In Vivo Near Infrared Techniques for Protein Drug Development

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INTRODUCTION

Near infrared (NIR) light (700 ~ 900 nm) possesses the capability of penetrating living tissues several centimeters due to the low absorbance of tissue intrinsic chromophores such as oxy- and deoxy-hemoglobin (the main absorber of visible light), melanin, water, and lipid (the principal absorber of infrared light). Featured with the deeper tissue penetration as well as nonionizing and nonradioactive, NIR light attracts extensive attentions on the development of noninvasive techniques for *in vivo* real time monitoring/tracing of biological signals in living tissues. Hitherto, *NIR techniques* have permeated to almost all aspects of health care, such as diagnosing disease (Nahum, Skippen, Gagnon, Macnab, & Skarsgard, 2006), designing the targeted molecular or drug carrier (Hsu et al., 2006), monitoring the response to therapeutic treatment (Tachtsidis et al., 2007), evaluating the rehabilitation, and so on. With the rapid development of various NIR techniques and more cooperation with clinic studies, more potential applications in health care will be exploited in the near future.

Characterized with the virtue of high physiological activity, specificity, and low toxicity, *protein drugs* are becoming mainstream therapeutic agents and constitute a substantial portion of the compounds under preclinical and clinical development in the global pharmaceutical industry (Baumann, 2006). And the *in vivo* evaluation of drug properties, from animal models to human subjects, becomes a crucial in the *protein drug* development. It involves drug screening, drug delivery, drug biodistribution, pharmacokinetics, pharmacodynamics, and so on. Each of these processes requires *quantitative* monitoring of *drug concentrations* in the specific organ/tissue or *qualitative imaging* of *drug distribution* in whole subjects. Thus, a number of analytical techniques were exploited to analyze the *protein drug* process within the experimental subjects. Immunoassay, bioassay, and isotope labeling are the most commonly used methods for quantification of macromolecule in biological fluid (Marshall, Macintyre, James, Krams, & Jonsson, 2006). Immunoassay is based on the specific antibody–antigen reaction. The response signal is generated from a label (e.g., enzymatic, fluorescent, and radio isotopic) attached to either antigen or antibody. This method, including radioimmunoassay (RIA), immunoradiometric assays (IRMA), and enzyme-linked immunosorbent assays (ELISA), is rapid and sensitive as well as economical, but it is heavily affected by a variety of endogenous and exogenous substances. Bioassay provides an indirect method by using living system to measure the biological activity of a drug. This method is expensive, time consuming, and lacks specificity and sensitivity. Isotope labeling is an alternative approach to *in vivo* quantification of protein drug (Liu, Dreher, Chow, Zalutsky, & Chilkoti, 2006). Although it is sensitive, some disadvantages regarding the complex preparation and radioactivity limited its broad application. Beside the aforementioned analytical methods, techniques such as chromatography and electrophoresis are usually used for *protein drug measurements*. However, all the listed techniques, except isotope labeling, are unable to perform the *in vivo* measurement of *protein drug* in biological matrices. They require the tedious and intricate process of sample preparation such as sample collection and purification. Furthermore, the clearance of protein drugs from the specific tissue/organ is quick and the above analytical techniques cannot catch up with its dynamic changes.

Therefore, a noninvasive *in vivo* real time monitoring modality for protein drugs in biological matrices will timely meet the needs and greatly expedite the step of *protein drug* development.

Near infrared techniques contributed considerably to the development of protein drugs by performing noninvasive *in vivo* real time *measurements* on experimental subjects. In this chapter, we will first present the fundamental theory of NIR techniques, followed by the summary of NIR *fluorescence probes*. Emphasis will be placed on the practical applications of NIR techniques on drug development. Future trends and conclusions will be given at the end.

BACKGROUND

Near Infrared Techniques: Spectroscopy and Imaging

Near infrared light (700 to 900 nm), where photon transport in tissue is dominated by scattering rather than absorption, has the maximal penetration depth of several centimeters, therefore enabling *in vivo* sampling of large tissue volumes, for example, breast, brain, skeletal muscles, or tumors. The absorptions of NIR light by endogenous chromospheres, that is, oxygenated and deoxygenated hemoglobins, and exogenous contrast agents have been used to determine important physiological characteristics of tissues.

In the last decade, extensive investigations in noninvasive NIR techniques have been conducted for both *quantitative* and *qualitative* determination of biological signal in tissues. Based on the profile of the incident light, three main near infrared systems, that is, time-domain (TD), frequency-domain (FD), and continuous wave (CW) photon migration system, have been developed for various *measurements*. Figure 1 illustrates the profiles of the incident light (red line) and tissue diffused light (blue line) for the three systems.

Usually, CW techniques employ illumination with a constant intensity. The diffused light attenuates exponentially with the source-detector (S-D) separation, section (a) of Figure 1, and with the depth of the tissue according to the tissue optical properties. Although having limited penetration depth and only intensity retrieved, CW system is the most easily implemented system and the most widely used in various *measurements* on small *animal models*. In contrast to CW techniques, TD approaches employ a source with a femto-picosecond pulse of light, which is broadened and attenuated as it propagates through the tissue, see section (b) of Figure 1. The diffused light intensity, transient time, and pulse shape are recorded to determine the tissue optical properties. The measurement from a TD system is more accurate and usually used as calibration for other newly developed systems. However, its high cost has limited its wide application. FD techniques offer a compromised approach to the determination of tissue optical properties. The incident light on an FD system is modulated at radiofrequency range (namely, 70 MHz—several hundred MHz). The diffused photon wave propagates through tissue and becomes amplitude-attenuated and phase-shifted relative to the incident light. After demodulation, the changes in amplitude and phase are retrieved for the determination of tissue optical properties. Various forms of heterodyne or homodyne demodulation systems have been utilized to implement FD system. Because the detected amplitude and phase are insensitive to the ambient light, FD system enables greater sensitivity and

Figure 1. Profiles of the incident light (red line) and the diffused light (blue line) for the continuous wave system, the time-domain system, and the frequency-domain system