Assessment of Antioxidant Copolymers for Improved ROS Scavenging in Post Traumatic Osteoarthritis

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Statement of Purpose: Post traumatic osteoarthritis (PTOA) remains one of the leading causes for mobility-related diseases.[1] PTOA is characterized by the progressive degradation of cartilage, driven by an inflammatory and oxidative environment. Current treatments merely reduce pain associated with the disease, and therapeutic disease-modifying OA drugs (DMOADs) that slow disease progression have thus far remained elusive. Reactive oxygen species (ROS) are a promising target for DMOADs as the overproduction of ROS are directly linked to the tissue damage caused in PTOA. TEMPO, a small molecule superoxide dismutase mimic, is a potential treatment, but due to its hydrophobicity, is susceptible to rapid synovial clearing. High density poly TEMPO grafting methods are water insoluble, exhibiting poor bioavailability. Therefore, we seek to develop a polymeric TEMPO that will be effectively retained in the joint, possessing high antioxidant power coupled with increased hydrophilicity. In the current study, random copolymers of hydrophilic dimethylacrylamide (DMA) and TEMPO were synthesized to optimize the TEMPO density and bioavailability of polymeric TEMPO.

Methods: DMA-co-pentafluorophenyl acrylate (PFPA) polymers were synthesized via reversible addition-fragmentation chain-transfer (RAFT) polymerization. TEMPO-amine was then grafted onto the polymer by substitution of the PFPA groups. A library of polymers was prepared by altering the molar ratios of DMA:PFPA during RAFT synthesis. Cellular uptake studies were performed by conjugating the fluorophore cy7-amine to DMA-co-TEMPO. RAW 264.7 macrophage cells were treated with the polymers for one hour and uptake was monitored by flow cytometry. A ferric reducing antioxidant power (FRAP) assay was utilized to assess antioxidant efficacy of the polymers under aqueous conditions. Finally, an air pouch model (APM) was used to simulate compartmentalized inflammation as previously described.[3] Carrageenan was co-injected with varying doses of 60:40 DMA-co-TEMPO, and ROS content was measured in the exudate using ROSStar®. Cell infiltrate into the air pouch was assessed using immunocytochemistry and flow cytometry.

Results: Cellular uptake studies indicated that the increase in internalization was proportional to an increase of hydrophilicity as demonstrated by 90:10 DMA-co-TEMPO providing maximum uptake (**Figure 1a**). FRAP results indicated an optimal reducing potential of polymers at a ratio of 60:40 DMA:TEMPO (**Figure 1b**). APM results indicated that increasing doses of 60:40 DMA-co-TEMPO provided a linear decrease in ROS content (**Figure 1c**). The distribution of cell phenotypes assessed within the air pouch were similar for all groups, however, the presence of the polymer led to a reduction in the overall cell number (**Figure 1d**).

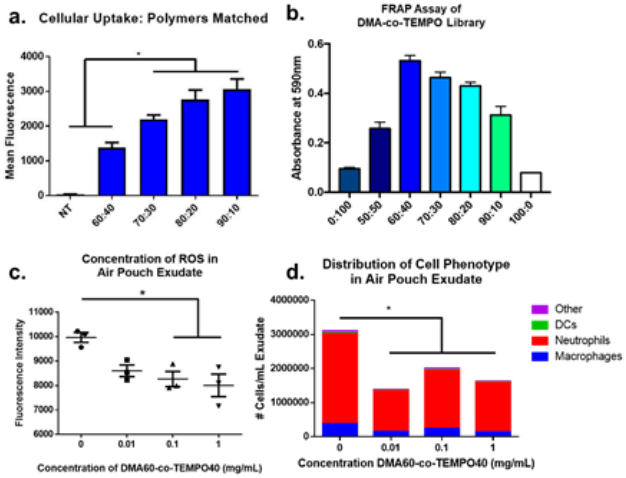

Figure 1: **a.)** Uptake of DMA-co-TEMPO polymers in RAW 264.7 cells assessed by flow cytometry **b.)** FRAP assay of DMA-co-TEMPO polymers **c.)** Concentration of ROS in air pouch exudate with varying doses of 60:40 DMA-co-TEMPO **d.)** Cell number and phenotype in air pouch exudate assessed by flow cytometry

Conclusions: These studies demonstrate the promising potential of DMA-co-TEMPO polymers as an antioxidant therapy for inflammation-driven PTOA. Hydrophilic modification and a balance of ROS scavenging in DMA-co-TEMPO polymers are necessary to provide bioactive efficacy in TEMPO-based PTOA delivery systems. Our data highlights the importance of compositional tuning, with increased water solubility driving cellular uptake while a more balanced ratio (60:40 DMA-co-TEMPO) optimized antioxidant potential. This random copolymer significantly reduced ROS and inflammatory cell presence in an *in vivo* model of inflammation. The *in vivo* results indicate that 60:40 DMA-co-TEMPO is a promising candidate as a PTOA therapy. Future studies will focus on the measurement of superoxide-specific scavenging by polymers in cell-free and activated immune cell-based assays.

References:

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