Supplementary material

Multimodal Layer-by-Layer nanoparticles: a breakthrough in gene and drug delivery for Osteosarcoma

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Table S1. Values of explanatory variables: homogenisation speed (rpm) (X_1), homogenisation time (s) (X_2) and response variable: size (nm) (Y_1) and ζ-potential value (mV) (Y_2). The table includes also the PDI value for each run.

Run	X_1, Homogenisation speed (rpm)	X_2, Homogenisation time (s)	Y_1, Size (nm)	Y_2, ζ-potential value (mV)	PDI (%)
1	20000	30.0	311.3	+38.4	30.1
2	16893	75.0	370.6	+28.6	61.9
3	27500	138.6	242.6	+34.2	20.9
4	20000	120.0	346.0	+23.0	27.2
5	27500	75.0	196.4	+30.6	11.1
6	27500	75.0	209.6	+36.5	16.7
7	27500	75.0	196.7	+38.9	19.3
8	35000	30.0	196.7	+38.9	32.6
9	27500	75.0	249.9	+11.1	18.5
10	38106	75.0	276.6	+25.0	31.5
11	35000	120.0	259.0	+30.7	42.1
12	27500	75.0	189.2	+30.2	17.6
13	27500	11.4	302.5	+17.0	50.3

The optimised conditions for manufacturing polyplexes with the minimum size (191.3 nm) were as follows: 29750 rpm of homogenisation speed and a homogenisation time of 79.5 seconds.

The optimised conditions for manufacturing polyplexes with the maximum ζ-potential value (+39.4) were as follows: 38107 rpm of homogenisation speed and a homogenisation time of 138.6 seconds.

Figure S1. Scheme of the procedure to manufacture the miR-NPs polyplexes.

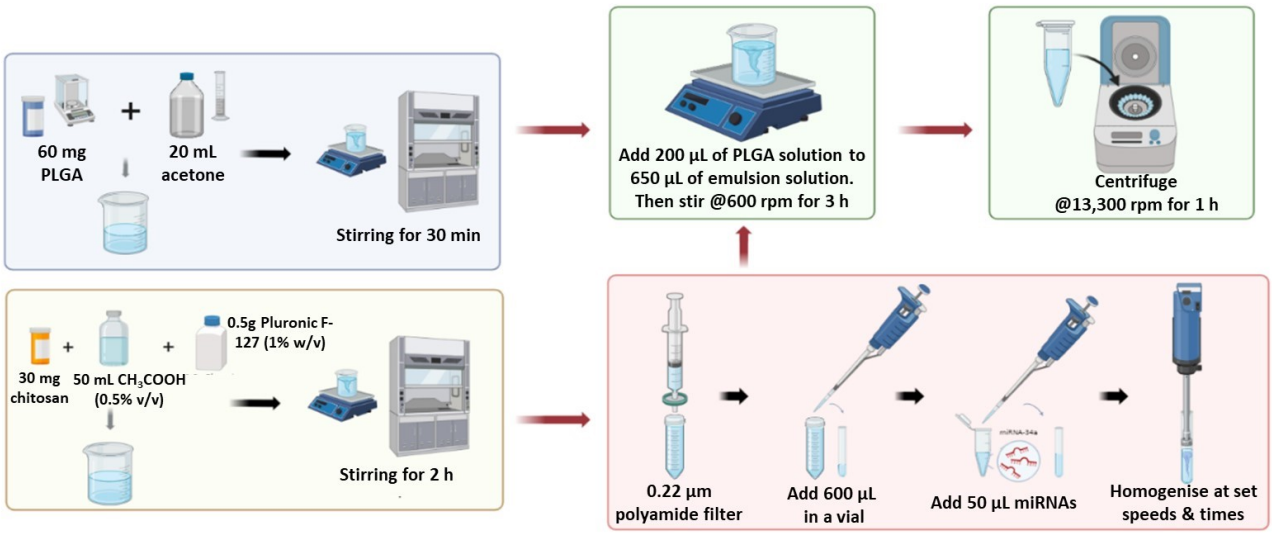

Figure S2. Scheme of the procedure to manufacture LbL-NPs by functionalisation of the preformed polyplexes using the LbL assembly.

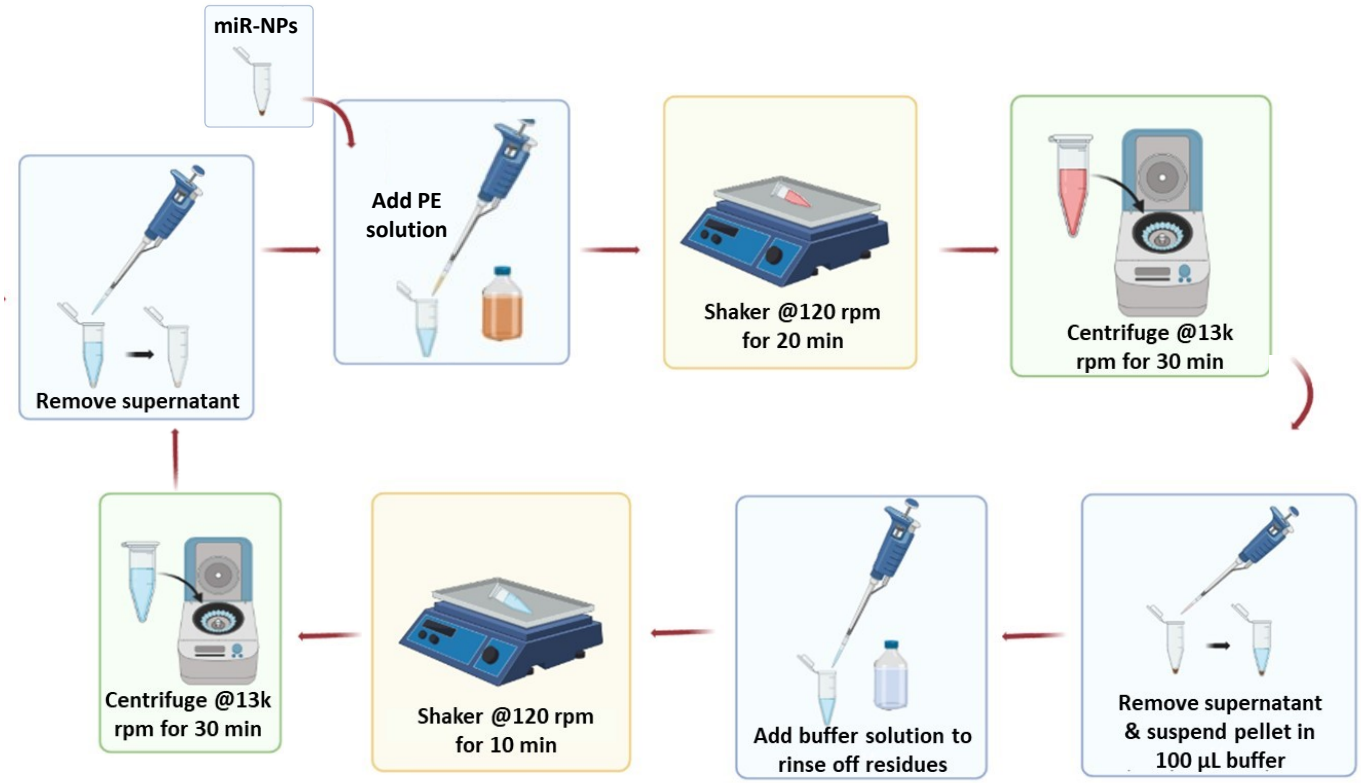

Figure S3. Live and dead images of non-treated Saos-2 and U2OS cells in 2D culture at different time points.