All materials, unless listed below, were obtained from Sigma and used as received.
Water used was obtained from a purification system where the resistivity was 18 Ω cm (Maxima USF, ELGA).

Preparation of Oxygen Responsive Sol-gel Nanosensors

In order to facilitate retention of the reference dye Oregon Green in the sol-gel matrix, the fluorophore purchased was a dextran conjugate with a molecular weight of approximately 10,000 Daltons. Oregon Green Dextran was used as received.
The fluorophores tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride ($Ru(dpp)_3^{2+}$) (0.005 g) (GFS Chemicals) and Oregon Green Dextran (5 mg/ml, 50 µl) were added to a round bottomed flask containing ethanol (6 ml) and ammonium hydroxide (30 wt %, 4 ml). The mixture was stirred for 1 hour. Tetraethyl orthosilicate (TEOS) (0.5 ml) was then added drop wise to the mixture, stirring continued for a further 2 hours. The nanosensors were collected by centrifugation (350 g, 20 minutes). The nanosensors were subsequently washed with ethanol (10 ml) and deionised water (10 ml) and further centrifuged followed by discarding of the supernatant. The washing process was repeated several times to remove any unreacted chemicals. Finally the nanosensors were collected by vacuum filtration using a Millipore anodised filter membrane (0.02 µm) and dried under vacuum.

Preparation of pH Responsive Sol-gel Nanosensors

A method outlined by Wang *et al* was modified to prepare pH responsive dyes covalently linked to 3-aminopropyltriethoxysilane (APTES)[1]. Amine reactive dye molecules, 5-(and-6)-carboxyfluorescein, succinimidyl ester (FAM-SE) (1.5 mg) (Invitrogen) and (6-carboxytetramethylrhodamine, succinimidyl ester) (TAMRA-SE) (1.5 mg) (Invitrogen) were individually dissolved in anhydrous dimethylformamide (DMF) (1 ml), combined with an excess of APTES (1.5 ml) and stirred under a dry nitrogen atmosphere for 24 hours in the dark.

The dyes attached to APTES (250 µl) were then added to ethanol (6 ml) and ammonium hydroxide (30 wt %, 4 ml) (Alfa Aesar) contained in a round-bottomed flask and stirred for 1 hour. A solution of TEOS (0.5 ml) was then added drop wise to the mixture; stirring continued for a further 2 hours. The resulting nanosensors were then collected by centrifugation and filtration as described previously for oxygen responsive sol-gel nanosensors.

Electrospinning PLGA Scaffolds

The polymer solution was prepared by dissolving PLGA (70:30 LA:GA ratio, 0.45 g) (Lakeshore Biomaterials) in dichloromethane (DCM) (2.97 ml)) (HPLC grade, Fisher Scientific) solvent with pyridinium formate (PF) (0.03 ml). The solution was placed into a 10 ml syringe (Becton Dickinson) with an 18G blunt fill needle (Becton Dickinson) and the syringe attached to a syringe pump (Harvard PHD 2200 syringe pump) set to a constant flow rate of 3.5 ml/min. A tension of 12 kV (Glassman High voltage Supply Series EL) was applied to the tip of the syringe and a distance of 20 cm from the syringe needle tip to grounded steel collector plate (25 x 35 cm) was used. Electrospinning continued for 1.5 hours resulting in a sheet of polymer being produced. The resulting scaffold was left to air-dry overnight in a fumehood to remove any solvent residue.
Self-reporting scaffolds were prepared as above but with the modification of adding nanosensors to the polymer solution (5 mg/ml) prior to the electrospinning process. The nanosensors were suspended in the PLGA solution with the assistance of ultrasonication.

Preparation of PLGA Scaffolds for Cell Culture

The PLGA scaffolds were cut into 2 cm^2 pieces, sterilized by exposure to UV light (15 minutes each side) and washed three times using phosphate buffered saline (PBS). Prior to the cells being seeded, the sterilised scaffolds were incubated at 37°C 5% CO_2 in standard culture media (SCM) for 2 days.

Culture of 3T3 Fibroblasts

The routine culture of 3T3 fibroblasts was performed using SCM. The 3T3 fibroblasts were cultured in a humidified incubator at 37˚C and 5 % CO_2. The media was refreshed every other day. Serial passages were carried out by trypsinisation following which cells were centrifuged and the pellet re-suspended before re-plating at a typical dilution of 1:4. For experimental analysis, cells were subcultured at a density of $4x10^5$ cells per ml onto sterilised PLGA scaffolds in 35 mm culture plates (Beckton Dickenson Labware) 3 days prior to experiments.
The cell/PLGA construct was maintained at 37°C in a humidified 5% CO_2 incubator with the medium replaced every other day.

Live/Dead® Staining

Cell viability was assessed using Live/Dead® reduced biohazard assay (Invitrogen). The Live/Dead® solution was applied to a population of 3T3 fibroblasts cultured upon PLGA scaffolds not incorporating nanosensors, termed blank scaffolds. Fibroblasts were incubated at 37 ^{o}C and 5 % CO_2 in a humidified atmosphere for 1 hour followed by aspiration of the Live/Dead® stain and washing twice using PBS (pH 7.4). The cells were fixed using paraformaldehyde (4 % w/v) before placing PBS (pH 7.4) (2 ml) into the cell culture plate to allow visualisation using CLSM utilising a 63X 0.9NA water immersion lens.

ECM Protein Assessment

Cell viability of 3T3 fibroblasts cultured upon blank PLGA scaffolds was assessed for the production of ECM proteins; the primary antibodies used were sheep antihuman – fibronectin FITC (diluted 1 in 20 in PBS) (Serotec) and rabbit antihuman – elastin (diluted 1 in 40 in PBS) (Abcam), the secondary antibodies used were donkey antisheep Alexa Fluor 488 (diluted 1 in 50 in PBS) (Invitrogen) and donkey antirabbit alexafluor 647 (diluted 1 in 50 in PBS) (Invitrogen), respectively. The manufacturers state that these antibodies are known to cross reactivity with other species including mouse. Cell samples that had been cultured in 35 mm culture plates were fixed with paraformaldehyde (4 % w/v) for 30 minutes at 21^{o}C then washed three times with PBS (pH 7.4). The samples were then incubated with primary antibody for 2 hours at room temperature followed by three washing stages with PBS (pH 7.4). The secondary antibody was then applied and incubated at room temperature for 1 hour. The samples were then washed a further three times with PBS before adding PBS (2 ml) prior to analysis by CLSM. Samples were kept in the dark at all stages which involved wrapping the samples in foil.

Response to Oxygen

Nanosensors were suspended in deionised water (5 mg/ml) by methods of ultrasonication and vortexing. The fluorophores Oregon Green Dextran (5 mg/ml, 20 µl) and $Ru(dpp)_3^{2+}$ (0.01 g, 30 µl) were dispersed into deionised water (1950 µl) and an excitation and emission spectra for the reference and oxygen responsive dye were collected.

To determine the response to a change in oxygen concentration, oxygen was displaced from the cuvette by purging the sample with argon before measuring the fluorescence emission intensity. The sample was allowed to equilibrate in atmospheric oxygen before measuring the fluorescence emission intensity again, to determine the reversibility of the dye. A calibration was performed whereby a gas blender (Signal Instrument Co) was used to purge the sample with different concentrations of oxygen ranging from 0 to 21 % and the emission spectra collected. The data were analysed utilising the Stern-Volmer equation to prepare a calibration curve.

Response to pH

The response of the fluorophores used to analyse changes in pH was determined by adding FAM (20 µl) and TAMRA (20 µl) to different Sørensen's phosphate buffer solutions (1600 µl) ranging from pH 5.5 to pH 7.5. The sodium phosphate buffers (0.1 M) are prepared by mixing specific ratios of monobasic (0.2 M) and dibasic (0.2 M) stock solutions. The final pH was checked using a pH meter (Jenway model 3510). Any minor adjustments to the pH were made using NaOH (4 M) or HCl (2 M). The response of pH nanosensors was assessed by preparing suspensions of nanosensors in different pH buffers (5 mg/ml) by methods of ultrasonication and vortexing.

Excitation and emission spectra for the pH responsive and reference dye for both the fluorophores alone or incorporated into nanosensors were collected. A ratio of the emission maxima produced at each pH value was used to produce calibration curves.

PLGA Scaffold Analysis

A 2 cm^2 scaffold sample was placed into a 35 mm culture plate and immersed in appropriate buffer solutions (2 ml). Observation of nanosensors located in the scaffold was carried out using CLSM with an argon ion laser (488 nm) and a 63X 0.9NA water immersion lens. The fluorescence intensities were taken from the whole image frame for each image acquired. A ratio of the emission responses was taken for each image and averaged for each scaffold.

The fluorescence emission for oxygen responsive scaffolds was collected at 500-530 nm (green) for Oregon Green Dextran and 600-630 nm (red) for $Ru(dpp)_3^{2+}$. The scaffold was immersed in PBS and gas mixtures at different oxygen concentrations were passed into the solution. An equilibrating period of 15 minutes was allowed before measurements were taken.

The fluorescence emission for pH responsive scaffolds intensity of FAM (λ_{ex} 488 nm) was collected at 500-530 nm and TAMRA (λ_{ex} 568 nm) at 558-580 nm. The scaffold was immersed in buffers of different pH and allowed to equilibrate for 15 minutes prior to analysis.

Preparation of Non-biological Samples for SEM Analysis

Nanosensors, PLGA scaffold or PLGA sensing scaffold samples were prepared for SEM (JEOL 6060L) analysis by placing directly on a carbon coated electron microscope stub. The samples were then sputter coated with gold for 4 minutes under an argon atmosphere using a Blazers SCD 030 gold sputter coater to increase conductivity of the sample (Blazers Union Ltd). To reduce ionisation of the sample during imaging the working distance, voltage and magnification were balanced.

Sample preparation of Cell seeded Scaffolds for SEM Analysis

Cell-seeded scaffolds were prepared for SEM by washing with PBS, fixing in paraformaldehyde (4 % w/v) at 21°C for 30 minutes before washing in PBS and secondary fixing in osmium tetroxide solution (1 v/v %) for 2 hours at 21^{o}C before dehydration through increasing concentrations of ethanol (25 % v/v, 50 % v/v, 70 % v/v, 90 % v/v, 95 % v/v and 100 % v/v). Finally scaffolds were cut, mounted on electron microscope stubs, sputter coated with gold for 4 minutes and observed by SEM. Desiccated samples were placed on carbon coated electron microscope stubs and sputter coated with gold for 4 minutes under an argon atmosphere.