Cationic, Multifunctional Dendrimers for Treatment of Osteoarthritis

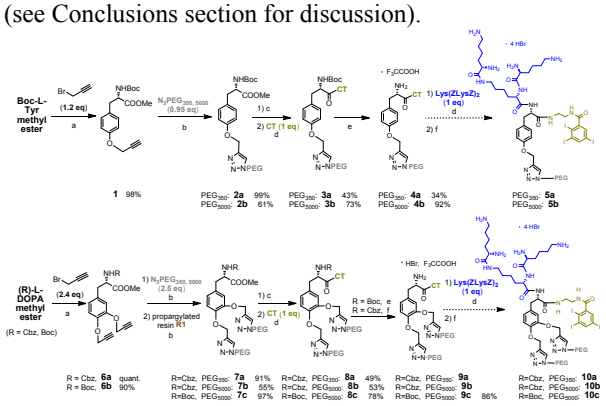
Benjamin G. Cooper, (Benjamin Lakin), (Rachel C. Stewart), Cynthia Ghobril, Mark W. Grinstaff.
Departments of Chemistry and Biomedical Engineering, Boston University, Boston, MA.

Statement of Purpose: Osteoarthritis is a degenerative disease of the hyaline articular cartilage that lines the ends of bones in diarthrodial joints. The disease is the leading cause of disability in the United States, affecting an estimated 27 million individuals,[1] and with the exception of total joint replacement, no biomaterial-based therapies have been shown to significantly improve patient outcomes. Intra-articular injection of viscosupplements intended to increase synovial fluid viscosity have found commercial success, but meta-analyses of multiple clinical trials have failed to conclusively demonstrate this strategy's efficacy.[2] Injection of surface-active supplements, or biomaterials intended to treat cartilage breakdown by attachment to the diseased tissue, have received less attention; native surface-active protectants, such as the mucin-like glycoprotein PRG4, feature a highly-hydrated brush-like conformation with hydrophilic oligosaccharide side-chains as well as sites for adhesion to the cartilage surface. With these structure-function relationships in mind, we present here the bio-inspired synthesis of a dendritic macromolecule for the treatment of osteoarthritis.

Methods: A series of multifunctional dendrimers were prepared starting from the methyl ester of either Boc-L-tyrosine or Boc- or Cbz-L-dihydroxyphenylalanine (Boc: *tert*-butyloxy; Cbz: carboxybenzyl) (AK Scientific, Union City, CA) (Scheme 1). Propargyl bromide (Sigma-Aldrich, St. Louis, MO) was conjugated to free alcohol moieties and subsequently coupled with azidated poly(ethylene glycol) (PEG) (Polysciences, Warrington, PA) (Scheme 2a). Further functionality was incorporated by addition of an iodinated fragment derived from 2,3,5-triiodobenzoic acid (Acros Organics, Geel, Belgium) and convergence with a cationic dendron derived from N,N'-bis(Cbz)-L-lysine and the dihydrochloride salt of L-lysine methyl ester (Chem-Impex International, Wood Dale, IL) (Scheme 2b,c). Compounds **7a-c** were isolated from excess azidated PEG by addition of a propargyl-functionalized resin[3] derived from poly(*para*-chloromethylstyrene-*co*-divinylbenzene) (EMD Chemicals, Darmstadt, Germany) followed by refluxing for 24 hours; the resin was then filtered and compound 7a was isolated by extraction into dichloromethane, while compounds 7b,c were isolated by precipitation in diethyl ether. Compounds **1-10** were characterized by ^{1}H nuclear magnetic resonance spectroscopy at 500 MHz (Varian, Palo Alto, CA), Fourier transform infrared spectroscopy (Thermo Scientific, Brookfield, WI), and mass spectrometry (Agilent Technologies, Santa Clara, CA)

Results: Primary amine-containing, hierarchically-branched polymers 5a,b and 10a-c were synthesized in high yields. The compounds feature either one or two PEG chains of molecular weight 350 or 5000 Daltons, and iodine content ranging from 3.4 to 20.9 wt%.

Compounds **4b** and **9b** were prepared as aqueous solutions of sodium chloride balanced to 400 milliosmolar and pH 7.4. A large excess of dendrimer solution was incubated with *ex vivo* bovine cartilage for 24 hours, and upon computed tomography imaging, no significant attention was observed compared to pretreatment controls (see Conclusions section for discussion).



Scheme 1. Synthesis of dendrimers **5a**,**b** and **10a-c**. a) K_2CO_3; b) CuI, DIPEA; c) $KOSiMe_3$; d) EDCI, HOBT, DIPEA; e) TFA; f) HBr, $HOOCCH_3$.

Conclusions: The objective of imparting three distinct functionalities to an injectable biomaterial has been achieved: i) the primary amines at the termini of the lysine-based dendron will exist, at physiological pH, as positively charged ammonium centers with the intended application of electrostatically interacting with the abundant fixed negative charges arising from carboxylate and sulfonate groups present on the glycosaminoglycans in cartilage; ii) the covalently attached iodine atoms will serve as contrast agents for CT imaging of the dendrimers' permeation into tissue, as related iodinated aromatic compounds[4] have shown high sensitivity in cartilage in several animal models; and iii) the linear PEG chains afford further sites of attachment of biomolecules as well as increased biostability, hydrophilicity, and mechanical robustness (due to the large number of water molecules associated with the lone pairs along the PEG backbone's oxygen atoms). The immediate next study that will be pursued is the CT imaging of *ex vivo* bovine cartilage in the presence of dendrimers **5b** and **10b**. These compounds feature four times as many positive charges as compounds **4b** and **9b**, and we hypothesize that the latter compounds elicited no significant attenuation because their weak level of attraction to the cartilage (with just a single positive charge) did not anchor them strongly enough to the tissue. Subsequent studies will involve investigating the effect of number and length of PEG chains on cartilage mechanical properties.

References: [1] Centers for Disease Control and Prevention. Morbid. Mortal. Weekly Rep. 2001;50. [2] AWS Rutjes. Ann. Int. Med. 2012;157. T Conrozier. Clin. Exp. Rheum. 2005; 23:711. [3] U Sirion. Bull. Korean Chem. Soc. 2010;31:1843. [4] NS Joshi. J. Am. Chem. Soc. 2009;131:13234. PN Bansal. J. Orthop. Res. 2011;29:704. PN Bansal. Osteoarthr. Cartil. 2011;19:970.