High-throughput screening of PLGA thin films utilizing hydrophobic fluorescent dyes

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Statement of Purpose: Paclitaxel is well known as an important treatment in various cancer pathologies and particularly limiting the growth of scar tissue, or restenosis. Drug eluting stents have incorporated paclitaxel into the slow release thin films, displaying reduced restenosis rates over several weeks. Thin films incorporating paclitaxel are under development to give various release profiles that control the burst and extended dosages from weeks to months. However, the quantification of these hydrophobic drugs such as paclitaxel or rapamycin by HPLC (Gold Standard) have major drawbacks and too laborious considering replicates, hourly or daily sample aliquots, filtering of samples, preparation of HPLC vials, etc.. The problem is that hydrophobic compounds such as paclitaxel or rapamycin are not fluorescent, so fluorescent analogues with similar logP values were chosen to model these drugs. Herein we describe a high-throughput assay using three model hydrophobic fluorescent compounds; fluorescein diacetate, coumarin-6, and rhodamine-6g that were incorporated into PLGA and PLGA/PEG films.

Methods: The fluorescent dye incorporated into the 6 mm diameter PLGA discs were immersed in 200 µL of PBS/2% Tween 80 solution, within a 96 well Costar flat black polystyrene flat bottom plate. Their subsequent release was screened in a high-throughput assay (96-well plate/M200 Tecan Spectrofluorometer) and directly compared to HPLC quantification of paclitaxel release.

Results: The drug release behavior of the hydrophobic fluorescent molecules: fluorescein diacetate (FDA), coumarin 6, rhodamine 6g and paclitaxel show biphasic pattern of release in pure PLGA films of intrinsic viscosity (i.v.) 0.2 and 1.03 dL/g. The fluorescein diacetate and paclitaxel revealed a similar drug release profile with no burst release. Fluorescein diacetate was found to mimic the paclitaxel release kinetics the most (Figure 1). The drug release behavior of paclitaxel and hydrophobic dye fluorescein diacetate, coumarin 6, and rhodamine 6G also showed biphasic pattern of release with the addition of PEG additives at PLGA i.v. of 1.03 dL/g. With the addition of PEG 8000 and 35000 at two percentages of 15% and 50% into the PLGA films, the release profiles of fluorescein diacetate and paclitaxel began to differ. As of the phase separation of crystalline PEG became more substantial release rates of the PEG co-localized paclitaxel increased resulted in a faster release. Phase separation was measure by Raman Microscopy, which allowed a qualitative distribution, over an 100 x 100 micron area.

Conclusions: Three fluorescent dyes with logP's similar to paclitaxel have been evaluated in PLGA films with Raman Microscopy for molecular distribution (Figure 2) and compared against paclitaxel release of < 30 days. Two of the dyes were found to be inadequate, as they were not soluble within the PLGA matrix, and tended to phase separate and crystallize. One hydrophobic dye, fluorescein diacetate (converted to fluorescein when treated with base) was found to correlate with paclitaxel release, and had similar diffusion coefficients when incorporated into PLGA films (intrinsic viscosity of 0.2 and 1.03 dL/g) where both fluorescein diacetate and paclitaxel were dissolved and completely homogenous. When PEG additives were present, fluorescein diacetate was still found to be correlated with paclitaxel release, when crystalline PEG was not present. As the MW and concentration of PEG increased, more crystalline PEG was present, and the co-localized paclitaxel had an increase in burst release, higher rate of release over the first five days, or both. Thus, a method for high-throughput screening of PLGA thin film formulations has been presented.

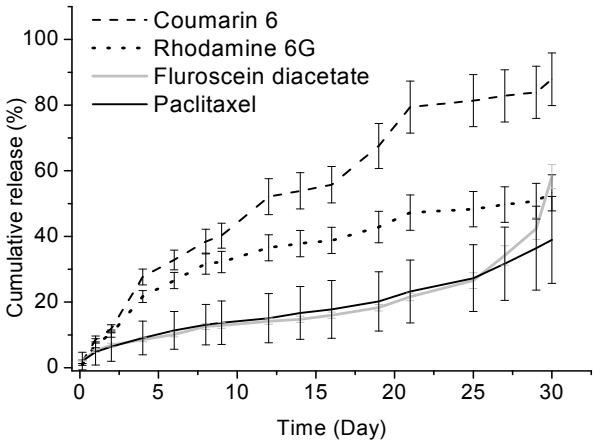

Figure 1- Release of hydrophobic fluorescent dyes from 0.2 (i.v.) PLGA.

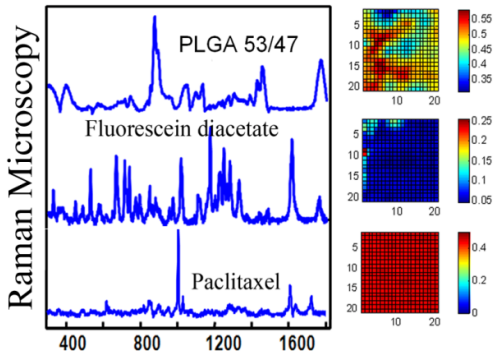

Figure 2 - Raman Microscopy of PLGA films with FDA and Paclitaxel.