3-D Visualization of Paclitaxel in Polymer Films by Coherent Anti-Stokes Raman Scattering Microscopy

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Introduction: Noninvasive chemical imaging is ideal for the study of drug distribution and release. Fourier transform Infra-red (FTIR) imaging or confocal Raman microscopy has low resolution or low sensitivity, which limit their applications in the physiological environment and in a dynamic process such as drug release in situ. On the other hand, coherent anti-Stokes Raman scattering (CARS) microscopy has chemical selectivity, inherent three-dimensional (3D) resolution and high sensitivity. In this study, CARS microscopy was used to investigate drug distributions in various polymer matrices and to examine drug release *in situ* from the polymer films.

Methods: ***CARS Microscopy.*** Two Ti:sapphire lasers (Coherent Inc, Mira900) were tightly synchronized to generate the 2.5-ps pump and Stokes beams at frequency ω_p and ω_s respectively ($\omega_p > \omega_s$). The two excitation beams were collinearly combined and were directed into a laser scanning microscope (Olympus Inc, FV300/IX70). CARS is a four-wave-mixing process in which a signal field at $\omega_{as}=2\omega_p-\omega_s$ is generated. The signal is enhanced when $\omega_p-\omega_s$ is tuned to the vibrational frequency of a Raman band, thus providing chemical selectivity. As a nonlinear process the signal is only generated at the laser focus, which provides inherent 3D resolution.

Film preparation. Films were prepared with a polymer/paclitaxel (PTX) mixture in chloroform using a spin coating method. Various polymers were used such as poly(hydroxyethyl methacrylate) (PHEMA), poly(lactic acid-co-glycolic acid) (PLGA), poly(ethyl vinyl acetate) (PEVA), polyurethane (PU), and poly(butyl methacrylate) (PBMA).

Results and Discussion: To identify the best Raman shifts to image the drug and the polymer matrix separately, the CARS spectra of pure drug and each polymer were measured point by point. The spectra are obtained by normalizing the CARS signal from pure drug or polymer films by the nonresonant signal from glass. The CARS spectra at 3000 and 1600 cm^{-1} regions are shown in Fig. 1-a and 1-b, respectively. A peak and a dip were observed at 3060 and 3090 cm^{-1} respectively for PTX from aromatic CH stretching, whereas signals are weak for the polymers at these Raman shifts. The image of PTX was constructed using the signal difference between 3060 and 3090 cm^{-1} to remove the nonresonant background. The images of the polymers were taken at 2840 cm^{-1}, CH_2 stretching vibration frequency, where polymer signal is much larger than PTX signal. The overlay of the PTX image (green) and polymer image (magenta) shows PTX distribution in polymer film. XY image in Figure 1-c shows that PTX particles were aggregated with a size ranging 0.5-2 μm in the mid-depth of a PEVA/7.5wt% PTX film. The PTX particles were evenly distributed inside the film as shown in the XZ image (Fig. 1-d).

To image PTX release in a medium (30% isopropyl alcohol (IPA)/PBS) *in situ*, we have to use a Raman band far away from the broad water band at 3200 cm^{-1}. A peak at 1590 cm^{-1} (C=O stretch) and a dip at 1620 cm^{-1} were observed for PTX while spectrum of polymer was dominated by nonresonant background in this region (Fig. 1-b). Images of PEVA film with 7.5wt% PTX during PTX release in the medium were taken at 1590 cm^{-1}. Figure 1-e shows time-dependent PTX release at different Z depth of the film. Holes were observed after PTX release. In the beginning stage of release in the medium, holes were seen only at the top layer, indicating that PTX release was started from the surface region.

Conclusion: CARS microscopy provided an excellent imaging tool to investigate drug distribution and release behavior in situ from a solid state polymer matrix. Drug release behavior from a polymer matrix was imaged in situ with 3D resolution using CARS microscopy. The unique ability of CARS microscopy to image drug molecules without labeling is extremely useful in the studies of various drug delivery systems and biomaterials.

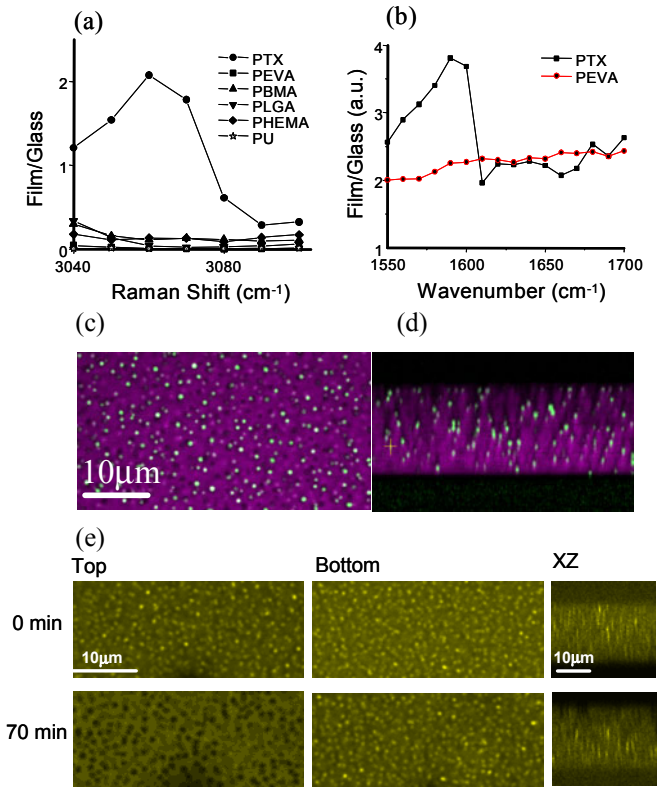

Figure 1. (a) CARS spectra of PTX and various polymers at 3060 cm^{-1} (b) CARS spectra of PTX and PEVA at 1600 cm^{-1} region (c) XY image of PTX distribution in PEVA film of 7.5wt% PTX/PEVA (Green; PTX and purple; PEVA) (d) XZ image of PTX distribution (e) XY images of time dependent PTX release from PEVA matrix along the Z axis in 50% IPA/PBS *in situ*. The images were taken at 1590 cm^{-1}.