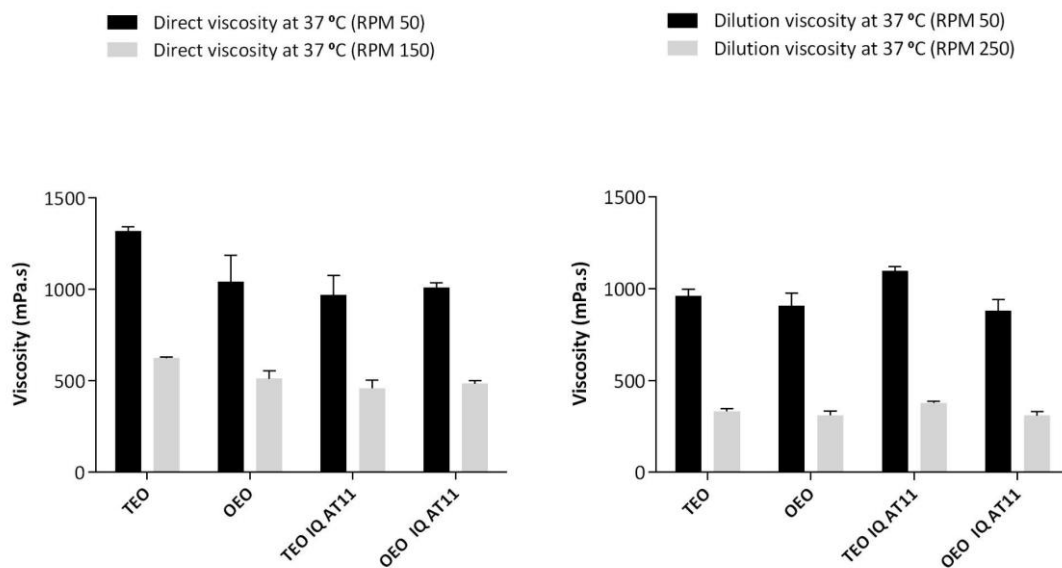

Figure S4 - Viscosity comparisons for direct and diluted measurements at 37 °C. The dilutions were made in VFS. The results are presented as mPa.s, corresponding to the mean and standard deviation of three measurements (n = 3).

Table S1: Osmolality results of the formulations studied, n = 3. BF – Base Formulation. TEO - Base Formulation with 0.25% Thyme. OEO - Base Formulation with 0.25% Oregano TEO IQ AT11 – Base Formulation with 0.25% Thyme and 1% of Lipo IQ AT11 and OEO IQ AT11 – Base Formulation with 0.25% Oregano and 1% of Lipo IQ AT11.**** Represents statistically different from BF used as control, two-way ANOVA, $p < 0.0001$.

	Osmolality ± SD value (mOsmol/kg)	
	Direct Formulation	**Diluted Formulation**
BF	1562 ± 47.6	838.0 ± 10.2****
TEO	1332 ± 39.8	1186 ± 22.7****
OEO	1366 ± 8.185	1184 ± 6.658****
TEO IQ AT11	1306 ± 54.2	1139 ± 28.3****
OEO IQ AT11	1367 ± 7.211	1165 ± 13.614****

Table S2: Bioadhesive parameters (Peak force adhesiveness (N), debonding distance (mm), and work of adhesion (N.mm)) determined for the base formulation (BF), Base Formulation with 0.25% Thyme and 1% Lipo IQ AT11 (TEO IQ AT11) and Base Formulation with 0.25% Oregano and 1% Lipo IQ AT11 (OEO IQ AT11), n = 4. Vaginal tissue without formulation was used as control.*Represents statistically different from control two-way ANOVA, $p < 0.05$.

	Peak Force Adhesiveness (N)	**Debonding Distance (mm)**	**Work of Adhesion (N.mm)**
Control	0.115 ± 0.149	0.443 ± 0.209	0.032 ± 0.035
BF	0.060 ± 0.017	2.332 ± 0.990*	0.070 ± 0.045
TEO IQ AT11	0.130 ± 0.060	1.160 ± 0.626	0.064 ± 0.040
OEO IQ AT11	0.338 ± 0.309*	0.608 ± 0.302	0.125 ± 0.111

Table S3- Linear range, calibration curve, correlation coefficients, and LLOQ (n=5).

Analyte	Weight	Linear range (mM)	Linearity*		R²*	LLOQ (ng/mL)
			Slope	Intercept		
IQ	1/x	0.0039-1	342.35 ± 34.35	1.108 ± 0.1776	0.998 ± 0.004	0.0039

*Mean values ± standard deviation

Table S4- Results of the visual MIC and MIC 50% for all the formulations in *Candida albicans*. BL- blank liposome, BL AT11- blank liposomes with AT11, TEO AT11 - base formulation with the thyme essential oil and BL AT11, OEO AT11 - base formulation with the oregano essential oil and BL AT11, TEO IQ AT11 - base formulation with the thyme essential oil and Lipo IQ AT11 and OEO IQ AT11- base formulation with the oregano essential oil and Lipo IQ AT11.

Concentration (mM)	Intra-day precision		Inter-day precision	
	CV (%)	RE	CV (%)	RE
0.0039	2.99	112.28	10.14	111.00
0.016	3.27	98.71	5.28	103.71
0.25	2.55	97.55	3.93	99.95
1	4.37	97.02	8.08	95.02

	MIC visual (% v/v)			MIC 50 (% v/v)		
	C.A	A.B	S.A	C.A	A.B	S.A
BL	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.
BL AT11	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.
TEO AT11	10%	2.5%	5%	5%	2.5%	5%
OEO AT11	N.O.	2.5	10%	5%	0.625%	5%
TEO IQ AT11	5%	5%	10%	5%	5%	10%
OEO IQ AT11	5%	2.5%	5%	5%	2.5%	10%

N.O. – not observed