APPLICATIONS OF PHOTONICS AND CERAMICS TO HEALTHCARE: THE FUTURE HAS BEGUN

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ABSTRACT

Dramatic improvements in our understanding of how biological systems work, diseases initiate, and illnesses progress have given rise to the expectation that many of these ailments can be cured, perhaps even in their precursor states, as new tools are developed that can take advantage of, and extend, our improved knowledge. This paper provides an overview of techniques currently in use or under development that combine optical techniques, properties of materials in various guises, e.g., fibers, light sources/detectors, nanoparticles, and improved understanding of cellular and tissue behavior that could potentially result in major advances in medical treatments. A variety of examples is presented, covering applications that range from basic science through possible clinical diagnostics and medical treatments. Potential benefits, characterization needs, and barriers to application are pointed out.

INTRODUCTION

Photonic applications in health care are increasing across the board, from basic research through diagnostics and treatment. These increases result from recent advances in light generation and detection, improved understanding of biological processes on the cellular level, and expanded use of non-traditional medical treatments in place of surgery or systemic medications. Advances in materials science, e.g., improved processing and characterization techniques, thin film technologies, nanotechnology, have been combined with, and have sometimes led to, many of the advanced photonic capabilities in medical understanding and treatment. This article addresses new and near-term uses of materials in photonic-based health care applications. The applications discussed are limited to those in which semiconductor and more traditional oxide ceramics are applicable, although in some of these applications, metals or polymers are also being used.

The driving forces for the increased use of optical approaches from the near-UV through the IR for medical applications include the following assumptions: optical techniques are rapid, non-contact, non-destructive, and non-invasive with approximately micrometer resolution. In addition, optical techniques can use multiple wavelengths simultaneously or sequentially, thereby potentially providing increased depth information and resolution as well as tunable absorption. Finally, optical techniques potentially provide both imaging and chemical information.

While each of the listed advantages is real in simple systems, in complex biological systems most of them are only approximately true and, even then, only over restricted ranges. Because biological systems are highly inhomogeneous, non-uniform transmission and absorption as well as a high level of light scattering limit penetration depth, reduce contrast, and degrade resolution, thereby reducing the signal to noise (S/N) ratio for optical measurements. Most of these difficulties can be overcome to some extent, but at the cost of reducing or eliminating benefits typically attributed to optical techniques. For example, one of the most common

methods of improving signal to noise (S/N) in spectroscopic techniques is to increase the signal integration time. This approach reduces the advantage of rapidity usually associated with optical measurements. In addition, because living tissue is very complex and usually not stationary on the micrometer and millimeter level, for many applications increased integration time will not overcome the S/N problem. Another simple approach to improving penetration depth and low S/N is to increase optical power. However, in biological material the upper limits to optical power are rather low before damage is generated. For some applications, tissue damage can be reduced or eliminated through use of short pulse width, high flux light sources, such as femtosecond lasers, perhaps including multiphoton spectroscopy (discussed below). With such tools, the average power is below the damage threshold but the individual pulses provide a high density of photons for imaging applications. Another approach to overcome S/N barriers is to move the light source or detector closer to the tissue being interrogated. However, for living creatures, this requires optical fibers and/or miniaturized sources and detectors to be inserted *in vivo*, compromising the anticipated advantages of non-contact and non-invasive procedures.

One of the greatest potential advantages of using optical techniques in healthcare applications is the possibility of obtaining image and chemical information on an identical volume of material either *in vitro* or *in vivo* on the cellular level. This advantage has not generally been realized for two interrelated reasons. First, the predominant constituent of most biological tissues, excluding bone and tooth enamel, is water. Consequently, most traditional optical imaging procedures provide very little contrast. *In vitro*, this difficulty has been overcome for years by fixing cells and staining them with organic dyes to highlight cellular regions of interest. Unfortunately, this process kills the cells, making it impossible to monitor cellular activity except as a series of discrete, isolated images. Moreover, such a process clearly cannot be applied to *in vivo* studies. Second, lack of understanding of cellular chemistry has made meaningful monitoring of chemical changes on the cellular level difficult until recently. However, in the past 15 years, tremendous strides have been made in decoding DNA, monitoring protein expression, and observing cellular interactions in real time. Part of this progress has involved development of fluorescent molecules and particles and techniques to bind them to biological components of interest, e.g., proteins. With these tools, it is becoming possible to monitor a specific protein expression and the resultant motion of the protein while reducing harm to the cells. Chemical interactions are still inferred because the fluorescent tags describe rate of production and subsequent motion of cellular elements but do not provide chemical bonding information.

The topic of photonic applications in health care can be divided into two categories: 1) applications of remotely generated light used alone to interrogate biological systems of interest and 2) applications of light combined with a non-biological material component to interrogate the biological system. This division is convenient because the first category allows a description of typical optical probes without the complications and limitations that arise in the presence of additional material components. For this purpose, optical interrogation of teeth for caries provides convenient examples that will be discussed below. There are numerous applications of the second category: fiber optics, photonic fiber optics, nanoparticles, miniature solid state optical sources and detectors, miniaturized spectroscopic systems. A detailed discussion of any of these applications is beyond the scope of this article, which is intended to provide a concise overview emphasizing current and potential future photonic/semiconductor/ceramic applications in health care. However, a summary of the status of each application is provided. Particular

emphasis is given to the nanoparticle application, because of the enormous capabilities that nanoparticles bring to the field of health care.

Specifically, this article discusses benefits of optical techniques in health care, barriers to implementation of those techniques, and how use of novel material properties or new devices can overcome those barriers. Few of these applications are in routine laboratory or clinical use; most of them are in the research and development stages. It should also be mentioned that while the applications described herein are promising and exciting, many materials issues, e.g., reliability, biocompatibility, manufacturability, characterization, remain to be addressed.

PHOTONIC APPLICATIONS TO DENTAL CARIES

Teeth are composite structures consisting of an enamel (hydroxyapatite) layer covering organic layers (dentine, pulp), surrounded by gum, and embedded in bone (Figure 1). The enamel has a columnar structure that interacts differently with vertically polarized light and horizontally polarized light. Caries (regions of demineralization leading to and including cavities) occur from a chemical attack on the enamel, with subsequent damage to the dentine and the pulp.[1] The attack on the enamel results in local demineralization that destroys the columnar structure of the hydroxyapatite, reducing the sensitivity to the incident light polarization.[2]

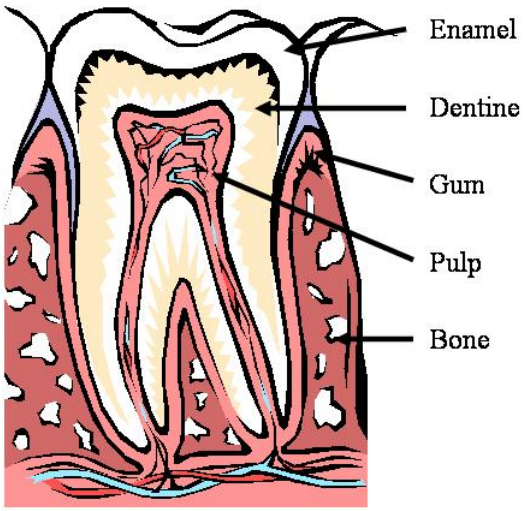

Figure 1: Schematic of tooth structure. Enamel is columnar ceramic structure. (after Microsoft Powerpoint 2003 Figures)

There are a number of light-based tools under investigation for the purpose of detecting and measuring dental caries. Many of the optical techniques discussed are under development not only to detect the presence but to evaluate the activity of the caries, as well. Because of the difficulties associated with directing and collecting light in the oral cavity and the length of time required for individual measurements, most of these techniques remain limited to measuring artificial dental material or teeth that have been removed from the oral cavity. They include light scattering,[1] multiphoton imaging,[1,3] fluorescence, optical coherence tomography (OCT),[4,5] and Raman spectroscopy.[2]

Scattering

Light scattering is the oldest optical procedure used to detect caries. Because the demineralization associated with caries degrades the columnar structure of the enamel, the caries scatter light in both transmission and reflectance geometries. Dentists routinely take advantage of this fact by observing teeth in visible light, looking for whitish regions corresponding to light scattering from demineralized regions. However, light scattering can also be caused by cosmetic features on the tooth surface. Therefore, techniques are needed that are insensitive to surface cosmetic effects for detecting and measuring caries.

Fluorescence

Fluorescence measurements provide one such technique. Fluorescence occurs when an absorbed photon excites an electron which then returns to the ground state via a (fluorescent) photon emission. Typically, the return to ground state includes nonradiative processes which result in the fluorescent photon having a longer wavelength than the excitation photon. When exposed to blue light (514 nm > λ > 488 nm), sound tooth structures fluoresce (λ > 520 nm) whereas caries do not, thereby producing a dark region on the image of the tooth.[1] Unfortunately, because of its short wavelength, blue light is highly scattered in the tooth, reducing penetration depth. High intensity sources would overcome this limitation, but blue light causes phototoxicity that could damage the pulp[1,3] (Figure 1). Longer wavelength light results in much less damage but, in conventional photoluminescence, does not generate the fluorescence.

Multiphoton imaging overcomes this problem. In multiphoton fluorescence,

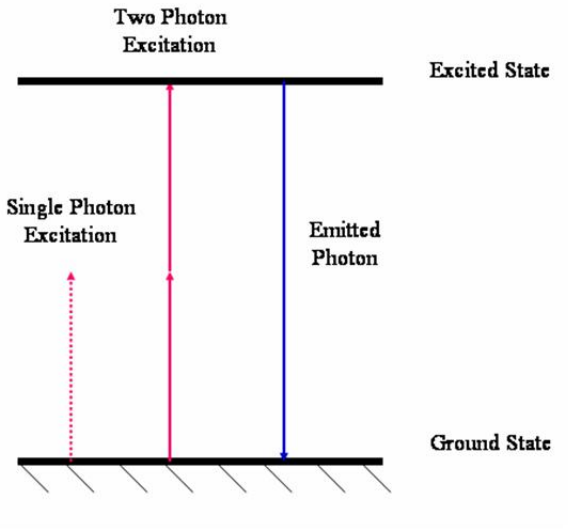

Figure 2: Two photon luminescence energy diagram. A single photon does not have the energy to excite the electron but two simultaneous photons at longer wavelength do.

shown schematically in Figure 2, two long wavelength (low energy) photons combine to move an electron from the ground state to an excited state with twice the single photon energy. This process has the advantage of reducing photon-generated damage in the material, due to the lower energy photons used to accomplish the excitation. However, the probability of a two photon process occurring is 10^{-6} that of a single photon process, so high photon flux is required for multiphoton fluorescence.[6] If a continuous wave light source is used for excitation, the

advantages of reducing the photon energy are negated by the large photon flux and concomitant specimen heating. However, with the advent of high intensity, short pulse-width sources, such as femtosecond lasers, instantaneous flux values can be high, generating multiphoton fluorescence while average flux remains low, minimizing tissue damage. With this approach, two photon fluorescence has been shown to detect caries readily *in vitro* and, by varying the focal depth of the excitation beam during scanning, the technique has provided three dimensional information regarding the subsurface structure of the caries. However, the technique has not been used *in vivo* both because of the difficulty directing the light in the oral cavity and because each three-dimensional image requires approximately one minute to be recorded.[1]

Optical Coherence Tomography (OCT)

Optical coherence tomography is an optical, interference-based imaging tool that provides high lateral and depth resolution.[7] The technique uses an interferometer with a moving reference mirror to obtain depth information; the depth resolution is inversely proportional to the band width. Consequently, limited coherence light sources, such as femtosecond lasers or superluminescent optical diodes, are typically used to increase bandwidth. The resultant high resolution is the principal benefit of OCT. Developed initially for inspection of the eye, OCT procedures are being extended to other biological systems. For imaging dental tissue, penetration depths up to 2 mm with depth resolution from 10 - 17 μm have been reported.[1,4]

Because OCT is an interference based system, changes in the intensity of the signal from the specimen cause changes in the contrast of the interference fringes. Since caries scatter light more than healthy teeth, they are detected by the reduced contrast. However, this means that cosmetic surface features could be mistaken for caries. This problem may be reduced by recent advances in OCT that monitor changes in the polarization of the light from the specimen.[4] At this time, no *in vivo* results have been reported for caries detections.[1]

Raman spectroscopy

The Raman effect is the inelastic scattering of incident light due to the energy transfer to (Stokes) or absorption from (anti-Stokes) vibrational modes in a specimen. Because the vibrational modes depend upon mechanical structure, including symmetry, chemical bonds, and stress state, and because the polarization of the scattered light is related to the symmetry of the vibrational modes of the tooth enamel and the polarization of the incident light, Raman scattering provides a potential tool to detect structural degradation and depolarization of dental enamel due to caries. Ko *et al.* have shown that both the depolarization ratio, ρ, and the polarization anisotropy, A, are highly sensitive to the presence of caries.[2] ρ and A are defined by the expressions

$$\rho = \frac{I_{\perp}}{I_{\parallel}} \qquad \text{and} \qquad A = \frac{\left(I_{\parallel} - I_{\perp}\right)}{\left(I_{\parallel} + 2I_{\perp}\right)},$$

in which $I_{\perp}$ is the Raman intensity observed when the incident polarization is perpendicular to the scattered polarization and $I_{\parallel}$ is the intensity observed when the polarizations are parallel. The results are relatively insensitive to the angle of the incident radiation relative to the tooth and, because ρ and A are ratios of intensities, the approach may be insensitive to cosmetic discoloration on the teeth, as long as the discoloration does not affect polarization. However,

both ρ and A appear to be highly sensitive to the presence of caries in teeth *ex vivo*. As was the case for OCT, no *in vivo* studies have been reported.

PHOTONIC/MATERIAL COMBINATIONS

Use of ceramics in photonic applications for health care in the near future fall into three categories: light transmitters, miniature light sources, and miniature light detectors. For *ex vivo* applications, the last two categories can be combined into miniature spectroscopic tools. Typically, for biological applications, light transmitters are fiber optics. Miniature light sources that are being developed are solid state lamps or lamp arrays. Most of the miniature detectors being developed for sub-cellular or in vivo applications are nanoparticles. The attribution of "detector" is a matter of definition, however, since the primary of the function of the nanoparticles is to absorb (i.e., detect) the incident photons and then to fluoresce.

Fiber optics

In health care, light is typically used in one of two historically distinct manners: imaging and therapy. As mentioned above, imaging in the visible region is difficult because tissue is highly scattering with poor contrast. The scattering limits the penetration depths for practical uses of visible light. Figure 3 shows the penetration depth in water and hemoglobin at which the light intensity, I, falls to half of its initial value, I_0, in the wavelength range between 400 nm (deep blue) and 950 nm (near infrared). The penetration depth is on the order of millimeters for incident wavelengths < 600 nm. However, penetration windows appear at about 700 nm (red) and 800 nm (near infrared) where the penetration jumps to the order of 10 cm. Consequently, excitation in the far red and near infrared (near IR) will reduce the resultant scattering. However, if the light can be moved to the region of interest without having to be transmitted through intervening tissue, scattering and absorption issues could be minimized.

Fiber optics are routinely used for this purpose. Although engineering advances continue to be made,[8] attributes and limitations of endoscopes based upon traditional optical fibers are well understood. However, advances being made in optical components have resulted in the discovery of new type of optical fibers, photonic fibers, composed of geometrically modified silica fibers or micro-fiber bundles that incorporate air gaps within the columnar structure of the macro-fiber. In photonic fibers, the light propagates along the voids in the fiber column through total internal reflection, due to the large optical index mismatch between air and SiO_2. The fibers accommodate multiple

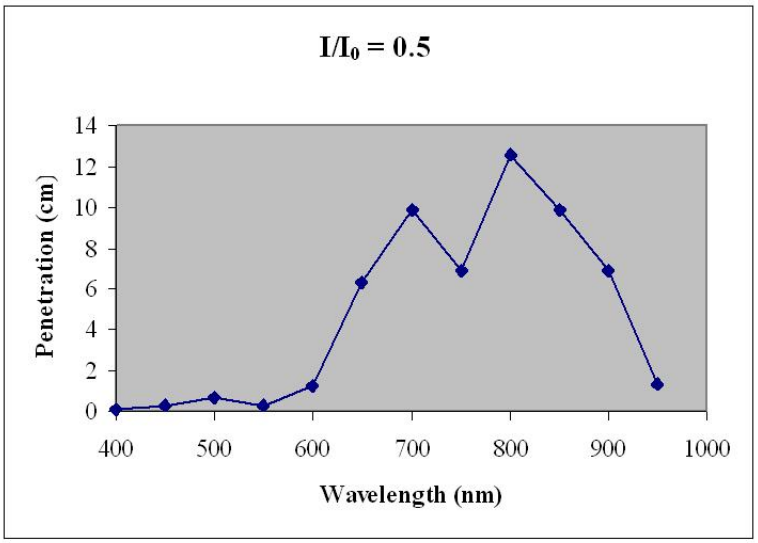

Figure 3: Penetration depth through water and hemoglobin across the visible range

wavelengths with minimal loss, and for applications like OCT the additional bandwidth results in greater depth resolution.[9] Values represent the depth at which the intensity is 50% of the original value.

Photonic fibers provide a wide range of valuable and novel optical properties but long term reliability issues are not yet known.

Miniature light sources

An alternative to optical fibers in moving the light close to the biological region of interest is to miniaturize the light sources themselves and transport them on a catheter. For such applications, solid state lamps, which require less power and produce less heat than incandescent lamps and are more rugged than fluorescent lamps, are the preferred solution. While this is an attractive possibility, there are a number of biological and materials hurdles that must be overcome. From the biological perspective, biocompatibility is the primary issue. Most solid state lamps under development are composed of toxic materials. In an aggressive biological environment, the lamps would need to be hermetically sealed. A second issue is heat generation. While light emitting diodes generate much less heat than incandescent lamps, their efficiency remains approximately 50%, meaning that 50% of the input power is converted to heat. Because the heat is generated in a very small volume, local heating can be severe, e.g., several 10's of degrees ^{o}Celsius. In addition, such light sources must overcome all the barriers associated with solid state lamp fabrication in general.[10]

For *ex vivo* applications, such as biochip assay arrays using vertical cavity laser arrays, development barriers involve traditional materials science processing and engineering issues: cost reduction, wavelength control, light path management, andbackground reduction.[11]

Detectors/absorbers

Recent advances in the understanding of cellular chemistry and sub-cellular control, e.g., protein expression, have resulted in the development of optical tracer and fluorophore tags that provide optical contrast via controlled fluorescence. Materials such as fluorescent particles,[12] fluorescent proteins[13] and quantum dots[14,15] have been and are being developed that will attach

to specific proteins or other cellular features and provide a signal for optical microscopy. On the cellular level *in vitro*, these tracers currently make it possible to monitor time-dependent processes in living cells. Indeed, tags can be targeted toward specific proteins, organelles, or, within a test animal, specific organs, inflamed joints,[16] or tumors, opening tremendous opportunities ranging from basic research into cell behavior and chemistry through applications in diagnostic and treatment monitoring. In addition, the increasing possibility for development of new markers that identify the onset of diseases afflicting specific subset populations holds the promise of "personalized medicine".

While fluorescent tags initially were organic, in the past few years there has been a trend toward metallic or oxide nanoparticles and, recently, semiconductor quantum dots (QDs). The trend reflects both limitations of organic fluorophores and strengths of nanoparticles and QDs.[17] Luminescence intensity of organic fluorophores diminishes with multiple excitations and can be quenched by high fluorophore density. More importantly, development of various organic fluorophores with different luminescence wavelengths for a common excitation is a difficult and time-consuming process. In contrast, luminescence from nanoparticles is relatively stable, and the wavelength of the emitted light is determined by the size of the particle. Consequently, nanoparticles of varying size can provide multiple wavelength output for the same excitation wavelength, allowing optical multiplexing. Finally, output brightness from nanoparticles, in addition to being more stable than that from organic fluorophores, is typically much higher.

The existence of fluorescing particles that can be targeted toward specific sites gives rise to a variety of imaging techniques for *in vitro* and, eventually, *in vivo* applications. The fluorescence tools described above, both single photon and multi-photon, can be used in real time to image sub-cellular activities, such as gene expression, or to map tumors or inflamed joints within a body. The nanoparticles also make more exotic optical techniques possible, such as fluorescent resonance energy transfer (FRET) and total internal reflectance (TIR).

FRET, shown schematically in Figure 4, uses two fluorescent markers and a laser source to image interactions between cellular components (e.g., two different proteins) on the nanometer scale. One cellular component is tagged with one fluorophore and a second is tagged with the second fluorophore. The wavelength of the laser source and the optical properties of the two fluorophores are chosen such that the laser wavelength, λ_l, will excite fluorescence λ_{f1} in the first fluorophore but will not excite the second fluorophore. However, when the two fluorophores are close enough together, a resonant energy transfer will occur between the excited first fluorophore and the non-excited second fluorophore, resulting in fluorescence from the second fluorophore at λ_{f2} and a decrease in the intensity of λ_{f1}. Monitoring the intensity of λ_{f1} and λ_{f2} during an experiment provides information regarding the conditions affecting interactions between the two cell components.[18] The primary advantage of FRET is its extreme sensitivity to the spacing between fluorophores. Because the energy transfer is a non-radiative resonance phenomenon, it is very short range and the efficiency of the transfer is strongly dependent upon the separation between the cell components. Measurements of the intensity of λ_{f1} or λ_{f2} can give very precise information regarding that separation;

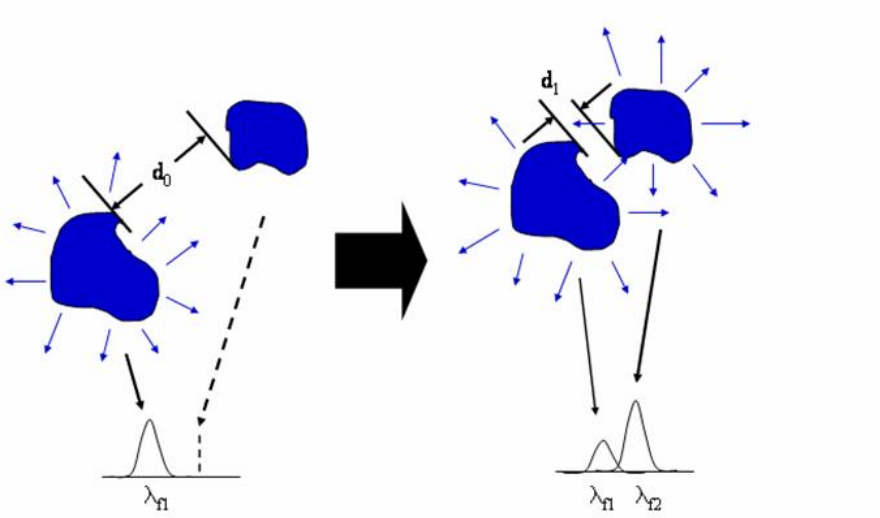

Figure 4: Schematic of FRET. As twoparticles approach, the laser-induced fluoresence in particle 1 induces a secondary fluorescence in particle 2 through a non-radiative resonance process.

monitoring the polarization of the emissions can give information regarding relative orientations.

TIR is being developed to provide images and information in very restricted regions of a cell. The technique involves placing the region of interest, e.g., a cell membrane, very near a planer waveguide. As light is transmitted through the waveguide, interactions between elements near the membrane can interact with the evanescent waves leaking from the waveguide; because the interactions are with evanescent waves, they are strong functions of their distance from the waveguide.[19] Typically, luminescent tags are attached to the cell components of interest and interactions are inferred from the intensity and distributions of the luminescence sites. Because the excitation of the fluorophores is via evanescent waves leaking from the wave guide, TIR provides information very analogous to that obtained by FRET.

It should be emphasized that in all of the imaging applications discussed thus far, the fluorescence is used to provide contrast only. No spectroscopic (i.e., chemical interaction) information is obtained, except through inference, because the fluorescence is generated by nanoparticles that are assumed to play no part in the interactions. The question of whether the biological functions are modified by perturbations arising from the attached materials remains largely unanswered and an object of concern.[20,21]

Photonic/nanoparticle tools for medical treatment lag behind those being developed for imaging. One treatment with potentially broad applications is photodynamic therapy (PDT). In localized PDT, light sensitive agents, e.g., QDs or nanoparticles, collect at a region of interest such as a tumor. A laser or high intensity diode lamp illuminates the region at a wavelength strongly absorbed by the nanoparticles, activating a toxin or generating local heating that kills the surrounding tissue. Although the technique has clear benefits, issues remain to be addressed. First, monitoring the concentration of the optically active agents - the nanoparticles - relative to the concentration[22] required for treatment both in absolute terms and as a function of time is difficult. Second, the localized target region must lie within the light penetration depth (see

Figure 1) for excitation intensities low enough not to damage healthy tissue. Third, the biological components that are attached to the nanoparticles to target the tumor must be very reliable and very specific, to prevent damage from occurring at healthy tissue.

While the potential benefits of nanoparticles for both imaging and treatment applications are great, there are a number of barriers that must be addressed before they can be used *in vivo*. From a safety standpoint, the primary concern is for biocompatibility.[23] What is the toxicity? What are the degradation mechanisms? What occurs at the interface between the body and the implant? What time frames are important? What are the failure probabilities and implications? Are there fouling mechanisms that need to be considered? The time frame for nanoparticles in the body is intended to be on the order of hours or, at most, days. Yet, some of the nanoparticles may become trapped and remain indefinitely. Therefore, long term toxicity issues are important. Some of the parameters that need to be quantified for toxicity assessment are:[24] composition, size, shape, deformability, stability, and coatings. In addition, the material composing most QDs is toxic. The QDs can be coated, but the lifetime and reliability of the coating in the chemically active environment of the body, as well as during optical heating, must then be ascertained. Nanoscale spheres made of Au, alumina, or polymers are more inert, although the long-term biological response to these materials is not known.

Besides safety issues, there are material issues that must be addressed. Nanoparticle dispersal in the body as well as optical absorption or fluorescence depends strongly upon particle size. In addition, particle dispersal depends upon surface charge, agglomeration, and reactivity. Therefore, material issues such as purity, size, size distribution, agglomeration, and reactivity must be well known, well understood, and well controlled. Finally, the mechanisms by which the properties interact with biological systems must be determined.

SUMMARY

There is a great deal of effort underway in the biology community to use optical techniques as probes, ranging from investigations of the fundamental properties of cell behavior to biomedical imaging and disease detection and treatment. To overcome limitations to resolution, contrast, and penetration depth caused by the inherent inhomogeneity and high absorption of biological systems, biophysicists are beginning to take advantage of newly developed areas of photonic materials, solid state lighting, and nanotechnology. While these areas show great promise as biological probes, many joint materials/biology issues remain to be overcome before that promise can be achieved.

REFERENCES

[1] A. Hall and J.M. Girkin, "A review of potential new diagnostic modalities for caries lesion," J. Dent. Res. **83** (Spec. Iss. C) C89-C94 (2004).

[2] A. C-T Ko, L-P Choo-Smith, M. Hewko, M. G. Sowa, C. C. S. Dong, and B. Cleghorn, "Detection of early dental caries using polarized Raman spectroscopy," Optics Express **14**, 1 203-215 (January 9, 2006).

[3] J.M. Girkin, A.F. Hall, and S. L. Creanor, "Multi-phonon imaging of intact dental tissue," in Proceeding of the 4th Annual Indiana Conference, G.K. Stookey, ed. (Indiana University School of Dentistry, Indianapolis, Indiana) 155-168 (1999).

[4] A. Baumgartner , S. Dichtl, C.K. Hitzenberger, H. Sattmann, B. Robl, and A. Moritz, "Polarization-sensitive optical coherence tomography of dental structures," Caries Res. **35** 59-69 (2000).
[5] B.T. Amaechi, S.M.Higham, A.G.Podoleanu, J.A. Rogers, and D.A.Jackson, "Use of optical coherence tomography for assessment of dental caries: quantitative procedure," J. Oral Rehabil. **28** 1092-1093 (2001).
[6] D.W. Piston, T.J. Fellers, and M.W. Davidson, "Fundamentals and Applications in Multiphoton Excitation," in Nikon MICROSCOPY, www.microscopyu.com/articles/fluorescence/.
[7] A.F. Fercher, W. Drexler, C.K. Hitzenberger and T. Lasser," Optical coherence tomography—principles and applications," Rep. Prog. Phys. **66** 239–303 (2003).
[8] V. Nadeau, K. Hamdan, J. Hewett, W. Sibbett, I. Tait, A. Cuschieri and M. Padgett, "Endoscopic fluorescence imaging and point spectroscopy system for the detection of gastro-intestinal cancers," J. Mod. Opt. 49, 731-742 (2002).
[9] I. Hartl, X. D. Li, C. Chudoba, R. K. Ghanta, T. H. Ko, J. G. Fujimoto, J. K. Ranka and R. S. Windeler, "Ultrahigh-resolution optical coherence tomography using continuum generation in an air–silica microstructure optical fiber," OPT. LETT. 26 # 9 608-610 (May 1, 2001).
[10] Brodrick, J. R., "DOE Solid State Lighting Status and Overview", DOE Solid State Lighting Program Review, San Diego, CA (2/3-4/2005).
[11] E. Thrush, O. Levi, W. Ha, G. Carey, L.J. Cook, J. Deich, S.J. Smith, W.E. Moerner, and J.S. Harris, Jr., "Integrated Semiconductor Vertical-Cavity Surface-Emitting Lasers and PIN Photodetectors for Biomedical Fluorescence Sensing," IEEE J. Quant. Elect. 40 #5 491-498 (May 2004).
[12] K. Robinson, Fluorescent Nanoparticles Provide Single-Bacterium Detection," Biophotonics International, 11 #11 23-24 (November, 2004).
[13] H. Hogan, "Fluorescence at the Flip of a Molecular Switch," Biophotonics International 12 #1 34-38 (January 2005).
[14] H. Hogan, "Dialing in Quantum Dots," Biophotonics International, 11 #9 27-28 (September, 2004).
[15] K. Robinson, "Quantum Dot Coating Improves Specificity of Tumor Targeting and Imaging," Biophotonics International, 11 #9 52-53 (September, 2004).
[16] "6th Annual National Forum on Biomedical Imaging in Oncology" NCI/NEMA (Bethesda, MD, April 7 – 8, 2005), http://imaging.cancer.gov/reportsandpublications/ReportsandPresentations/6th-BIO-forum-presentations.
[17] A.P. Alivisatos, M. Sauer, and S. Weis, "Time-gated biological imaging by use of colloidal quantum dots," Opt. Lett. 26 #11 825-827 (June 1, 2001).
[18] R.M. Clegg, "The Vital Contributions of Perrin and Forster," Biophotonics International 11 #9 42-45 (September, 2004).
[19] K. Hassler, T. Anhut, R. Rigler, M. Gösch, and T. Lasser, "High Count Rates with Total Internal Reflection Fluorescence Correlation Spectroscopy," Biophys. J. LO1-LO3 (2005).
[20] H. Hogan, "Differential Interference Contrast Microscopy Images DNA," Biophotonics International, 11 #10 52-53 (October, 2004).
[21] L. Esterowitz, National Science Foundation, private communication (3/24/2005).
[22] S.M. Reiss, "Laser System Helps Quantify PDT Drug Concentrations," Biophotonics International, 11 #9 16-17 (September, 2004).

[23] R.E. Swaja, Senior Science Advisor, National Institute of Biomedical Imaging, National Institutes of Health, private communication (5/5/2005).
[24] N. Walker, "Evaluating the Safety of Materials Produced Through Nanotechnology: A Big Issue in a Small World", Workshop on Biomedical Applications of Nanotechnology, NIBIB/DOE (Bethesda, MD March 2005).