Monomer Sequence-Controlled PLGA for Controlled Release Applications

Samruddhi Patil

Prof. You-Yeon Won

Sequence control is a defining feature of biomacromolecules such as DNA, RNA, and proteins, allowing them to perform highly specific and diverse functions in living systems. Inspired by this, polymer scientists have recognized repeat-unit sequence as an important design parameter in synthetic copolymers. However, for widely used biopharmaceutical polymers such as poly(D,L-lactic-co-glycolic acid) (PLGA), the influence of monomer sequence on polymer and formulation properties remains largely unexplored, mainly due to the lack of scalable methods for synthesizing sequence-controlled PLGA copolymers. To address this research gap, this dissertation focuses on the development and preclinical evaluation of statistically monomer sequence-controlled PLGA copolymers for controlled release applications.

In the first part of this work, a MATLAB-based kinetic population balance model was developed for DBU-catalyzed PLGA copolymerization, providing mechanistic insight into the reaction pathway and the copolymerization kinetics of lactide (LA) and glycolide (GL). Parameterization of the model using experimental data enabled the estimation of previously unknown rate constants and LA/GL comonomer reactivity ratios. The resulting model was then used to guide the design of a semi-batch synthesis strategy for producing sequence-tailored PLGA, including polymers with constant monomer-sequence characteristics along the chain.

A major outcome of this research is the development of a facile, scalable feed rate-controlled polymerization (FRCP) strategy that enables statistical control over comonomer sequence distribution. Using this patented platform, PLGAs with comparable molecular weights and monomer compositions but different monomer sequence distributions were synthesized across various LA/GL ratios. These materials were then used to systematically evaluate the effect of monomer sequence distribution on the glass transition behavior of PLGA, a property commonly considered an important factor influencing drug release. The experimentally measured glass transition temperatures (T_g) and the monomer sequence lengths showed excellent agreement with theoretical frameworks established by Johnston and Barton, highlighting the complex interplay

between monomer composition and sequence distribution in determining the T_g of PLGA copolymers.

In the final part of this work, compositionally equivalent PLGA copolymers with different sequence distributions—relatively more uniform ("uniform") and relatively less uniform ("gradient") were synthesized using the FRCP technique and formulated into paclitaxel (PTX)-loaded microparticles to investigate the effect of monomer sequence distribution on PTX release kinetics. Despite having comparable bulk properties, the uniform and gradient PLGA formulations exhibited significantly different *in vitro* release profiles, as quantified by high performance liquid chromatography (HPLC). To further elucidate the underlying mechanisms, comprehensive *in vitro* degradation studies were performed using gel permeation chromatography (GPC), scanning electron microscopy (SEM), and modulated differential scanning calorimetry (MDSC), while molecular simulations were employed to examine the evolution of copolymer nanostructure during formulation. The findings reveal that PTX release is governed by a cascade of interconnected mechanisms, beginning with sequence-dependent formation of LA-rich and GL-rich domains that promotes PTX partitioning into GL-rich regions, followed by water uptake and cluster formation that displace PTX into less hydrated LA-rich domains where diffusion is facilitated. Overall, the combined experimental and computational analyses show that sequence-dependent differences in domain size and distribution play a central role in determining the release kinetics.

In summary, this work presents significant advances in the development of sequence-controlled PLGA and demonstrates its potential to tailor polymer properties and drug release kinetics. These findings highlight monomer sequence distribution as an important critical quality attribute (CQA) in the rational design of PLGA-based biomaterials for controlled-release applications.