Novel Stabilization of Calcium Phosphate Nanoparticles for Drug Delivery

Jessica Woodman[1], Glenn Prestwich, Ph.D.[2], Diane Burgess, Ph.D.[3] & Liisa T. Kuhn, Ph.D.[4]
Materials Science and Engineering, University of Connecticut[1], Medicinal Chemistry, University of Utah[2]
Pharmaceutical Sciences, University of Connecticut[3], Reconstructive Sciences, University of Connecticut Health Center[4]

Statement of Purpose: Calcium phosphate (CaP) is an attractive biomaterial for drug delivery because it is biocompatible, resorbable and inexpensive to manufacture. During precipitation, CaP nanoparticles can be stabilized with carboxylate containing molecules that limit their crystal growth; however some stabilizers negatively impact the bioactivity of the drug being delivered or are toxic themselves. Based on the negative effects we observed previously with sodium polyacrylate[1] we investigated the stabilization of nanoCaP by new a molecule: CMHA[2].

Methods: All chemicals were purchased from Sigma Aldrich unless otherwise stated. NanoCaP^{CMHA} was precipitated using 30 mM calcium lactate pentahydrate to which potassium phosphate dibasic was added at equal volume and molar concentration with moderate mixing. CMHA (34 kDa) synthesized at the University of Utah in Dr. Glenn Prestwich's lab[1], is a natural polysaccharide that has been modified with carboxyl groups which enhance interaction with CaP. NanoCaP stabilization was achieved with 2% (w/v) CMHA at 20% of the total volume of precipitation. NanoCaP^{CMHA} was collected via centrifugation (20,000 g) and washed with ultrapure water. The amount of CMHA incorporated into the nanoCaP^{CMHA} was determined using Alexa Fluor® labeled CMHA, used at 1% (w/w) of the CMHA in precipitation. Aquated CDDP (Aq CDDP) allows for electrostatic binding[1] to nanoCaP^{CMHA}. Binding was performed overnight in 20 mM potassium phosphate buffer (KPB pH 6.0) with gentle rocking at 37°C. Binding solution was removed via centrifugation, followed by two washes in 10 mM KPB. NanoCaP^{CMHA}CDDP was suspended in ultrapure water via sonication at 50 mg particles per mL for testing. NanoCaP^{D}CDDP stabilized with sodium polyacrylate was prepared as previously reported[1]. Particle size and zeta potential analysis were performed using a 90Plus with ZetaPlus Analyzer (Brookhaven Instruments Corp., Holtsville, NY). Platinum content was determined with an Optima™ 5300 DV Inductively Coupled Plasma-Optical Emission Spectrometer (Perkin Elmer, Waltham, MA). CDDP loading was determined by analysis of CDDP in a known weight of NanoCaP^{CMHA}CDDP. Transmission Electron Microscopy (TEM) was performed using a HITACHI H-7650.

Human breast cancer cells in an agressive mesenchymal stem cell phenotype (LMS)[3] were used for *in vitro* studies to examine cytotoxicity of NanoCaP^{CMHA}CDDP, and controls: CDDP in saline, co-mixture of Aq CDDP-CMHA, and nanoCaP^{D}CDDP via an MTS assay, CellTiter 96® AQueous One (Promega Corp, Madison, WI). The inhibitory concentration at which 50% of the cells have died (IC_{50}) was calculated using a nonlinear regression curve fit with a variable slope (four parameters). Each IC_{50} value represents 4-6 replicates. A one-way ANOVA with a Tukey post-test was used to determine significance.

Results: CMHA is able to effectively stabilize CaP as nanoparticles evidenced by particle size analysis (PSA) and zeta potential in Table 1 (SD in parentheses).

Sample	PDI	Particle Size (nm)	Zeta Potential (mV)
NanoCaP^{CMHA}	0.120	215 (3)	-19 (3)
NanoCaP^{CMHA}CDDP	0.117	252 (8)	-16 (1)

The TEM image of nanoCaP^{CMHA}CDDP is in contrast to the PSA in part due to drying artifacts, and shows 20-50 nm size particles agglomerated into larger micron sized clusters. The thin needle-like projections (arrow) are CMHA, confirmed with TEM images of CMHA alone. CMHA contributes 30% (w/w) of nanoCaP^{CMHA}. The drug loading was 158 ug CDDP/ mg nanoCaP^{CMHA}. The IC_{50} values (μg/mL) calculated from curves in Figure 2 are as follows: CDDP in saline 4.1, nanoCaP^{CMHA}CDDP 9.5, nanoCaP^{D}CDDP 17.8 and Aq-CMHA 6.9. Significant differences were found between all groups. Importantly, NanoCaP^{CMHA}CDDP is significantly more cytotoxic against LMS cells than NanoCaP^{D}CDDP. NanoCaP^{CMHA}CDDP did not show enhanced toxicity over CDDP alone at the same dose which is expected given the delayed release of CDDP observed in our other stabilized nanoCaP formulations.

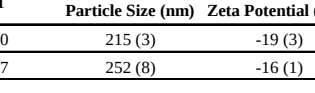

Figure 1: TEM image

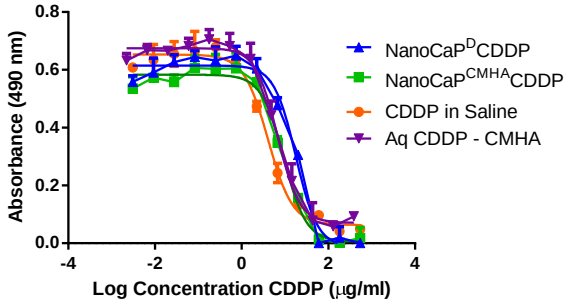

Figure 2: Cytotoxicity results in LMS cells

Conclusions: This data shows for the first time that CMHA is capable of stabilizing nanoCaP with less adverse effects on cisplatin delivery than sodium polyacrylate. NanoCaP^{CMHA}CDDP shows enhanced toxicity against an aggressive cancer cell phenotype, LMS, compared to NanoCaP^{D}CDDP indicating CMHA is promising for future investigations such as *in vitro* release and *in vivo* anticancer efficacy studies.

References:

1. Cheng X. Int J Nanomed. 2007:2(4): 667-674.
2. United States Patent # *7981871*.
3. Guttilla IK. Breast Cancer Res Treat. 2012:132:75-85.

Acknowledgements: Support from a 2012 UCHC/Storrs and Regional Campus Incentive Grant.