Development of a Poly(lactic acid) Poly(ethylene glycol) Nanoparticle for Delivery of Vitamin D_3 to Severe Asthmatics

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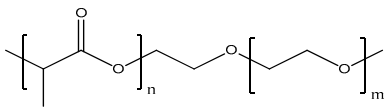
Statement of Purpose: Asthma is a chronic condition which induces airway constriction, inflammation and shortness of breath in more than 22 million Americans. A majority of patients with mild to moderate asthma are able to manage their symptoms with the use of inhaled glucocorticoids (GCs) and bronchodilators yet the same does not hold true for severe asthmatics. Despite treatment with high dosages these drugs, nearly 25% of patients continue to experience profound airflow limitation and inflammation. Compelling data from collaborators at the University of Pennsylvania suggests that vitamin D_3 (vitD) differentially modulates airway inflammation from that of GCs and may provide therapeutic relief in the treatment of severe asthma. In order to deliver vitD to inflamed lung cells we have developed a block copolymer nanoparticle utilizing poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) while employing the bronchodilator albuterol as a targeting ligand. PLA and PEG were chosen because of their proven biocompatibility and biodegradability as well as their hydrophobic and hydrophilic natures.

Methods: Synthesis of the copolymer involved the ring opening polymerization of D,L-lactide to form PLA with PEG350 used as the hydrophilic end. The polymerization was catalyzed by tin(II) oxide in the presence of toluene. The block copolymer was then purified in selected solvents which were evaporated off and then analyzed with H^1NMR. The average molecular weight was determined through gel permeation chromatography (GPC). Because of the hydrophilic and hydrophobic properties of PLA and PEG the copolymer forms self assembling micelles in the presence of water. VitD was first dissolved in an organic solvent and then added to the copolymer solution so as to be encapsulated inside the nanoparticle. Encapsulation efficiency is determined by reversed-phase chromatography (RPC). In order for attachment of albuterol the end group of PEG was modified from a hydroxyl to carboxylate group. This was accomplished by use of chloroacetic acid, yielding a stable ether bond terminating in a carboxymethyl group with results being analyzed with H^1NMR. The attachment of albuterol is completed through an esiterifcation reaction using N,N'-dicyclohexylcarbodiimide as a coupling agent and 4-dimethylaminopyridine as a catalyst. Cytotoxicity tests were conducted on unloaded PLA-PEG samples by inoculating human airway smooth muscle (HASM) cells with small amounts of polymer. Cells were allowed to incubate for 48 hours and results were analyzed using a Vi-Cell instrument (Beckman Coulter).



n = number of poly(lactic acid) repeat units
m = number of poly(ethylene glycol) repeat units

Figure 1. Chemical structure of PLA-PEG copolymer

Results: The synthesis of PLA-PEG copolymer was determined to be successful by H^1NMR. The molecular weight of the copolymer ranged from 500-100 g/mol with an average of 606 g/mol over 15 samples. Scanning electron microscope images of the formed micelles conformed nanoparticle formation and showed an approximate radius of 300nm. H^1NMR has shown the carboxylation of the hydroxyl group of the PEG to be successful. The percent recovery of the carboxylated PEG is currently 10%. Cytotoxicity test showed a cell viability of 89% of HASM cells after an inoculation of unload PLA-PEG and an incubation time of two days.

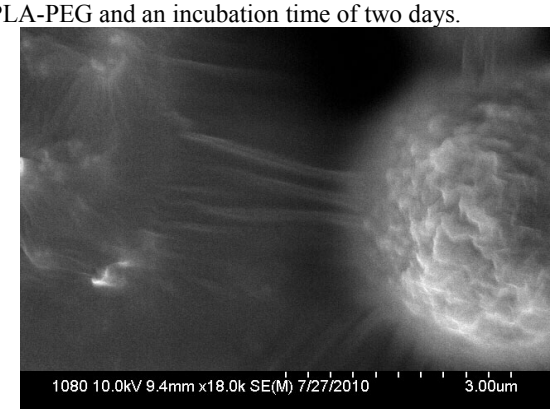


Figure 2. SEM image of formed PLA-PEG nanoparticle

Conclusions: The PLA-PEG block copolymer was successfully synthesized and purified for HASM cell application, as verified by H^1NMR and the Vi-Cell analyzer. The molecular weight of the polymer was averaged 6oo g/mol. SEM verified formation of the micelle-like nanoparticles consistent spherical shape and size. Stability of the nanoparticles was observed through SEM imaging over several months which allows for extended storage of the drug loaded polymer. Though carboxylation of the hydroxyl end group of PEG was successful, percent recovery remains low at 10%. The carboxylation allows for an easy attachment of albuterol but recovery of the carboxylated PEG needs to be increased. Further studies need to focus on optimizing the ratio of PLA to PEG in the copolymer. The degradation rate determines the release rate of vitD, which is determined by the ratio of PLA to PEG. Since it has been proven that synthesis of PLA-PEG nanoparticle is achievable and cell viability is good, optimizations of the copolymer as a delivery agent for vitD in HASM warrants further studies.

References: Banerjee, A., et al., *Vitamin D and Glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells.* British Journal of Pharmacology, 2008. **155**(1): p. 84-92.

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