

## Poly(trimethylene carbonate) Molecular Weight Affects Enzymatic Adsorption and Conformation, and Macrophage Behavior

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**Statement of Purpose:** High molecular weight poly(trimethylene carbonate) (pTMC) and its copolymers are commonly used in biomaterials research and development. pTMC degrades *in vivo* by macrophage mediated surface erosion during which macrophages attach to the material surface and secrete reactive oxygen species (ROS) and enzymes to degrade the material<sup>1</sup>. Interestingly, *in vivo*, 100 kg/mol or higher pTMC degrades faster than 60 kg/mol pTMC<sup>2</sup>. The reasons for this difference in degradation rate are still unclear and may be related to both macrophage attachment and ROS secretion or enzymatic adsorption and activity. Identifying this mechanism may have significant implications for the design of future p(TMC) based biomaterials. The following study examines macrophage behavior, enzymatic adsorption and viscoelastic properties of the adsorbed enzyme layer on 100 kg/mol and 60 kg/mol pTMC to determine possible causes of the molecular weight dependent degradation rate.

**Methods:** PTMC with targeted molecular weights of 60 kg/mol and 100 kg/mol was synthesized from TMC monomer by ring opening melt polymerization in the presence of tin(II) 2-ethylhexanoate catalyst. 1-pentanol and residual water served as the initiator for 60 kg/mol and 100 kg/mol pTMC, respectively. The polymerized pTMC was purified by precipitation in -20°C methanol, dissolved in chloroform (0.5 g/mL) and pipetted into the wells of 96 well TCPS plates. RAW 264.7 murine monocyte derived macrophages were seeded in the wells at 10,000 cells/well and cultured for 14 days in supplemented media. Cells were analyzed for cell number and ROS secretion (n=6). Surface plasmon resonance (SPR) was used to quantify mass of enzyme adsorption to the pTMC surfaces. SPR sensor chips (n=3) were coated with 0.0125 mg/mL solution of pTMC in chloroform and dried overnight. SPR baselines in PBS were measured for the coated sensors. 380  $\mu$ L of cholesterol esterase (CE) or lipase solution (1 mg/mL) was injected and enzymatic adsorption monitored. Viscoelastic properties of adsorbed enzyme layers were quantified by quartz crystal microbalance with dissipation (QCM-D). pTMC coated QCM-D sensors (n=3) were inserted into the QCM-D module and conditioned in PBS overnight. CE or lipase solution was then flowed over the sensor and the enzyme was allowed to adsorb for 12 hours. Enzyme layer viscoelastic properties were calculated by fitting the raw data to the Voigt viscoelastic model. Significance was determined at  $p < 0.05$ .

**Results:** Macrophages cultured on the 100 kg/mol pTMC surface secreted more superoxide anion compared to 60 kg/mol pTMC surface over 14 days and significantly more on day 10 (Figure 1). Significantly more CE adsorbed to the 100 kg/mol pTMC while no difference was observed for lipase adsorption (Figure 2). CE adsorbed in a significantly stiffer layer on the 60 kg/mol

pTMC, suggesting a difference in the adsorption orientation or conformation of the enzyme on the 60 and 100 kg/mol pTMC surfaces (Figure 3).

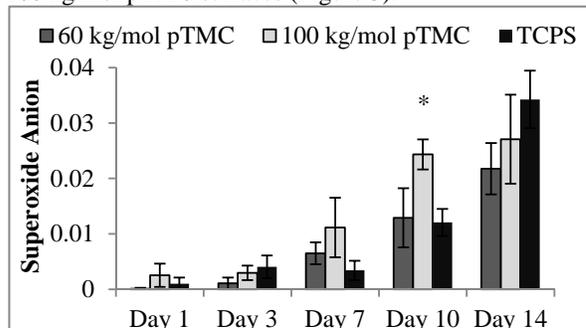


Figure 1. Macrophage O<sub>2</sub><sup>-</sup> secretion (counts/cell)

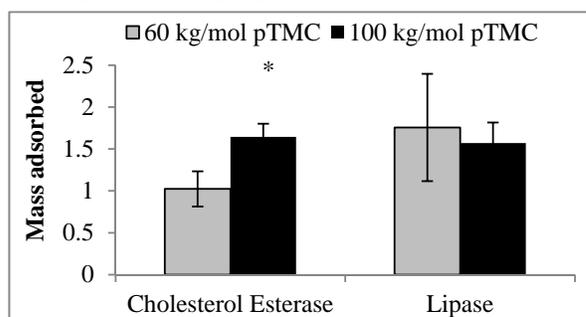


Figure 2. Mass of enzyme adsorbed on pTMC (ng)

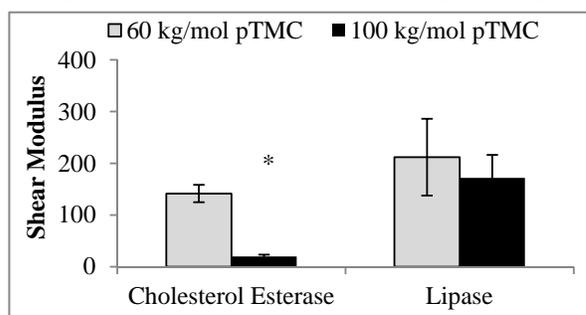


Figure 3. Shear Modulus (kPa) of enzyme layer on pTMC

**Conclusions:** CE adsorbs in lower amounts and in a stiffer layer on the surface of 60 kg/mol pTMC suggesting that the enzyme may be more denatured on that surface. 60 and 100 kg/mol pTMC differ only in bulk modulus and polymer chain mobility. As enzymatic adsorption and activity are surface phenomena, the CE results suggest that polymer chain mobility may be influencing enzyme configuration or availability of active sites. Additionally macrophages cultured on 100 kg/mol pTMC secreted more O<sub>2</sub><sup>-</sup> despite there being no difference in cell attachment. This change in activity is possibly in response to a difference adsorbed serum proteins such as IgG between the two pTMC surfaces.

**References:** 1. Bat E. Acta Biomater. 2011;7:1939-48.  
2. Zhang Z. Biomaterials. 2006;27:1741-1748.