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NAD(P)H and Collagen as *in Vivo* Quantitative Fluorescent Biomarkers of Epithelial Precancerous Changes[1]

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ABSTRACT

During the development of neoplasia, epithelial tissues undergo biochemical and structural changes that can manifest in tissue fluorescence. There have been several reports on different *in vivo* fluorescence characteristics between normal and precancerous (dysplastic) tissues. However, it has been difficult to identify and quantify the origins of these changes, mainly because of distortions introduced in measured tissue fluorescence spectra by tissue scattering and absorption. Such distortions can be removed by combining information in simultaneously measured fluorescence and reflectance spectra. Thus, we can recover the intrinsic (undistorted) tissue fluorescence. In this report, we show that extraction of the intrinsic fluorescence allows us: (*a*) to determine the fluorescence spectra of NAD(P)H and collagen in an *in vivo* environment, and (*b*) to use these NAD(P)H and collagen spectra to describe, quantitatively, diagnostically significant biochemical changes between normal and dysplastic tissues. Specifically, by analyzing intrinsic fluorescence of human epithelial tissue as it becomes deoxygenated *in vivo*, we can resolve the fluorescence spectra of NAD(P)H and collagen, two of the major tissue fluorophores. This is important because fluorescence depends on the local environment of the chromophore. Then, we extract the intrinsic fluorescence spectra of sites from 35 patients with suspected cervical lesions and 7 patients with Barrett's esophagus and describe them accurately as a linear combination of NAD(P)H and collagen contributions. In both tissue cases, we find that low collagen and high NAD(P)H fluorescence characterizes the high-grade dysplastic lesions when compared with nondysplastic tissues. These data present evidence for the presence of detectable levels of NAD(P)H fluorescence in human epithelial tissues in an *in vivo* setting and demonstrate that NAD(P)H and collagen may be used as quantitative fluorescence biomarkers for *in vivo* detection of dysplasia in the cervix and the esophagus.

INTRODUCTION

Carcinomas, which comprise almost 90% of all human cancers, arise in the epithelium, the superficial layer lining the surfaces of organs and tissues (1). During the development of premalignant (dysplastic) and malignant changes, epithelial cells undergo transformations that result in modified rates of metabolic activity, cellular proliferation, and/or death. Additionally, the communication pathways among epithelial cells and between epithelial and stromal cells can be impaired (2). Such changes are likely to alter both tissue morphology and biochemistry and could be detected by fluorescence spectroscopy. Indeed, fluorescence spectroscopy has been under investigation as a tool for the detection of precancerous and early cancerous lesions in a number of organs (3, 4). However, although there have been a number of promising reports on the presence of different fluorescence emission characteristics between normal and dysplastic or cancerous tissues, it has been difficult to identify and quantify reliably the origins of these changes.

One of the factors hindering the extraction of quantitative biochemical information from measured tissue fluorescence spectra is the presence of potentially significant distortions introduced by tissue scattering and absorption. Although a number of methods have been proposed for the recovery of the intrinsic (undistorted) tissue fluorescence (5–9), they are either not easily implemented in a clinical setting or they have limited applicability in the 400- to 500-nm region of the spectrum because of high hemoglobin absorption levels. Recently, we presented a method for extracting the line shape and intensity of intrinsic tissue fluorescence spectra even when absorption is significant (10, 11). To achieve that, we combine information in simultaneously measured tissue fluorescence and diffuse reflectance spectra.

In this article, we use this novel technique to extract intrinsic (undistorted) tissue fluorescence spectra during the induction of ischemic changes in esophageal tissue *in vivo*. As the tissue becomes deoxygenated, its redox state changes and we expect an increase in NAD(P)H fluorescence (12). Using chemometric tools, we can analyze the observed spectroscopic changes to isolate and quantify the spectral contributions of NAD(P)H and collagen, the two major human epithelial tissue fluorophores in the examined wavelength regime, in an *in vivo* environment. This is important, because the fluorescence spectrum of a chemical depends highly on its milieu (13). Furthermore, we demonstrate that the relative contributions of these two chromophores to the intrinsic tissue fluorescence spectra are modified during the development of precancerous lesions in tissues such as BE[3] and the uterine cervix. Thus, they can serve as quantitative *in vivo* biomarkers of premalignant change, without the need for tissue removal.

MATERIALS AND METHODS

All studies involving human subjects were approved by the Brigham & Women's Hospital Institutional Review Board and the Massachusetts Institute of Technology Committee On the Use of Humans as Experimental Subjects. Informed written consent was acquired from each patient before the procedure.

Chemicals. Collagen type I powder was purchased from Sigma Chemical Co. (St. Louis, MO).

Spectroscopic Measurements of Esophageal Varices. Esophageal varices are swollen blood vessels that can rupture, resulting in significant bleeding and possibly death. To prevent rupture, a nylon loop or rubber band is placed around a section of the varix and the surrounding epithelial tissue to stop the blood flow. Sets of fluorescence emission spectra at 11 different excitation wavelengths in the 337- to 610-nm range (*i.e.*, fluorescence EEMs) and a white light (350–750 nm) reflectance spectrum were acquired from esophageal tissue before, and at 2, 3, 5, and 15 min after ligation of esophageal varices. An optical fiber probe approximately 1 mm in diameter was passed through the

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[3] The abbreviations used are: BE, Barrett's esophagus; EEM, excitation-emission matrix; HSIL, high-grade squamous intraepithelial lesion.

biopsy channel of the endoscope and brought in contact with the tissue for light delivery and collection. Each data set was recorded in less than 1 s using a FastEEM instrument described previously (14). Spectra were obtained from two patients and three different esophageal varices. A dilute rhodamine solution was used to calibrate the observed fluorescence intensities. The diffuse reflectance spectrum of a 20% reflectance standard (Labsphere, Sutton, NH) was used as a reference to calibrate the spectral characteristics and the overall intensity of the white light xenon lamp used to measure the reflectance spectra. The wavelength response of the detector was calibrated using a National Institute of Standards and Technology Halogen-Tungsten calibration lamp.

Extraction of Intrinsic Tissue Fluorescence Spectra. To determine the tissue fluorescence spectra that are not distorted by scattering and absorption, we combined information in fluorescence and white light reflectance spectra measured at the same time and with the same light delivery and collection geometry. The processing of the spectral information was performed using a photon migration-based model (10, 11). The basic premise of this model is that the photons contributing to the measured diffuse reflectance spectrum have experienced similar scattering and absorption events while traveling in tissue as the fluorescence photons. Thus, we can use the scattering and absorption information included in the reflectance spectrum to remove these distortions from the corresponding fluorescence spectrum. Intrinsic fluorescence EEMs were extracted in this manner for spectra collected with the first six excitation wavelengths between 337 and 425 nm. Low signal-to-noise levels in the fluorescence acquired at longer excitation wavelengths limited our capability to describe these spectra reliably in a quantitative manner. To generate the intrinsic fluorescence EEM contour plots included in Figs. 2 and 3, cubic spline interpolation was performed using standard Matlab (The MathWorks, Inc, Natick, MA) functions to estimate the fluorescence emission spectra at smaller incremental excitation wavelength steps than the recorded data.

Extraction of *in Vivo* NAD(P)H and Collagen Fluorescence Spectra. To determine the relative contributions and corresponding spectra of the individual biochemicals that constitute the bulk intrinsic tissue fluorescence of the esophageal varices, the extracted intrinsic fluorescence EEMs were analyzed using the multivariate curve resolution algorithm incorporated in the Chemometrics Matlab toolbox (Eigenvector Research, Manson, WA). The algorithm was implemented with non-negativity constraints for the lineshape intensities and concentrations of the spectral components.

Fluorescence Measurements of Isolated Cervical Epithelium. Using the FastEEM, we acquired fluorescence and reflectance spectra of freshly excised human cervical epithelial tissue separated from the stromal layer after treatment with dispase II (2.4 units/ml; Roche Diagnostics Corp., Indianapolis, IN). Cells from cervical epithelium isolated in this manner have been shown to be viable and capable of forming cell cultures (15).

Spectroscopic Measurements of the Uterine Cervix *in Vivo*. Data were recorded with the FastEEM (14) from 35 patients undergoing colposcopy. The optical fiber probe was passed through an aluminum tube handle and brought into contact with the tissue. Spectra were acquired after the application of 3% acetic acid from colposcopically abnormal sites, which were biopsied immediately after spectral acquisition, and from colposcopically normal cervical squamous epithelium. Spectra were analyzed from 43 colposcopically normal ectocervical tissue sites (not biopsied) and 22 colposcopically abnormal tissue sites (biopsied). Twelve of the biopsied sites were classified by an experienced pathologist as squamous metaplasia, *i.e.,* they represented benign changes, and the remaining 10 were classified as HSILs, *i.e.,* they were precancerous lesions.

Spectroscopic Measurements of BE *in Vivo*. Data were recorded with the FastEEM (14) during endoscopy from nondysplastic and dysplastic BE sites. The procedure followed was the same as in the case of the esophageal varix measurements. However, all sites examined spectroscopically were biopsied immediately after data acquisition and evaluated by an experienced gastrointestinal pathologist. In the seven patients studied, spectra from 10 nondysplastic BE sites and 5 high-grade dysplastic lesions were obtained.

RESULTS

Spectroscopic Changes Observed during Asphyxiation of Esophageal Tissue. Changes in oxygenation of the esophageal tissue as a function of time from the onset of asphyxiation were readily detectable in white light reflectance measurements (Fig. 1*a*). Analysis of the reflectance spectra included in this figure, using the method described by Zonios *et al.* (16), revealed that the oxygen saturation value of tissue before and at 3 and 15 min after ligation decreased from 0.7 to 0.6 to 0.5, respectively. These changes are consistent with oxygen deprivation resulting from the blockage of blood flow through the tissue.

We also observed changes in the intrinsic (undistorted) tissue fluorescence after variceal ligation (Fig. 1, *b* and *c*). These spectra were extracted by combining information in measured fluorescence and reflectance spectra to remove distortions introduced by tissue scattering and absorption (10, 11). At 337-nm excitation, we noted a significant decrease in the overall intensity, with a concomitant shift in the line shape of the intrinsic fluorescence toward the red region of the spectrum as a function of time from the onset of asphyxiation of the esophageal tissue. Similar changes in intrinsic fluorescence intensity were also observed at excitation wavelengths between 358 and 425 nm. However, the lineshape shift observed at 337-nm excitation was not present.

NAD(P)H and Collagen Fluorescence Spectra *in Vivo*. Using multivariate curve resolution analysis, we established that the esophageal varix intrinsic fluorescence EEMs could be attributed to two component EEMs (Fig. 2) and that a linear combination of these could accurately account for the observed changes during asphyxiation. We assign the first component to stromal collagen (Fig. 2*a*). The shift in its fluorescence emission peak observed as a function of excitation wavelength is characteristic of collagen fluorescence (17). For comparison, a fluorescence EEM of commercially available collagen type I powder is shown (Fig. 2*c*).

The maximum fluorescence intensity of the second component occurs at approximately 460 nm, irrespective of excitation wavelength, whereas the overall intensity of the fluorescence signal de-

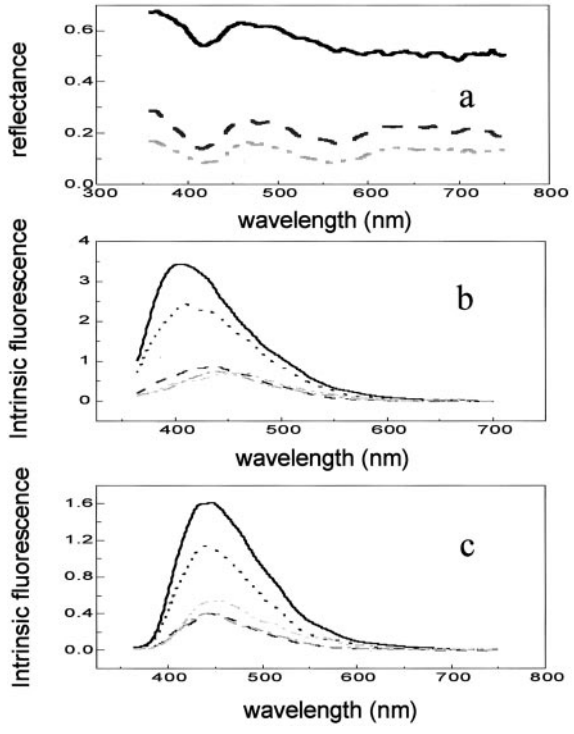

Fig. 1. Spectral changes observed during asphyxiation of esophageal varices *in vivo*. *a,* reflectance spectra measured before (———), at 3 min (— — —), and at 15 min (—·—) after ligation of a varix. *b* and *c,* intrinsic fluorescence spectra acquired before (———), at 2 min (····), 3 min (— — —), 5 min (—·—), and 15 min (—··—) after variceal ligation. *b,* 337-nm excitation; *c,* 358-nm excitation.

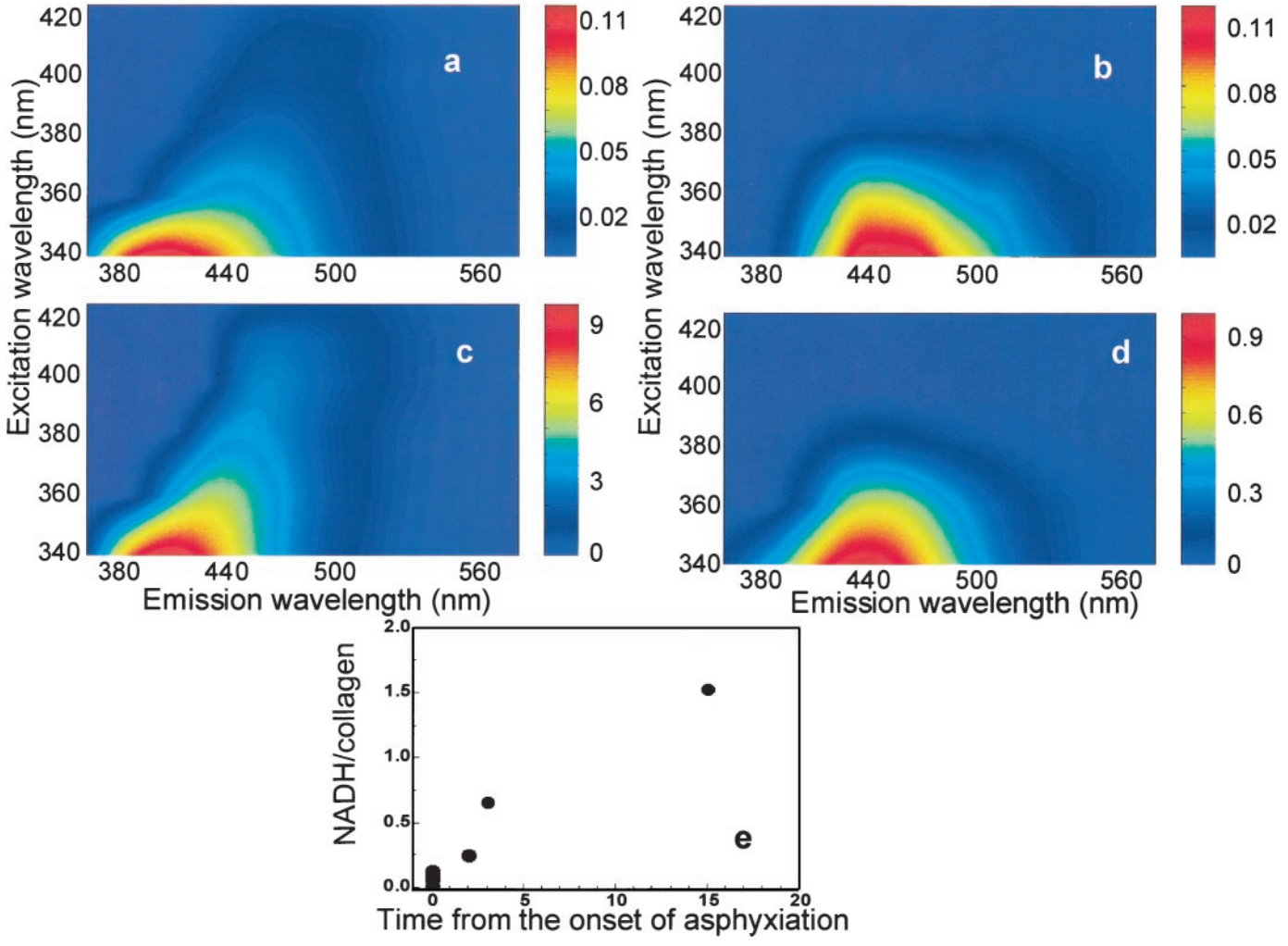

Fig. 2. Tissue collagen and NAD(P)H intrinsic fluorescence EEMs are extracted from variceal asphyxiation measurements. *a* and *b*, extracted intrinsic fluorescence EEMs of two components that describe the changes observed during tissue asphyxiation. Different *colors* represent different fluorescence intensities, as indicated by the adjacent *color bar scales*. *c*, intrinsic fluorescence EEM of collagen I in powder form. *d*, NAD(P)H fluorescence from isolated epithelium of freshly excised cervical tissue. *e*, extracted NAD(P)H/collagen ratios as a function of time from the onset of variceal asphyxiation.

creases to the noise detection levels of our system for excitation wavelengths beyond 381 nm (Fig. 2*b*). These spectral features are in good agreement with the fluorescence emission of NAD(P)H (12). The EEM of this second component is similar to the fluorescence EEMs of freshly excised human cervical epithelial cells (Fig. 2*d*). It is well established that the fluorescence of epithelial cells in this excitation-emission range is attributable to NAD(P)H (12).

An initial rapid increase followed by a plateau was observed for the relative contribution of NAD(P)H to collagen as a function of time from the onset of asphyxiation (Fig. 2*e*). This behavior is consistent with the expected changes in the redox state of the tissue resulting from oxygen deprivation (12).

Quantitative Biochemical Decomposition of *in Vivo* Normal, Benign, and Dysplastic Cervical Tissue Fluorescence. Spectra were acquired from colposcopically normal and abnormal sites of the uterine cervix. Intrinsic fluorescence EEMs corresponding to each one of the examined sites were extracted by combining the measured tissue fluorescence and reflectance spectra (10, 11). These intrinsic fluorescence EEMs were decomposed using a linear combination of the collagen and NAD(P)H EEMs recovered from the esophageal varix measurements. Thus, we could estimate the contributions of these two biochemicals to the bulk tissue fluorescence. The collagen and NAD(P)H EEMs describe the extracted intrinsic fluorescence EEMs of cervical tissue with excellent agreement, as indicated by the low residual levels of fluorescence, *i.e.*, the fluorescence spectral features remaining after a linear combination of the collagen and NAD(P)H EEMs has been subtracted from the data (Fig. 3).

Using this linear decomposition scheme, we determined the relative contributions of collagen and NAD(P)H to the tissue fluorescence of colposcopically normal and abnormal sites from 35 patients (Fig. 4*a*). Biopsied squamous metaplasias and HSILs exhibit reduced collagen fluorescence relative to normal squamous epithelium. Furthermore, there is a substantial increase in the relative NAD(P)H contribution for the HSILs, which are precancerous lesions, compared with the benign squamous metaplasia sites.

Quantitative Biochemical Decomposition of *in Vivo* Nondysplastic and Dysplastic BE Tissue Fluorescence. Fluorescence EEMs and reflectance spectra were acquired *in vivo* from seven patients with BE. Intrinsic fluorescence EEMs were extracted from columnar dysplastic and nondysplastic BE tissue sites and described accurately with a linear combination of the collagen and NAD(P)H fluorescence EEMs obtained from our varix ligation study. Thus, we determined the NAD(P)H and collagen fluorescence contributions (Fig. 4*b*). We find that collagen fluorescence decreases significantly and NAD(P)H fluorescence increases significantly when tissue becomes dysplastic.

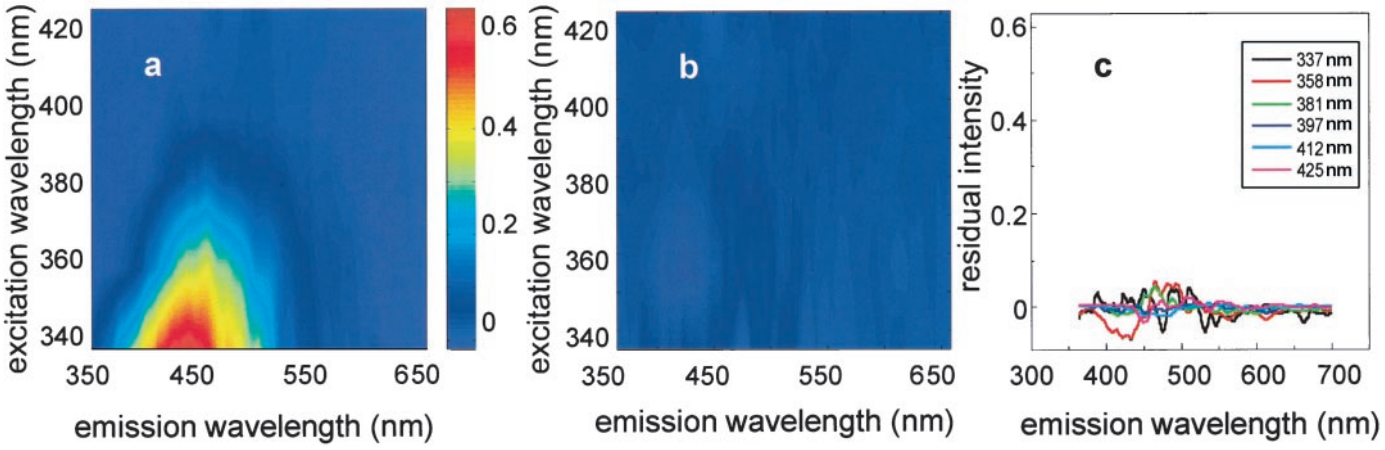

Fig. 3. Collagen and NAD(P)H are the main tissue fluorophores in the visible spectral range. The intrinsic fluorescence EEM of a cervical HSIL, shown in *a*, can be described very well by a linear combination of collagen and NAD(P)H EEMs, as indicated by the very small residual, shown in *b*. Different *colors* represent different fluorescence intensities as indicated by the adjacent *color bar scale*. The same intensity scale was used to create the images shown in *a* and *b*. The individual residual fluorescence emission spectra at six excitation wavelengths between 337 and 425 nm are shown in *c*.

DISCUSSION

To characterize tissue biochemically in a quantitative manner, we used a novel clinical technique that extracts biological tissue intrinsic fluorescence spectra over a wide range of excitation and emission wavelengths by combining information incorporated in fluorescence and white light reflectance spectra acquired simultaneously from the same light delivery/collection probe (10, 11). In contrast to directly measured tissue fluorescence spectra, in which fluorescence, scattering, and absorption are all entangled, intrinsic tissue fluorescence spectra consist of linear contributions of the fluorescence spectra of individual tissue biochemicals. To understand the origins of the intrinsic tissue fluorescence and to characterize quantitatively the fluorescing biochemicals, it is important to identify the fluorescence line shapes and relative intensities of the major contributors. Because fluorescence depends on the local environment of the chromophore (13), it is necessary to acquire a basis set of tissue fluorophore spectra in an environment consistent with that of the bulk tissue *in vivo*. To achieve this, we performed *in vivo* tissue fluorescence and reflectance measurements before and after ligation of esophageal varices. Elimination of blood flow through the tissue enclosed by a ligating band results in tissue deoxygenation, which in turn should lead to an increase in the NAD(P)H content (12). Thus, ligated varices provide a unique system for observing specific contributions of NAD(P)H to bulk tissue fluorescence *in vivo* in human epithelium.

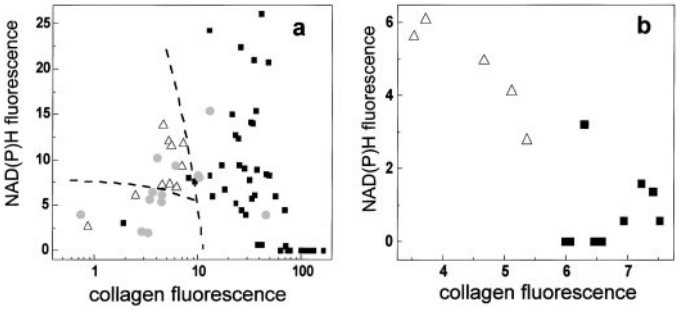

Fig. 4. Quantitative biochemical tissue characterization based on analysis of intrinsic fluorescence spectra. *a*, NAD(P)H and collagen contributions to the overall intrinsic fluorescence for 43 colposcopically normal ectocervical tissue sites (■), 12 biopsied squamous metaplasia sites (*gray circles*), and 10 HSILs (△) from 35 patients. — — —, drawn based on logistic regression analysis of the data to illustrate the spectroscopic classification of the three different tissue types. *b*, NAD(P)H and collagen contributions to the overall intrinsic fluorescence of 10 nondysplastic (■) and 5 high-grade dysplastic (△) BE tissue sites from 7 patients.

The observed changes in tissue intrinsic fluorescence and reflectance spectra as a result of asphyxiation were consistent with oxygen deprivation and changes in the tissue redox state (Fig. 1). Using multivariate curve resolution analysis of the esophageal varix intrinsic fluorescence EEMs, we extracted the fluorescence EEMs of collagen and NAD(P)H in an *in vivo* environment (Fig. 2, *a* and *b*). Agreement between the two *in vivo* EEMs with EEMs of commercially available collagen (Fig. 2*c*) and of epithelial cells isolated from human cervical excised tissue (Fig. 2*d*) is good but not perfect, indicative of the roles played by contributions from multiple collagen types and environmental spectral shifts in actual tissue.

To determine whether NAD(P)H and collagen contributions to the bulk intrinsic tissue fluorescence can be used as quantitative biomarkers for detecting biochemical and/or architectural changes that occur during the development of dysplastic lesions, we analyzed spectra acquired *in vivo* from sites of the uterine cervix and BE. Such biomarkers could aid in diagnosing dysplasia in these organs and lead to improved detection.

In the case of the uterine cervix, spectroscopic measurements were performed during colposcopy, *i.e.*, visualization of the cervix under ×15 or ×30 magnification. A solution of 3% acetic acid is typically applied to the surface of the cervix during this procedure to enhance contrast between normal and abnormal tissues. In particular, abnormal tissues appear white and are biopsied to confirm the presence of precancerous or cancerous change histopathologically. Although this method for detecting cervical lesions has very high sensitivity (94%), it yields a very high rate of false positives (51% specificity; Ref. 18). This means that a significant number of nondysplastic tissue biopsies are taken, increasing the risks and costs associated with colposcopy. Thus, it would be useful to have a tool that could assist the physician in performing biopsies more selectively on diseased tissue sites.

Our analysis of the intrinsic cervical tissue fluorescence indicates that it can be described with excellent agreement using a linear combination of the collagen and NAD(P)H EEMs extracted from the esophageal varix measurements (Fig. 3). A decrease in collagen fluorescence is observed for the biopsied squamous metaplasias (benign changes) and HSILs relative to the colposcopically normal squamous epithelium. In addition, there is a substantial increase in the NAD(P)H contribution for the HSILs, which are precancerous lesions,

compared with the benign squamous metaplasia sites. This change could be the result of an increased number of cells and/or increased levels of metabolic activity in the epithelial cells (12). It should be mentioned that NAD(P)H fluorescence is successful in separating most of the benign squamous metaplasias, which were biopsied because they were colposcopically abnormal, from the HSILs, demonstrating the potential of this technique to guide clinicians to lesions in a more specific manner, and hence to improve the yield of biopsy.

As noted, the biopsied squamous metaplasia and HSIL sites exhibit a significant decrease in the relative contribution of collagen fluorescence as compared with the normal ectocervix sites. This consistent decrease in *in vivo* collagen fluorescence of cervical tissue within a data set that includes multiple patients has not been reported previously. However, a trend toward decreased collagen fluorescence levels has been presented by Ramanujam *et al.* (19) when normal and abnormal cervical tissue sites from the same patient were compared.

Colposcopically abnormal sites typically are found in the transformation zone of the cervix, which consists of tissue that undergoes constant change as the squamous epithelium gradually replaces the columnar epithelium. Collagenases have been known to play an important role in processes that take place during significant tissue architectural changes, as in the case of wound healing and tissue regeneration (20). Therefore, this decrease in collagen fluorescence observed within sites of the dynamically changing transformation zone of the cervix may be attributable to the presence of collagenases, such as matrix metalloproteinases, degrading the fluorescing collagen crosslinks. This hypothesis is consistent with very recent fluorescence microscopy measurements performed *ex vivo* with fresh normal and dysplastic cervical tissue slices (21).

Fluorescence EEMs and reflectance spectra were also acquired *in vivo* from patients with BE, a condition defined by the replacement of normal squamous esophageal epithelium by metaplastic columnar epithelium (22). Because these patients are at higher risk for developing adenocarcinoma of the esophagus, they undergo yearly endoscopic surveillance procedures. Because dysplastic changes are not usually visible endoscopically, such procedures generally necessitate acquiring a significant number of random biopsies (23).

We find that the intrinsic fluorescence EEMs of columnar nondysplastic and dysplastic BE sites are described accurately by a linear combination of the collagen and NAD(P)H intrinsic fluorescence EEMs obtained from our varix ligation study. The fact that the varix EEMs, obtained from squamous esophageal tissue, provide good fits to columnar tissue fluorescence suggests that the architectural differences between these two types of epithelial tissue do not introduce significant differences in the line shapes and intensities of the extracted intrinsic fluorescence EEMs. Linear decomposition of the intrinsic fluorescence BE spectra suggests that dysplastic lesions are characterized by decreased collagen and increased NAD(P)H fluorescence (Fig. 4b). Such changes could be attributed to processes similar to the ones mentioned for the cervical lesions.

Previous studies performed *in vitro* (24), *ex vivo* (21), and *in vivo* with mitochondrion-rich (7, 12) or optically transparent (25) animal tissues have reported detectable NAD(P)H signals. Qualitative differences in the collagen fluorescence between normal and diseased tissue have been reported for a number of tissues. Fluorescence spectroscopy studies of normal skin and nonmelanoma skin cancers performed *in vivo* at 290 and 350 nm excitation reported lower levels of collagen fluorescence in the tumor region than in the surrounding normal skin of each patient (26). Previous analyses of human fluorescence spectra acquired *in vivo* from colon (27) and cervical tissues (19) at 337 nm excitation have also provided insights into the origins of the observed changes in tissue fluorescence. The current work advances the previous clinical studies in two significant ways. First, the combined information from simultaneously measured tissue fluorescence and reflectance spectra enables us to remove significant distortions introduced in measured tissue fluorescence by scattering and absorption and, thus, to extract the intrinsic fluorescence and quantitate the contributions from individual chromophores. In the colon study (27), absorption and scattering effects were not accounted for before biochemical decomposition, whereas the biochemical model used in the cervix study (19) used a light propagation model that does not incorporate rigorously the effects of tissue scattering. As a result, spectra had to be normalized either to their peak intensity (27) or to the spectra of normal tissue from the same subject (19). It has been shown that removal of scattering and absorption artifacts is essential for extracting quantitative information about NAD(P)H fluorescence and tissue metabolic activity (12). Next, rather than using fluorescence spectra from commercially available NAD(P)H and collagen dissolved in saline (19, 27), the present study obtained the relevant component spectra from *in vivo* measurements, performed in an environment that is as similar as possible to that of the tissue from which biochemical information is extracted. This is important because the line shape and intensity of a fluorescence spectrum are highly dependent on its milieu (13). Thus, acquisition of reliable quantitative biochemical information necessitates the use of spectra acquired in the appropriate local environment. Because of these two differences, we can observe consistent changes in the absolute levels of NAD(P)H and collagen fluorescence of multiple patients and can accurately correlate these biochemical markers with the degree of neoplastic change.

In summary, this study reports a method for extracting the fluorescence spectral features of collagen and NAD(P)H *in vivo* over a wide range of excitation and emission wavelengths. We present substantial evidence for the presence of detectable levels of NAD(P)H fluorescence *in vivo* during the induction of redox state changes in nontransparent human epithelial tissues that do not exhibit high metabolic activity. We demonstrate that NAD(P)H and collagen fluorescence show promise as biomarkers for the detection of dysplastic changes in both the squamous epithelium of the uterine cervix and the columnar epithelium of BE. Similar changes may also be present in other organs, such as the colon, bladder, breast, lung, and oral cavity. Our ability to quantify tissue biochemical changes *in vivo* relies heavily on the combination of fluorescence and reflectance spectroscopic information and the extraction of the intrinsic tissue fluorescence, which is free from artifacts introduced by tissue scattering and absorption. Inasmuch as this technique can be applied clinically in a noninvasive manner, it can serve as a guide to biopsy of invisible or ambiguous lesions in the uterine cervix and BE. In addition to its diagnostic value, extraction of quantitative biochemical information from *in vivo* tissue intrinsic fluorescence spectra provides a unique opportunity to study tissue changes without the introduction of excision and processing artifacts. We believe that continued intrinsic fluorescence studies will enhance our understanding of the biochemical relationships between the epithelium and the stroma during cancer development. Furthermore, we anticipate that detection of biochemical changes within tissue in its native state will prove useful as a novel and effective noninvasive means of assessing the effects of chemopreventive/immunotherapeutic treatments for various epithelial tissues (28).

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