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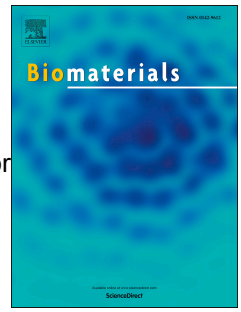

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PII: S0142-9612(18)30610-0

DOI: 10.1016/j.biomaterials.2018.08.050

Reference: JBMT 18856

To appear in: *Biomaterials*

Received Date: 18 June 2018

Revised Date: 20 August 2018

Accepted Date: 21 August 2018

Please cite this article as: Krishnan Y, Rees HA, Rossitto CP, Kim S-E, Hung H-HK, Frank EH, Olsen BD, Liu DR, Hammond PT, Grodzinsky AJ, Green fluorescent proteins engineered for cartilage-targeted drug delivery: Insights for transport into highly charged avascular tissues, *Biomaterials* (2018), doi: 10.1016/j.biomaterials.2018.08.050.

Green Fluorescent Proteins Engineered for Cartilage-Targeted Drug Delivery: Insights for Transport into Highly Charged Avascular Tissues

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Abstract

Osteoarthritis (OA), the most common form of arthritis, is a multi-factorial disease that primarily affects cartilage as well as other joint tissues such as subchondral bone. The lack of effective drug delivery, due to the avascular nature of cartilage and the rapid clearance of intra-articularly delivered drugs via the synovium, remains a major challenge in the development of disease modifying drugs for OA. Cationic delivery carriers can significantly enhance the uptake, penetration and retention of drugs in cartilage by interacting with negatively charged matrix proteoglycans. In this study, we used "supercharged" green fluorescent proteins (GFPs), engineered to have a wide range of net positive charge and surface charge distributions, to characterize the effects of carrier charge on transport into cartilage in isolation of other factors such as carrier size and shape. We quantified the uptake, extent of cartilage penetration and cellular uptake of the GFP variants into living human knee cartilage and bovine cartilage explants. Based on these results, we identified optimal net charges of GFP carriers for potential drug targets located within cartilage extracellular matrix as well as the resident live chondrocytes. These cationic GFPs did not have adverse

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