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Implantable fluorescent nanoparticles for "smart tattoo based" dissolved oxygen monitoring

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INTRODUCTION AND OBJECTIVES

Oxygen (O_2) is an important dissolved gas component in the human body which mediates several physiological, pathological, and atmospheric reactions. O_2 measurement could serve in determining fate of several processes. Continuous monitoring of O_2 is possible with the advent of implantable biosensors. "Smart Tattoo" biosensors allow for minimally invasive method for determination of *in vivo* analytes (McShane 2007).

O_2 can be measured optically using different luminescent indicator dyes that exhibit a dynamic quenching of their fluorescence intensity in the presence of O_2. When an indicator dye molecule is excited by an energy-rich photon the molecule would, under anoxic conditions, return to its ground state by emitting a longer wavelength photon. If O_2 is present however, it can collide with the excited indicator molecule, transferring excitation energy to O_2 causing quenching of the fluorescence intensity. The relationship of such reaction is given by Stern-Volmer equation :

$$\frac{I_0}{I} = K_{sv}[O_2]$$

Photostable oxygen sensing materials like Ruthenium compounds or porphyrins like platinum octaethylporphyrin (PtOEP) can be used for dissolved oxygen measurement (Chu 2007).

Smart tattoo concept It uses near infra red fluorescene emission scanning in response to reaction with the analyte.

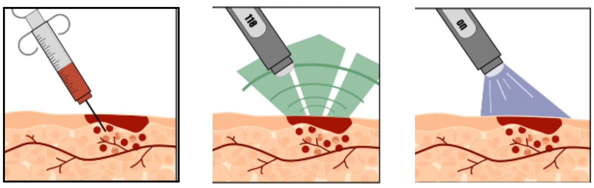

Figure 1: "Smart tattoo" concept: implantation, interrogation, and readout [McShane 2007].

The research aims at developing a "Smart Tattoo biosensor" responsive to dissolved O_2 for continuous measurement sensing assay based on near infra red range fluorophores.

MATERIALS AND METHODS

Platinum octaethyl porphine (PtOEP), Poly Lactic acid (PLA) were purchased from Sigma-Aldrich, India. Acetone, PVA and DCM were purchased from S. D. Fine chem. pvt Ltd., Mumbai. All chemicals were reagent grade and used as received.Polymeric nanoparticles of poly lactic acid (PLA) were prepared using conventional emulsification method (Desgouilles 2003), and Platinum octaethyl porphine (PtOEP) (an oxygen sensitive fluorophore) was loaded in nanoparticles and characterized using DLS, TEM and fluorescence spectroscopy. Dye loading efficiency was also determined using UV spectroscopy. This was done usin an indirect method of determination where in dye is extracted using DCM and measured in THF. The amount was determined using a previously made calibration curve. The response of PtOEP was determined in response to dissolved O_2 at different concentrations using fluorescence spectroscopy with excitation and emission maximum of 500 and 640 nm, respectively. The values were then correlated using Orion Oxygen sensing electrode. The dye loaded nanoparticles were tested for biocompatibility using sulphorhodamine B (SRB assay) against control samples.

RESULTS AND DISCUSSION

DLS, SEM and TEM measurements show that, PLA nanoparticles and dye loaded PLA nanoparticles showed a particle size of 280 nm and 450 nm with a poly-dispersity of 0.151 and 0.24, respectively.

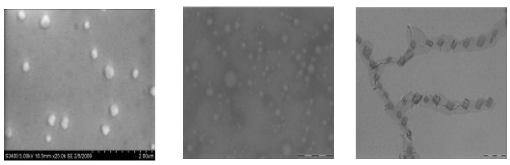

Figure 2: SEM image of PLA nanoparticles (left), TEM image of PLA nanoparticles (middle) and dye loaded PLA nanoparticle (right)

SEM and TEM images showed the presence of nearly spherical nanoparticles with similar sizes of 250 nm and 400 nm for unloaded and loaded nanoparticles (Fig. 2). Zeta potential analysis for determining the surface charge and stability showed that dye loaded nanopartices are stable as indicated by an electrophoretic mobility value of -15 mv ± 5 mV. Dye loading efficiency was determined by a calibration curve having equation (y = 86.155 x +

0.0362) at 530 nm was made. The results show that dye was encapsulated to an extent of ~78 % w/w.

Oxygen sensing The basis of O_2 measurement is quenching of intensity due to presence of oxygen. The fluorophore loaded Sensors were used in a concentration range of 0-15 mg % O_2. Stern-Volmer plot of linear range was 0-5mg/L in contrast to a logarithmic plot in range 0-14 mg/L (Fig. 3). Sensitivity (Ksv) value for oxygen is found to be 0.098/mg/L of oxygen (Gillanders 2004).

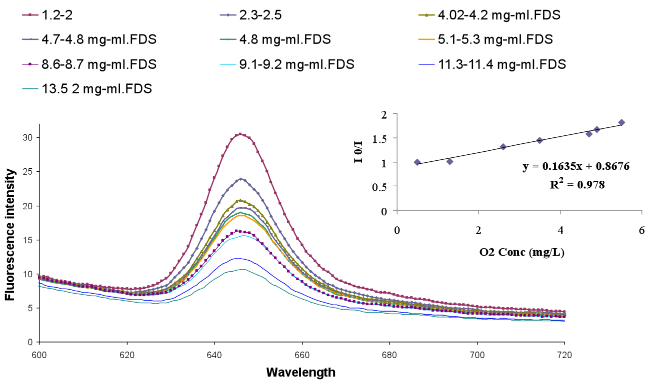

Fig. 3: Fluorescence emission scans of PtOEP loaded PLA nanoparticles in response to oxygen concentration (Inset shows the stern-volmer plot for oxygen measurement).

The mechanism of oxygen sensing is on the basis of collisional relaxation of excited state molecules in contact with oxygen analyte.

Biocompatibility study (SRB) assay The results of biocompatibility (Fig. 4) suggest that the percentage cell viability of increases as the fluorophore is encapsulated in a biocompatible matrix and the cells are not exposed to the dye.

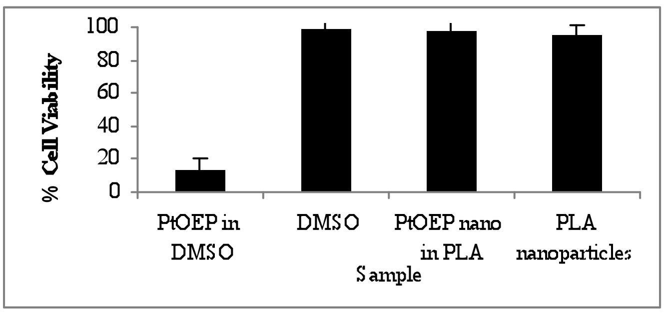

Fig. 4: % Cell viability calculated using SRB assay for different samples on L929 mouse fibroblast cell lines.

In all samples where in dye is encapsulated in nanoparticles the percentage viability is more than 80% when compared against the free dye which shows a higher percentage of cell death.

CONCLUSIONS

Polymeric nanoparticles of PLA containing PtOEP dye can be produced using conventional emulsification technique with uniform size. These particles can be used to determine oxygen concentration in the range of 0-15 mg/L at ambient conditions. The encapsulation of dye in PLA nanoparticles renders them suitable for *in vivo* applications. These nanoparticles can amplify the prospects of implantable "smart tattoo" biosensors.

ACKNOWLEDGEMENTS

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