SUPPLEMEMTARY INFORMATION

Materials and Methods

Formulation development and optimization. Cisplatin was dissolved in hot sterile de-ionized water (~90ºC) at the concentration of 15 mg/ml and hyaluronan was dissolved in sterile de-ionized water at the concentration of 5 mg/ml. The molecular weight of the hyaluronan was 0.6-1.2 x 10^6 g/mole. Different weight ratios of hyaluronan and cisplatin (0/15, 2/15, 5/15, 5/7.5, 5/5, 4/3) were mixed and incubated at 90-95ºC for 1 h with the final concentration of cisplatin fixed at 3 mg/ml as shown in Supplementary Table 2, followed by cooling on ice for 10-30 min. The materials were not stirred either during incubation at high temperature or during cooling. Precipitates were found in formulations of ratios <4/3 (w/w, hyaluronan/cisplatin) due to insoluble and un-incorporated cisplatin (solubility of cisplatin in water at 4ºC is ~ 1 mg/ml). Therefore, a ratio of 4/3 was chosen for subsequent formulation development. Briefly, 200 µl of the cisplatin solution (15 mg/ml) and 800 µl of the hyaluronan solution (5 mg/ml) were mixed and incubated at either 45 ºC or 90ºC for 10 min - 6 h followed by cooling on ice for 10-30 min. The formulations were analyzed at 25 ± 0.1 ^{o}C for intensity averaged size and zeta potential using a Zetasizer ZS (Malvern Instruments, Malvern, Worcestershire, UK) and the CONTIN model.

Determination of the incorporation efficiency during formulation development. The method was adopted from our published work with minor modifications (1). Forty µl of the nanoparticle samples were diluted with 360 µl of de-ionized water before being added to Microcon® centrifugal filter devices (molecular weight cut-off 3,000, Millipore, Bedford, MA). After centrifugation at 10,000 rpm for 10 min, 50 µl of the flow-through was collected and mixed with 450 µl de-ionized water, followed by the addition of 50 µl sodium diethyldithiocarbamate solution (100 mg/ml in 0.1N NaOH). The mixture was incubated at 37ºC for 30 min and then on ice for 2 min. The hydrophobic complex of cisplatin- diethyldithiocarbamate was then extracted from the mixture with 200 µl $CHCl_3$, and 100 µl of the chloroform layer was mixed with 100 µl dimethyl sulfoxide and the absorbance at 350 nm was determined by a NanoDrop UV/VIS spectrometer (ND 1000, NanoDrop Technologies Inc.). The concentration of the un-incorporated cisplatin was then calibrated from a standard curve and the incorporation efficiency was calculated.

Knockdown of CD44 in A2789 and OV2008 cells. Sublines of A2780 and OV2008 in which CD44 was constitutively knocked down were generated using the CD44-shRNA-lentivirus (Sigma-Aldrich, St. Louis, MO) to infect the cells that were then selected by exposure to puromycin (5-10 µg/ml) following the manufacturer's protocol.

References:

1. P.A. Andrews, W.E. Wung, S.B. Howell, A high-performance liquid chromatographic assay with improved selectivity for cisplatin and active platinum (II) complexes in plasma ultrafiltrate, Anal Biochem 143 (1) (1984) 46-56.

Tables

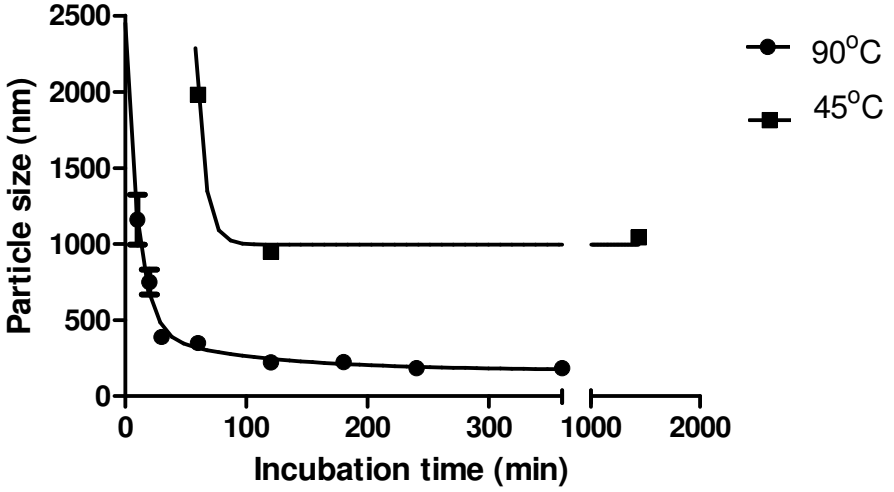
Supplementary Table 1. Electron diffraction spectroscopic analysis of Hyplat.

Element	Weight %	Atomic %
C	38.98 ± 1.96	52.71
O	41.88 ± 2.06	42.52
N	2.20 ± 3.71	2.55
Pt	15.66 ± 1.57	1.3
Na	1.29 ± 0.27	0.91

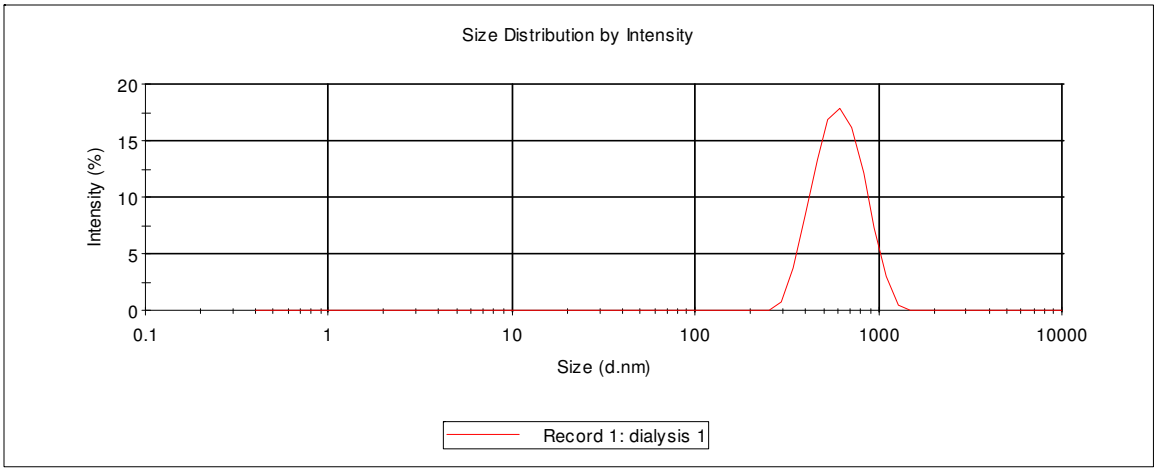
Supplementary Table 2. Formulation of hyaluronan-cisplatin microparticles

Hyaluronan/cisplatin (weight ratio)	0/15	2/15	5/15	5/7.5	5/5	4/3
Hyaluronan (5 mg/ml)	0 µl	40 µl	100 µl	200 µl	300 µl	400 µl
Cisplatin (15 mg/ml)	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Heated water	400 µl	360 µl	300 µl	200 µl	100 µl	0 µl

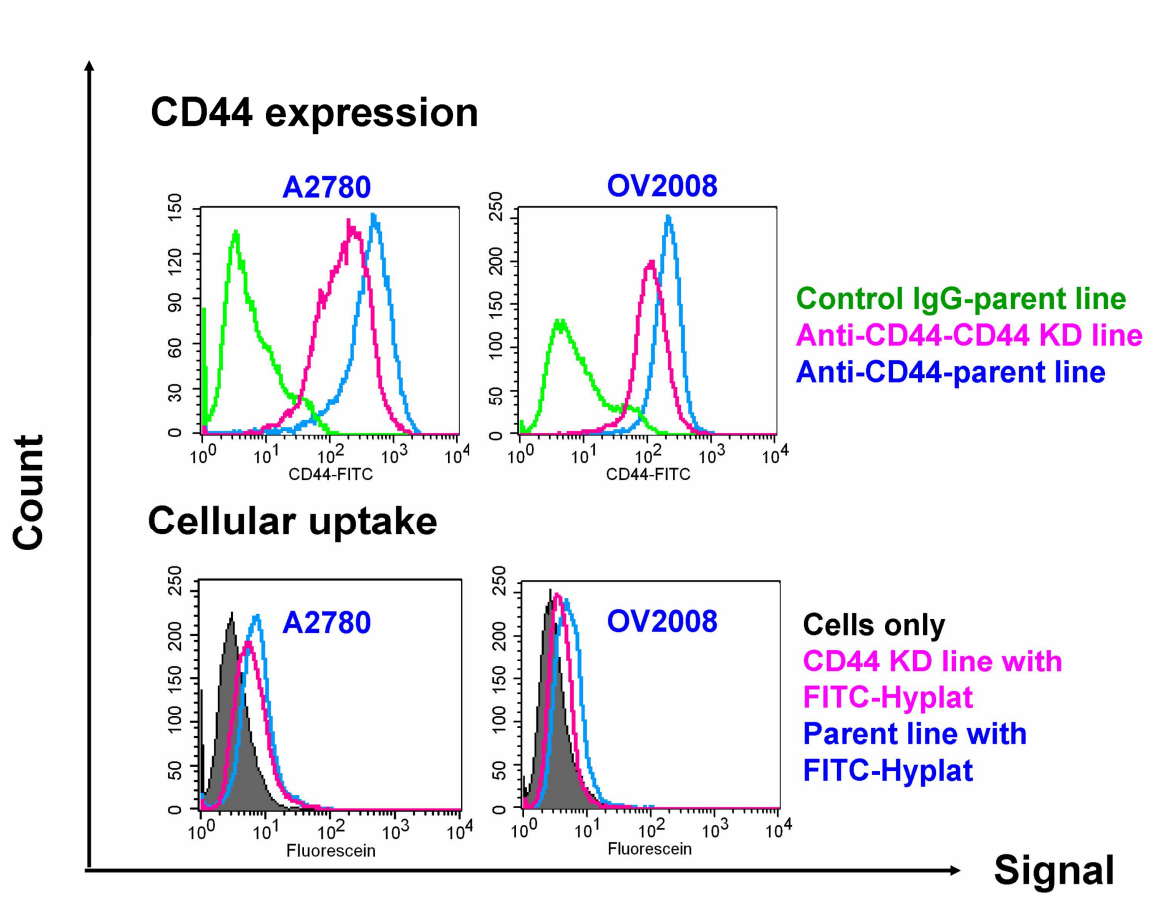
Figures



Supplementary Figure 1. Average diameter of the hyaluronan-cisplatin particles as a function of incubation temperature and time. Vertical bars, ± SD. Where bars are missing, SD was less than the size of the symbol. N≥3.



Supplementary Figure 2. Particle size distribution of Hyplat after removal of un-incorporated cisplatin by dialysis (mean size = 552.6 nm, PDI = 0.142)



Supplementary Figure 3. Cellular uptake of FITC-Hyplat in parent lines and the CD44 knockdown (KD) lines.