Dynamic noninvasive monitoring of renal function in vivo by fluorescence lifetime imaging

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Abstract. Kidneys normally filter the blood of excess salts and metabolic products, such as urea, while retaining plasma proteins. In diseases such as multiple myeloma and diabetes mellitus, the renal function is compromised and protein escapes into the urine. In this study, we present the use of fluorescence lifetime imaging (FLI) to image excess serum protein in urine (proteinuria). The near-infrared fluorescent dye LS-288 has distinct lifetimes when bound to protein versus free in solution, providing contrast between the protein-rich viscera and the mostly protein-free bladder. FLI with LS-288 in mice revealed that fluorescence lifetime (FLT) differences in the bladder relative to surrounding tissues was due to the fractional contributions of the bound and unbound dye molecules. The FLT of LS-288 decreased in the case of proteinuria while fluorescence intensity was unchanged. The results show that FLI can be useful for the dynamic imaging of protein-losing nephropathy due to diabetes mellitus and other renal diseases and suggest the potential use of the FLI to distinguish tumors from fluid-filled cysts in the body.

Keywords: optical imaging; fluorescence lifetime; near-infrared; proteinuria; albumin; nephropathy.

1 Introduction
Proteinuria (PU), the presence of abnormally high levels of protein in urine, is a marker of chronic kidney disease (CKD), which can lead to end-stage renal disease. A recent study has shown that 11% of Americans over 20 years of age have CKD and 63% of these have microalbuminuria. Early detection of kidney dysfunction can be useful in treating diabetes mellitus, urinary tract infections, polycystic kidney disease, autoimmune diseases, transplant rejection, and acute toxicity.

Nuclear imaging has been a mainstay of urology for years but remains expensive, hazardous, and time consuming. Op-
3 Results and Discussion

3.1 Dynamic Bladder Imaging in Real Time

We used LS-288 for this study because it has distinctly different FLTs when free versus interacting with proteins in solution. Using a series of FLT map images, the change in bladder size was visualized over time (Fig. 2 and Video 1). FLI provided excellent contrast between the low-protein urine in the bladder and the surrounding protein-rich tissues. In addition to imaging the bladder and abnormalities in the region, this method could be used to monitor urine flow rate or frequency of urination without invasive procedures such as surgery or urethral catheterization.

3.2 Fluorescence Lifetime Data Analysis

A single exponential fitting of the TPSF decay provides a single value $\bar{\tau}$, which can be considered a weighted average of the values obtained through biexponential fit corresponding to the unbound ($\tau_1$) and protein-bound ($\tau_2$) fractions ($f_1$ and $f_2$, respectively) of LS-288 [Eq. (1)]. The measured values of $\tau_1$ and $\tau_2$ for LS-288 remained relatively constant after intravenous administration in living mice despite a large change in $\bar{\tau}$ (Table 1). Dye molecules in the blood and other tissues are largely protein bound ($\bar{\tau}$, with only slight contribution from $\tau_1$, as demonstrated by FLT gating of fluorescence intensity values for each FLT from the biexponential model mapped over the bladder (Fig. 3). Thus, the single exponential fitting of FLI data can be used to simplify analysis and data representation [Fig. 3(c)]. FLT of the unbound LS-288 shown in Table 1 is closer to the lifetime of the dye in water (0.44 ns).

$$\bar{\tau} = f_1 \tau_1 + f_2 \tau_2.$$  

3.3 Detection of Proteinuria In Vivo

Variations in $\bar{\tau}$ values are expected between the individual PU mice because each mouse is hydrated differently, has a different metabolic status, and urinates at different times. The BSA
solution concentration of 1.0 mg/mL used in this study corresponds with macroalbuminuria, but the combination of continuous glomerular filtration, varying initial urine volume, and sporadic urination allows for a wider range of albumin levels to be examined. This is reflected in the PU results for individual mice, and despite the variability between each of them, the PU $\bar{\tau}$ values are significantly different from those of the control mice. In both treatment sets, the bladder was distinguishable from the body by using $\bar{\tau}$. Statistical analysis shows that there is a significant difference between PU and control bladders and between both bladder ROI sets and their respective body ROIs (Table 1).

<table>
<thead>
<tr>
<th>$\bar{\tau}$/ns</th>
<th>$\tau_1$/ns</th>
<th>$\tau_2$/ns</th>
<th>$f_1/f_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control bladders</td>
<td>0.682 ±0.029$^a$</td>
<td>0.451 ±0.034</td>
<td>0.966 ±0.084</td>
</tr>
<tr>
<td>PU bladders</td>
<td>0.794 ±0.050$^a$</td>
<td>0.427 ±0.043</td>
<td>1.084 ±0.062</td>
</tr>
<tr>
<td>Control bodies</td>
<td>0.90 ±0.12</td>
<td>0.465 ±0.050</td>
<td>1.061 ±0.066</td>
</tr>
<tr>
<td>PU bodies</td>
<td>1.037 ±0.078</td>
<td>0.510 ±0.076</td>
<td>1.172 ±0.042</td>
</tr>
</tbody>
</table>

$^a$Denotes $p<0.01$

4 Conclusions

NIR fluorescent dye-mediated FLI is an exciting optical imaging method with a great potential to unravel physiological and molecular processes in cells and living organisms. Particularly, the early detection of nephropathy due to diabetes mellitus and other causes would improve treatment for patients. Our results show that the FLI of mouse bladder with LS-288 allows for dynamic imaging of bladder physiology and proteinuria. Because the bladder is a superficial organ in humans, clinical translation of the FLI method using a mouse model of proteinuria described herein is feasible. The FLI strategy is applicable to other organs penetrable by NIR light to improve diagnostic information. For example, the distinct FLT behavior of LS-288 in different environments suggests the potential use of FLI to distinguish tumors from fluid-filled cysts in the body or to identify solid cancer in the bladder.

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References