Evaluation of the particles abiotic degradation and of the internalization rate

Material and methods

J774 cells were seeded in 12 well plates at 500,000 cells/ml, left for 24 hours and then treated with Blue 14-labelled PCL nanoparticles or fluorescent PS nanoparticles at 80µg/ml for 24 hours. At the end of the incubation period, the culture medium was removed, the cell layer rinsed twice with PBS, and then lysed in 400µl of 10mM Hepes pH 7.5. The lysate was centrifuged at 15,000g for 30 minutes to pellet the beads. The beads pellet was the resuspended in 400µl of 10mM Hepes pH 7.5 and the fluorescence of the suspension was measured on a DeNovix QFX fluorimeter (excitation 635 nm, emission 665-740 nm).
As controls, the same concentration of beads (80µg/ml) was suspended in complete culture medium for either 60 minutes or 6days, prior to centrifugation and resuspension as described above.

Results

PS beads	Measured value
Beads alone 60 min	1270±19
Beads in cells	743±13
% internalization	53%

PCL beads	Measured value
Beads alone 60 min	574±26
Beads in cells	219±253
% internalization	38%
Beads alone, 6 days	584±17
% fluorescence loss	0

Conclusions

The PCL beads did not degrade abiotically in complete culture medium (with serum) for 6 days at 37°C and neutral pH.
The internalization rate was different for PS and PCL beads, which allowed to calculate the average beads number per cell.