審 査 の 結 果 の 要 旨

氏 名　フアン　ジョージ　ロ

Immunotherapy is a promising strategy for treating cancers by employing the immune system to identify and kill malignant tumors. However, many tumors have immunosuppressive microenvironments that inhibit immunotherapy. There is significant interest in developing novel immunotherapeutics that can modulate the immunosuppressive environment to promote anticancer immunity. We have identified two amino acid based small molecules that have been demonstrated to modulate the tumoral microenvironment to inhibit the immunosuppressive environment found in cancers. One such immunotherapeutic is N-ω-hydroxy-arginine (NOHA), which is an Arginase 1 (Arg1) inhibitor and inducible nitric oxide (iNOS) substrate. This small molecule has the potential to significantly alter macrophage function by inhibiting protumor biological activity while simultaneously functioning as a substrate for synthesizing nitric oxide, which is used by macrophages to kill cancer cells. Arg1 inhibition has been demonstrated to slow tumor growth and metastasis which may work well in combination with other drugs for synergistic anticancer efficacy. Another immunotherapeutic is methyl tryptophan, which is showing promising activity in clinical trials. However, the drug suffers from poor pharmacokinetics, including rapid clearance which results in low serum concentration. Polymeric micelles are a promising drug delivery system (DDS) which may avoid renal clearance and increase serum concentrations. Thus, novel polymeric micelles were synthesized from methyl-L-tryptophan (MLT), allowing the polymer to be constructed from the drug itself. This strategy significantly reduces the inactive incipient material used to deliver the drug. Additionally, by constructing the polymeric micelle from MLT, a novel DDS platform has been synthesized that allows for loading of other therapeutics in the micelle core for combination therapy.

In **Chapter 1**, the biological and pharmacological background for understanding this study was introduced. First, an introduction to immunotherapy and cancer immunology was introduced, with a discussion on the various key cell types that play a role in cancer immunology. Additionally, the microenvironmental cues of toll like receptors (TLR), Indoleamine 2,3-dioxygenase (IDO), and Arg1 on cancer immune phenotype was introduced. Next, an introduction into polymeric micelles will be introduced, with a discussion of EPR effect and polymer chemistry. The synthesis strategy of PEG-polyamino acids through the use of N-carboxy-anhydrides (NCA) and N-phenyl-carbamates NPC will be introduced as well. Lastly, an introduction into how polymeric micelles could be designed to modulate these microenvironmental cues was introduced.

In **Chapter 2** the successful synthesis of block copolymers of polyethylene glycol and poly methyl tryptophan (PEG-P(MLT)) and block copolymers of polyethylene glycol and

N-ω-hydroxy-arginine (PEG-P(NOHA)) for IDO and Arg1 inhibitors were presented. Polymers of MLT and MDT were first synthesized to elucidate proteolytic activity. As MLT is significantly more responsive to proteases, MLT was selected for developing polymeric micelles. NCAs of MLT were synthesized from triphosgene, and the presence of NCA was confirmed by NMR and IR spectroscopies. However, polymers synthesized from MLT were not soluble in any solvent, suggesting crosslinking. Thus, NPCs of MLT were synthesized by another strategy to avoid crosslinking during MLT polymerization. Uniform block copolymers of PEG-P(MLT) were successfully synthesized from MLT-NPC, as confirmed by SEC and NMR. Next, successful synthesis of PEG-P(NOHA) from PEG-P(Ornithine) were presented and discussed.

In **Chapter 3** the biological activity of PEG-P(MLT) was characterized in vitro. First, successful enzymatic degradation of PEG-P(MLT) was confirmed in vitro by a chymotrypsin digestion assay. Next, PEG-P(MLT) micelles were self-assembled and confirmed to be non-cytotoxic against immune cells. Alexa Fluor labeled micelles were then incubated with THP-1 human monocytes and demonstrated to have significant cellular uptake over 24 h. Micelles were also confirmed to reduce the amount of kynurenine synthesized from THP-1 monocytes, suggesting successful IDO inhibition. Lastly, micelles were confirmed to be non-immunogenic by TNF-a expression in THP-1 cells and NF-kB expression in RAW 264.7 cells.

In **Chapter 4**, attempts to load the TLR agonist Imiquimod into MLT based micelles are presented. As Imiquimod failed to load into PEG-P(MLT) micelles, PEG-P(Glu(Otbu)-MLT) was synthesized for direct drug conjugation of small molecule TLR agonists to polymer. Additionally, PEG-P(Lys(Cbz)-MLT) was also synthesized for polyion complexation of polynucleotide based TLR agonists. PEG-P(Glu) was used as a model polymer for steiglich esterification of resiquimod to the polymer. Results of TLR loading are still limited, and a point of future work.

In **Chapter 5** the results and significance of each chapter was summarized and future perspectives of this study for clinical applications is discussed.

In this dissertation, novel block copolymers are synthesized in which the therapeutic is used as a building block to construct the polymer. This strategy reduces the amount of inactive excipient material in the drug delivery system while allowing for codelivery of two drugs. Future work will focus on loading drugs such as TLRs that can stimulate the immune system to generate an antitumor effect. The findings of this thesis will promote the development of bioactive materials based on the polymerization of drugs for controlled activation in the body. These materials could be applied in different fields of bioengineering, such as tumor targeting or tissue regeneration.

According to the referees' comments, this dissertation is eligible for applying for a diploma of Ph.D. (engineering).