REVIEW

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Nanoscale fluorescent sensors for intracellular analysis

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Abstract There is a growing interest in the development of submicron optochemical sensing devices. Miniaturization of sensors to nano-dimensions decreases their typical response time down to the millisecond time scale. Their penetration volume is reduced to a few cubic micrometers and they exhibit a spatial resolution at the nanometer scale. In this review the fabrication of submicron optical fiber fluorescent sensors and particle-based fluorescent nanosensors is described. The functional characteristics of these exciting miniaturized fluorescent sensors and their applications for quantitative measurement of intracellular analytes are demonstrated.

Introduction

Biological studies at the single cell level have attracted the attention of researchers from various disciplines for decades. Two of the most popular cellular analysis techniques are fluorescence microscopy and flow cytometry [1–6]. Fluorescence microscopy is used for real time continuous observation of cells over time while flow cytometry is used to individually analyze a large number of cells at the single cell level. The rate of analysis in flow cytometry can reach 10 000 cells/second. However, unlike in fluorescence microscopy, each cell is observed only once as it flows through the detection region. A common feature of these two complementary and well-advanced technologies is the employment of molecular fluorescent probes to label the observed cells.

The field of fluorescent probes for cellular analysis has been growing steadily over the last 20 years resulting in a variety of fluorescent probes that are available commercially in a cell permeable form. Most notable are the numerous fluorescent probes for intracellular pH and free calcium ions [7, 8]. Fluorescent probes for other cellular components (reactive oxygen species, inorganic and metal ions) and for such cellular properties as viability, morphology and fluid flow, and membrane potential are also available [9]. Nevertheless, the number of cellular constituents that can be quantified using fluorescence microscopy and flow cytometry is limited due to the cytotoxicity of most organic chromophors. Furthermore, because of cytotoxicity, it is difficult to label the entire cell with bioactive molecules such as enzymes and antibodies for selective detection.

Other problems associated with cellular labeling include possible alteration of cellular functions, false readings because of the heterogeneous intra-cellular distribution of fluorophors within the cell, and the possible cross-talk between adjacent cellular zones.

Miniaturized fluorescent sensors have been developed in the last few years for single cell analysis. To realize true non-invasive intracellular analysis, the sensor must be about 100 times smaller than the analyzed cell. To prevent cytotoxicity upon insertion of the sensor into the cell, the sensing reagent must be isolated from the cellular environment by a biocompatible matrix that is selective to the intracellular analyte. In principle, it is possible to fabricate a sensor that contains several fluorophors and bioactive macromolecules such as enzymes, protein receptors and antibodies for multiple analyte sensing. Furthermore, it is possible to incorporate ligands on the sensor that are not soluble in aqueous solution. Overall, the scope of analytes that can be detected with these miniaturized sensors by far exceed the coverage of fluorescence microscopy and flow cytometry. In addition, miniaturized fluorescent sensors can be used for site specific intracellular measurements since problems associated with heterogeneity of the cellular environment and in the distribution of fluorescent probes in the cell are eliminated.

This review covers major developments in the area of fluorescence-based nanosensors over the last decade. First, we describe the fabrication of submicron optical fiber sen-

Dedicated to the memory of my late father, Victor Rosenzweig (1923–1999), who never ceased to inspire me to pursue a scientific career

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sors and their advantages and drawbacks. Secondly, we describe the fabrication and application of particle-based nanosensors for intracellular analysis. Finally, we list the main problems associated with the employment of nanosensors for intracellular analysis and suggest possible solutions.

Submicron-sized optical fiber fluorescence-based chemical sensors

To realize optochemical sensing in nanometer dimensions one must overcome the theoretical resolution of light microscopy, which is limited to the diffraction limit. The diffraction limit is equal to $\lambda/2$, where λ is the fluorescence excitation wavelength. The basis for the fabrication of fluorescence sensors with resolution exceeding the diffraction limit is the understanding that while light cannot be focused to a spot smaller than $\lambda/2$ it can be apertured to achieve a spot of these miniaturized dimensions. This principle was previously used in the development of near-field optical microscopy (NSOM). As a result, submicron fluorescent sensors are sometimes termed near-field optochemical sensors. In 1991, Lewis et al. [10] fabricated for the first time a submicron fluorescent sensor by incorporating the sensing reagent into a micropipette tip tapered to submicron dimensions by a micropipette puller. Shortly after that, Kopelman et al. [11–12] fabricated the first submicron optical fiber sensor. They used the same instrument to taper optical fibers instead of micropipette tips to submicron dimensions. However, pulling optical fibers could damage the core-cladding interface and could cause light leakage through the side of the tip. To prevent light leakage, the tapered optical fiber tip is coated with a thin layer of aluminium without blocking the active aperture of the fiber tip. The sensing reagent is attached to the tip of the fiber using a method that was developed by Walt et al. in 1986 [13]. To fabricate the sensor, the fiber tip is first activated with a silanization agent containing a primary amine functionality. Then, the fiber tip is shortly dipped into a solution of benzophenone, a photopolymerization initiation agent. Finally the tapered fiber is immersed in a solution containing acrylamide, the cross-linker bisacrylamide, and the fluorescent-sensing reagent. A light source, which may be a continuous wave lamp or laser, is coupled to the proximal end of the fiber. The growth of submicron sensing layer on the tapered distal end of the fiber is initiated by photopolymerization. The acrylamide polymer is covalently attached to the silane-activated tapered fiber. The thickness of the sensing layer is controlled by the light intensity, illumination duration and composition of the solution used for photopolymerization. The use of tapered optical fibers greatly simplified the fabrication and application of submicron sensors and improved their analytical properties compared to the previously used micropipette tips, mainly because the light source is directly coupled to the sensor, allowing higher photon flux to be transmitted to it.

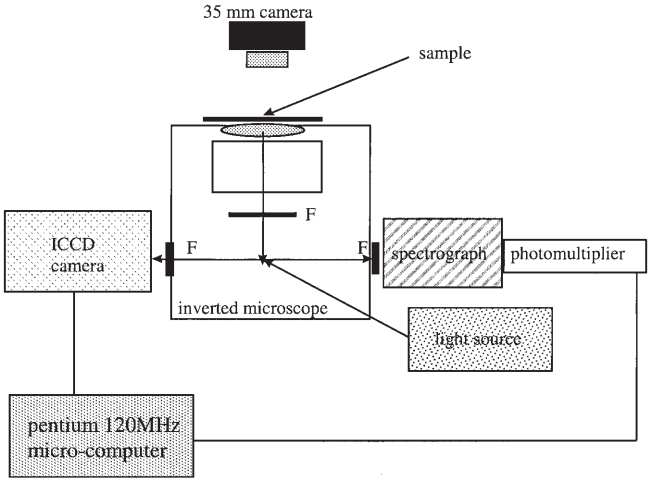

Fig. 1 A typical detection system for fluorescence measurements of submicron optical fiber sensors. The instrument consists of an argon ion laser as a light source, optical fiber sensor, inverted fluorescence microscope, and a spectrograph attached to a CCD camera for fluorescence measurements. A microcomputer is used for data acquisition and analysis

The limited photo flux dictates the use of relatively sophisticated detection systems for fluorescence measurements of submicron optical fiber sensors. A typical detection system for these measurements is shown in Fig. 1. Typically, a continuous-wave source, such as an argon ion laser, is used as a light source. The laser beam is directed to the proximal end of the fiber via an optical fiber coupler. The optical fiber sensor is positioned in the sample cell viewed by an inverted fluorescence microscope. The fluorescence signal is collected through a microscope objective. The emitted photons are reflected by a dichroic mirror (Chroma DCLP500), then filtered through an appropriate emission filter and focused on the entrance slit of a spectrograph that is used to disperse the fluorescence light. A high-performance charge-coupled device (CCD) camera is employed for digital imaging of the emission spectrum with a resolution of around 0.5 nm. The exposure time needed for image collection varies depending on the sensitivity of the employed camera (usually from 0.1 to 2 s). A microcomputer equipped with image analysis software is used for data acquisition and analysis.

The first submicron optical fiber sensor that was developed by Kopelman and co-workers in 1992 [11, 12] was for pH using fluorescein as a pH indicator. A polyacrylamide layer containing the pH indicator was grown at the end of the silanized tapered fiber tip via photopolymerization. The fluorescein derivative acryloylfluorescein was used allowing co-polymerization of fluorescein with polyacrylamide matrix. Co-polymerization of fluorescein with the polyacrylamide prevented leakage of the highly lipophilic fluorescein molecules from the polyacrylamide support, thus improving the chemical stability of the sensor. The high spatial resolution of the sensor was demonstrated by measuring the pH of buffer solutions inside single 10 μm pores of a polycarbonate membrane. Kopelman

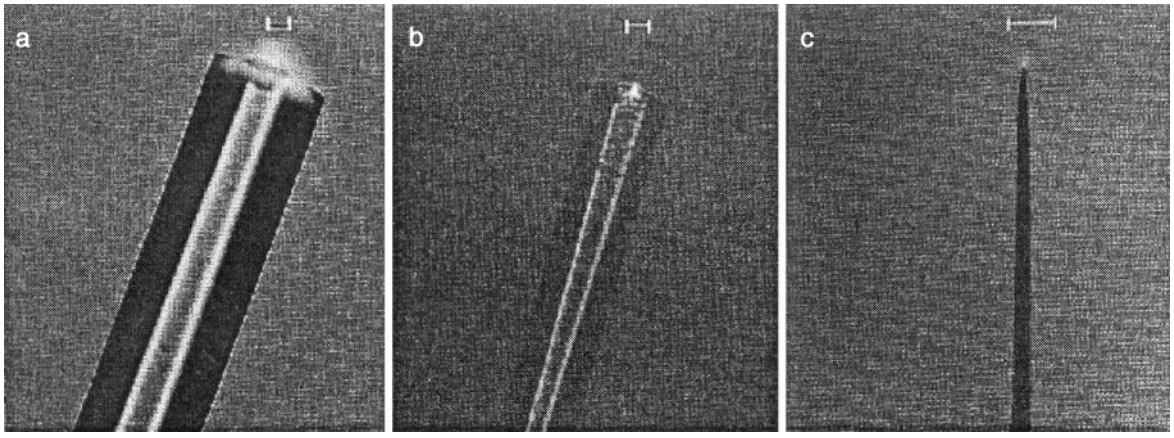

Fig. 2 Images of optical fiber oxygen sensors. *(a)* Sensor prepared from unpulled, 100 μm core, multimode fiber; *(b)* sensor prepared from an unpulled, single mode, 3–5 μm core, fiber; *(c)* sensor prepared from pulled, submicron optical fiber tip. The scale bars represent *(a)* 25, *(b)* 10, and *(c)* 10 μm

and co-workers employed the pH sensor to measure the pH in the yolk sac of rat embryos at different stages of gestation. Later, McCulloch et al. [14–16] used a different fabrication technique to produce nanometric optical fiber sensors. They tapered the optical fiber in a conventional fusion splicer instrument instead of using a CO_2 laser and a puller. Sol-gel synthesis was then used to trap the pH sensitive reagent fluorescein or oxygen sensitive indicator tris-(1,10-phenanthroline)ruthenium chloride within a SiO_2-based porous glass film, which was deposited on the surface of the fiber tip. They demonstrated the utility of their sensors by conducting pH measurements in pores of a polycarbonate membrane and in fibroblasts taken from a mouse embryo. The main advantages of this method are the increased simplicity of fabrication and the use of a sol-gel as a support instead of acrylamide. Sol-gels show higher mechanical stability and lower toxicity compared to acrylamide. However, it is more difficult to control the size of these sensors in a consistent manner because a dip coating technique is used to grow the sensing element on the pulled tip. Both sensors mentioned above are based on the measurement of a single wavelength. As such, quantification is usually complicated and affected by many parameters of the measuring equipment, including fluctuations in excitation power, efficiency of fluorescence collection and photobleaching. Plaschke et al. [17] recently fabricated submicron optical fiber pH and calcium sensors by incorporating a rhodamine derivative as a reference dye. Additionally, larger molecular weight dextran-fluorophore conjugates of fluorescein and rhodamine were used to increase the chemical stability of the sensors with respect to dye leaking. The reproducibility of the sensors improved significantly as a result of these refinements. Recent developments in pH nanosensors include the fabrication by Song et al. [18] of a single-excitation, dual-emission high-performance optical fiber pH sensor for practical physiological measurements. Additionally, Walt et al. [19] developed an optical fiber chemical sensor microarray for the detection of pH and O_2 in aqueous samples with a sub-second response time.

In 1995, Rosenzweig and Kopelman developed a submicron optical fiber oxygen sensor based on the fluorescence quenching of tris-(1,10-phenanthroline) ruthenium chloride by dissolved oxygen [20, 21]. The ruthenium complex was incorporated into an acrylamide polymer layer that was covalently attached to a silanized optical fiber tip by photo-initiated polymerization. Figure 2 shows typical optical fiber oxygen sensors of different dimensions fabricated using the controlled photopolymerization approach: 100 μm (Fig. 2 a), 3 μm sensor (Fig. 2 b) and 0.8 μm pulled optical fiber sensor (Fig. 2 c). The polymer tip grown at the distal end of the fibers contains the oxygen indicator tris-(1,10-phenanthroline)ruthenium chloride. When a 488 nm laser beam is coupled to the aluminium-coated fiber tip, a bright spot of fluorescence at the tip is observed. The emission peak of the fluorophore used is at 610 nm. The polymer tip itself is not seen because of the limited spatial resolution (1 μm) of the microscope. Leakage of the sensing reagent from the polymer host matrix was minimized by the optimization of the ratio between the monomer and the cross-linker. The sensor was fully reversible and highly reproducible. The sample volume required for measurements was only 100 femtoliter. To further improve the stability of this sensor, Rosenzweig and co-workers later synthesized a modified ruthenium complex, suitable for acrylate co-polymerization with acrylamide while maintaining its spectral properties and oxygen sensitivity [22]. A significant improvement in the leakage stability of oxygen sensors was realized when the modified oxygen indicator was used as a sensing reagent.

In 1997, Shalom et al. [23] reported the first successful development of a submicron fluorescent sensor for calcium ions in aqueous solution. It was based on a pulled micropipette, filled with a conducting porous sol-gel glass, which was doped with the fluorescent dye Calcium Green-1. The sensor was used to probe Ca^{2+} concentrations in mouse myococytes. In 1998, Xin and Wightman [24] attached a dextran conjugate of Calcium Green-1 to the tip of a miniaturized carbon electrode by cross-linking

with glutaraldehyde, and fabricated a dual microsensor to measure extracellular level of calcium ions and catecholamine simultaneously following their secretion from individual neuron cells. In 1998, Tan et al. inserted near-field optical probes into vascular smooth muscle cells to monitor calcium ion level fluctuations during cell stimulation [25]. The penetration of the probe into a cell was achieved by using manipulators commonly used for microinjection of solutes into cells.

In the sensors described above, the indicator changes its optical properties upon interaction with the analyte [26]. The scope of analytes is further expanded by the immobilization of receptor molecules capable of specific molecular recognition along with a universal fluorescence indicator with environmental sensitivity in the sensing support. This principle was first demonstrated by Simon and co-workers in 1989 [27–29]. They fabricated sensing thin films using highly selective ionophores for recognition and nonspecific pH indicators for signal transduction. One of the characteristics of these sensors is that components dissolved in the organic liquid polymer diffuse randomly throughout the entire volume of the film. Shortreed and Kopelman [30] found that the use of such a liquid membrane increases the photostability of submicron sensors and their working lifetime. The increased photostability was attributed to self-recovery of the membrane from photobleaching by random and continuous diffusion of fluorophores throughout the membrane. Barker and Kopelman [31] used a similar approach to fabricate an optical fiber sensor for nitrite and chloride ions in aqueous solution. A vitamin B_{12} derivative and an indium porphyrin were used as selective ionophores, respectively. The chloride sensor was utilized to determine chloride levels on the surface and inside the visceral yolk sac of organogenesis-stage rat conceptuses. Using a similar approach, Koronczi et al. developed a submicron optical fiber fluorescent sensor for potassium ion measurements in aqueous solutions [32]. A plasticized PVC membrane containing valinomycin for potassium complexation and a fluorescein derivative for pH measurement was used to determine the potassium ion levels in aqueous solutions. To enhance the signal reproducibility, the highly photostable dye, Nile red, was incorporated into the membrane as a reference dye. The experimental system was modified to achieve maximum spectral separation between the analyte and reference signals. Additionally, Kurihara et al. [33] recently prepared a micrometer-sized sodium ion-selective optode using a 16-crown-5 derivative as the neutral sodium ionophore and a dibromofluorescein or a coumarin derivative as the anionic dye.

Submicron optical fiber fluorescent biosensors

Like the ionophore-transducer approach described above, biosensors contain a bioselective component (e.g. enzyme or antibody) for molecular recognition and a non-specific signal transduction component. The first micron-sized optical fiber fluorescent biosensor for glucose was fabricated by Rosenzweig and Kopelman in 1996 [21, 34]. It was based on the enzymatic reaction of glucose oxidase that catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide while consuming oxygen. The ruthenium complex and glucose oxidase were co-immobilized in acrylamide polymer that was attached covalently to an optical fiber tip. Due to its small size, the response time of the sensor was only 2 s and an absolute detection limit of around 1×10^{-15} mol was achieved.

In 1998, Barker et al. reported the fabrication of three submicron optical fiber sensors for nitric oxide [35–37]. The pulled fiber was first modified with a self-assembled monolayer of gold, and dye-labeled cytochrome c' or guanylate cyclase was then adsorbed on the gold surface. The sensor was used to measure extra- and intracellular nitric oxide production by BALB/c mouse macrophages. The quantification of nitric oxide was based on the fluorescence change of the dye-labeled cytochrome c' or guanylate cyclase upon binding with nitric oxide.

Recently, Tan et al. [38] developed a submicron optical fiber sensor for glutamate ions. A tapered aluminium coated optical fiber surface was initially activated with amine functional groups. The enzyme glutamate dehydrogenase was then immobilized on the optical fiber probe surface by using glutaraldehyde as a bifunctional cross-linking reagent. The sensor was used to measure the level of glutamate ions in brain cells. Glutamate analysis was carried out by monitoring the fluorescence of NADH, a product of the enzymatic reaction between glutamate and NAD^+.

The miniaturization of the sensors results in at least a million-fold reduction of the sample volume and response times of milliseconds or less. The faster response of these sensors is due to the extremely small size and thickness of the sensing layer. Analyte diffusion to the sensing region is controlled by radial diffusion as opposed to lateral diffusion. These small sensors offer us a microscopic view of the world of biochemical, geochemical and technologically important chemical reactions. Kopelman et al. [39] recently discussed the principles behind this work in detail and outlined the advantages and disadvantages of sensor miniaturization. Submicron optical fiber sensors have indeed revolutionized the field of optical fiber sensors offering excellent absolute detection limits and fast response, as well as high spatial resolution. Nevertheless, the application of submicron optical fiber sensors for intracellular measurement has remained problematic.

The fabrication of submicron optical fiber sensors is technically involved and time consuming. Like patch clamp or capillary electrophoresis based measurements, cellular analysis is limited to one cell at a time. The penetration of the sensor into the cell may disrupt the membrane. The sensor occupies about 1% of the cell volume, which may induce severe biological perturbation and potentially lead to cell death. The sensor is fragile and may crash during positioning. To address these problems, a new generation of nanoparticle-based fluorescent sensors was developed in recent years for intracellular measurements.

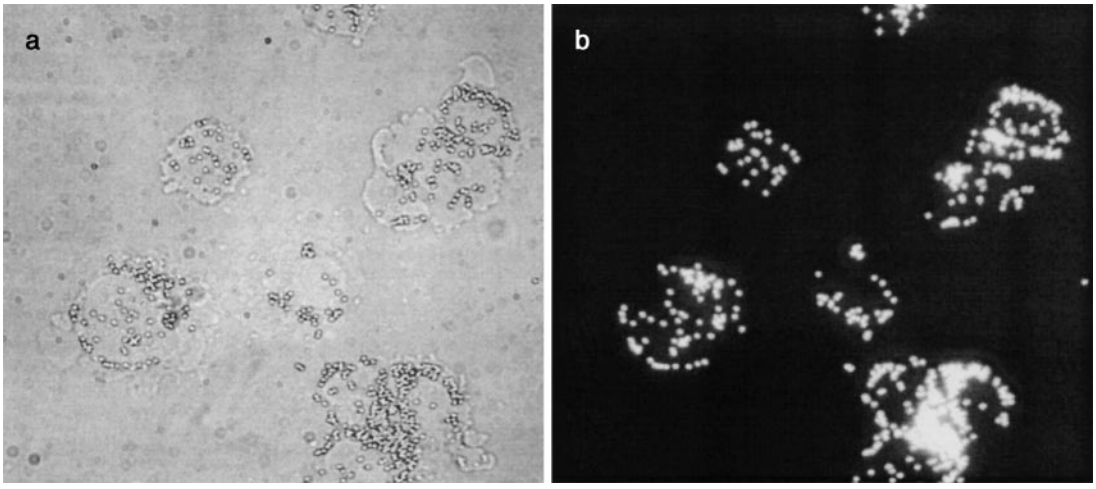

Fig. 3 Phagocytosis of 1 μm sized particle-based fluorescent pH nanosensors by murine macrophages. *(a)* A bright field image shows that the particles distribute evenly in the cells; *(b)* fluorescence image of the particles 1 hour after phagocytosis shows that the particles maintain their structural integrity and their signal is not degraded. The particles were used to measure the pH in the lysosomes and to monitor the effect of anti-malarial drugs on the pH in the lysosomes

Polymer nanoparticle-based fluorescent sensors

The employment of a single fluorescent nanoparticle as an optochemical sensor was first demonstrated by Sasaki et al. in 1996 [40]. The pH-sensitive dye fluorescein was entrapped in a polyacrylamide nanoparticle that was used to measure the pH distribution in a water/glass interface. In 1998, Kopelman et al. prepared a new type of nanosensor named PEBBLEs (Probes Encapsulated By Biologically Localized Embedding). PEBBLEs ranging from 20 to 200 nm in diameter for pH, molecular oxygen, calcium ions, glucose and nitric oxide were fabricated and applied for intracellular measurements in macrophages [41]. These new nanosensors show very high selectivity and reversibility, fast response time, and reversible analyte detection. They were delivered into the observed cells by a variety of minimally invasive techniques, including picoinjection, gene-gun delivery, liposomal incorporation and natural ingestion. The new technique offers several important advantages over optical fiber nanosensors. First, chemical information can be obtained on multiple cells simultaneously. Second, because of their small size the particles can be used to detect analytes in cellular organelles. Third, the technique is truly non-invasive allowing intracellular measurements while maintaining cellular viability. Lastly, the confinement of the sensor reporter dyes to the PEBBLE avoids dye compartmentation and enables the differentiation of the nanosensor location from autofluorescence centers in the observed cells. Rosenzweig et al. have recently developed nanometric fluorescent sensing beads for intracellular pH measurements. To increase the accuracy of the sensor, the particles were dually labeled with succinimidyl derivatives of the pH sensitive dye, Oregon Green and the pH insensitive dye Texas Red that was used as a reference standard. The two probes were covalently coupled to the surface of amino-modified polystyrene beads through the formation of amide bonds between the amino-modified surface and the fluorescent dyes. Figure 3 demonstrates the application of these fluorescent nanosensors for site specific intracellular pH measurements in Murine Macrophages known for their phagocytotic activity. A bright field image (Fig. 3a) reveals that the particle-based 1 μm sized sensors are internalized by the cells. The particles are directed into lysosomes, which are acidic intracellular organelles that protect the cells from the effect of foreign invaders. A digital fluorescence image taken 1 h after the internalization of the particles (Fig. 3b) shows that the particles maintain their structural integrity and are not degraded in the cells. The new particle-based nanosensors were applied to measure the pH in the lysosomes and to monitor the concentration dependent effect of anti-malarial drugs on the pH in the lysosomes.

In a related area, Rosenzweig et al. recently demonstrated the use of unilamellar phospholipid vesicles, liposomes, as fluorescent nanosensors for pH and molecular oxygen [42, 43]. The sensing reagents were encapsulated in the internal aqueous compartment of the liposomes and maintained their free solution properties. This improves the response characteristics of the sensors since matrix effects common to covalent bonding or entrapment of sensing reagents in solid state supports are eliminated. Depending on the cellular system, the liposomes may fuse into the cell membrane or be internalized by the cells while maintaining their structural integrity. In contrast, microinjection or optical fiber sensor penetration may cause cell injury and cytoplasmic acidification. Current research in this area focuses on the formulation of liposomes that exhibit high stability to degradation in the cellular environment.

Another type of fluorescent nanosensor was recently developed by Rosenzweig et al. The sensors were fabricated by coating polystyrene nanoparticles with fluorescent phospholipids with optochemical sensing capabilities. Covalent bonding of the phospholipids to the parti-

cles was accomplished by first acylating the surface of hydroxylated polystyrene nanoparticles with palmitoyl chloride. Upon dispersing the acylated polystyrene nanoparticles in a liposome suspension the hydrophobicity of the acylated bead leads to a transfer of fluorescent phospholipids onto this surface and to the formation of a fluorescent bilayer shell around the polystyrene core particles. To date, phospholipid coated nanoparticles, named lipobeads, with pH, oxygen and calcium ion measurement capabilities were fabricated and applied for intracellular measurements in Murine Macrophages. The newly prepared fluorescent sensing lipobeads show significant improvement in mechanical stability and size homogeneity compared to liposomes, while maintaining their biocompatibility. The technique is highly versatile since it is possible to incorporate biomolecules such as antibodies, enzymes and receptors into the membrane of the particles and use them as selective and sensitive intracellular biosensors.

Conclusion and outlook

The pioneering work of Kopelman in the area of fluorescent nanosensors provided a new analytical tool for single cell measurements. His work inspired many researchers worldwide, including the author of this review, and efforts to further develop this technique continue to grow. Early studies focus on the miniaturization of sensors using microfabrication techniques and on the ability to detect low levels of fluorescence from the sensor. The field of fluorescent nanosensors was greatly assisted by recent advances in digital fluorescence imaging instrumentation [44]. For example, it is now possible to detect single fluorescent molecules and monitor their diffusion kinetics at room temperature in aqueous solution [45]. These advances made the task of observing signal changes of fluorescent nanosensors feasible. The analytical properties of fluorescent nanosensors allow accurate analysis of biological systems. For example, the pH sensitivity of these sensors is within 0.1 pH units, the limit of detection for dissolved oxygen is around 0.5 ppm and the limit of detection for calcium ion levels is around 0.1 μM. The response time of fluorescent nanosensors is in the millisecond time scale. The photostability of these miniaturized sensors is still a problem in spite of the development of highly sensitive fluorescence detectors and the use of low light levels for excitation. Current research focuses on the development of nanosensing particles with multi-analyte detection capabilities and on the incorporation of reference standards into the sensing nanoparticles to increase the accuracy of the measurements. Another research direction is the expansion of the scope of analytes that can be detected with this technology, specifically by the attachment of bioactive molecules such as enzymes, antibodies and receptors to the surface of the particles. It is expected that artificial receptors that mimic bioactivity will be incorporated into the technology of fluorescent nanosensors in the near future. Another important issue that needs to be addressed is the biocompatibility of fluorescent nanosensors. While the technique is minimally invasive, the presence of a fiber optic sensor tip or a nanoparticle in the cell over a long observation period may induce toxic effects resulting in carcinogenesis or cell death. It is therefore important to investigate the effect of sensing materials on cells and to select sensing materials with minimal effect on the cell viability and function.

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References

1. Ploem JS (1986) In: Taylor DL, Waggoner AS, Lanni F, Murphy RF, Birge RR (eds.) Applications of Fluorescence in the Biomedical Sciences, Alan R. Liss, New York, Chap. 13
2. Melamed MR, Mullaney PF, Shapiro HM (1990) In: Melamed MR, Lindmo T, Mendelson ML (eds.) Flow Cytometry and Sorting, 2nd Ed, Wiley: New York, Chap. 1
3. Dunn K, Maxfield FR (1998) Methods Cell Biol 56:217–236
4. Bastiaens PIH, Squire A (1999) Trends Cell Biol 9:48–52
5. Darzynkiewicz Z, Bedner E, Li X, Gorczyca W, Melamed MR (1999) Exp Cell Res 249:1–12
6. Oheim M, Loerke D, Chow RH, Stühmer W (1999) Phil Trans R Soc Lon B 354:307–318
7. Johnson I (1998) Histochem J 30:123–140
8. de Silva AP, Eilers J, Zlokarnik G (1999) Proc Natl Acad Sci USA 96:8836–8837
9. Haugland RP (1996) Handbook of Fluorescent Probes and Research Chemicals, 6th Ed, Molecular Probes Inc, Eugene, Chap 15, 16, 21
10. Lewis A, Lieberman K (1991) Anal Chem 63:625A–638A
11. Tan W, Shi ZY, Smith S, Birnbaum D, Kopelman R (1992) Science 258:778–781
12. Tan W, Shi ZY, Kopelman R (1992) Anal Chem 64:2985–2990
13. Munkholm C, Walt DR, Milanovich FP, Kainer SM (1986) Anal Chem 58:1427–1430
14. McCulloch S, Uttamchandani D (1997) IEE, 162–167
15. McCulloch S, Uttamchandani D (1995) Meas Sci Technol 6:1157–1162
16. McCulloch S, Uttamchandani D (1997) SPIE 2980:228–239
17. Plaschke M, Geyer M, Reichert J, Ache HJ (1997) SPIE 3105:31–37
18. Song A, Parus S, Kopelman R (1997) Anal Chem 69:863–867
19. Healey BG, Walt DR (1997) Anal Chem 69:2213–2216
20. Rosenzweig Z, Kopelman R (1995) Anal Chem 67:2650–2654
21. Rosenzweig Z, Kopelman R (1996) Sensors Actuators B35–36:475–483
22. McNamara KP, Li X, Stull AD, Rosenzweig Z (1998) Anal Chim Acta 361:73–83
23. Shalom S, Strinkovski A, Peleg G, Druckmann S, Krauss A, Lewis A, Linial M, Ottolenghi M (1997) Anal Biochem 244:256–259
24. Xin Q, Wightman RM (1998) Anal Chem 70:1677–1681
25. Tan W (1998) Trends Anal Chem 17(8+9):501–513
26. Wolfbeis OS (1991) Fiber Optical Sensors and Biosensors, CRC Press, Boca Raton, Vol 1 and 2
27. Morf WE, Seiler K, Lehman B, Behringer C, Hartman K, Simon W (1989) Pure Appl Chem 9:1613–1618
28. Spichiger UE, Freiner D, Bakker E, Rosatzin T, Simon W (1993) Sensors Actuators B11:263–271
29. Lerchi M, Bakker E, Simon W, Pretsch E (1994) Anal Chem 66:1713–1717
30. Shortreed M, Monson E, Kopelman R (1996) Anal Chem 68:4015–4019

31. Barker SLR, Thorsrud BA, Kopelman R (1998) Anal Chem 70:100–104
32. Koronczi I, Reichert J, Heinzmann G, Ache HJ (1998) Sensors Actuators B51:188–195
33. Kurihara K, Ohtsu M, Yoshida T, Abe T, Hisamoto H, Suzuki K (1999) Anal Chem 71:3558–3566
34. Rosenzweig Z, Kopelman R (1995) Anal Chem 68:1408–1413
35. Barker SLR, Kopelman R, Meyer TE, Cusanovich MA (1998) Anal Chem 70:971–976
36. Barker SLR, Clark HA, Swallen SF, Kopelman R, Tsang AW, Swanson JA (1999) Anal Chem 71:1767–1772
37. Barker SLR, Zhao Y, Marletta MA, Kopelman R (1999) Anal Chem 71:2071–2075
38. Cordek J, Wang X, Tan W (1999) Anal Chem 71:1529–1533
39. Kopelman R, Dourado S (1996) SPIE 2836:2–11
40. Sasaki K, Shi Z, Kopelman R, Masuhara H (1996) Chem Lett 141–142
41. Clark HA, Barker SLR, Brasuel M, Miller MT, Monson E, Parus S, Shi Z, Song A, Thorsrud B, Kopelman R, Ade A, Meixner W, Athey B, Hoyer M, Hill D, Lightle R, Philbert MA (1998) Sensors Actuators B51:12–16
42. McNamara KP, Rosenzweig Z (1998) Anal Chem 70:4853–4859
43. McNamara KP, Rosenzweig N, Rosenzweig Z (1999) Mikrochim Acta 131:57–64
44. Rizzuto R, Carrington W, Tuft RA (1998) Trends Cell Biol 8:288–292
45. Xu X, Yeung ES (1997) Science 275:1106–1109