Quantum Sensing in a Physiological-like Cell Niche Using Fluorescent Nanodiamonds Embedded in Electrospun Polymer Nanofibers

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Fluorescent nanodiamonds (fNDs) containing Nitrogen Vacancy (NV) centers are promising candidates for quantum sensing in biological environments. This work describes the fabrication and implementation of electrospun Poly Lactic-co-Glycolic Acid (PLGA) nanofibers embedded with fNDs for optical quantum sensing in an environment, which

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recapitulates the nanoscale architecture and topography of the cell niche. A protocol that produces uniformly dispersed fNDs within electrospun nanofibers is demonstrated and the resulting fibres characterized using fluorescent microscopy and Scanning Electron Microscopy (SEM). Optically detected magnetic resonance (ODMR) and longitudinal spin relaxometry results for fNDs and embedded fNDs were compared. A new approach for fast detection of time varying magnetic fields external to the fND embedded nanofibers is demonstrated. ODMR spectra were successfully acquired from a culture of live differentiated neural stem cells functioning as a connected neural network grown on fND embedded nanofibers. This work advances the current state of the art in quantum sensing by providing a versatile sensing platform that can be tailored to produce physiological-like cell niches to replicate biologically relevant growth environments and fast measurement protocols for detection of co-ordinated endogenous signals from clinically relevant populations of electrically active neuronal circuits.

1. Introduction

The Nitrogen-Vacancy (NV) color center in diamond has emerged as a high performance quantum sensor with application areas including photonics, quantum information science and the life sciences.^{[1] [2], [3]} The NV color center is a naturally occurring paramagnetic impurity comprising of a substitutional nitrogen atom adjacent to a vacant lattice site, which in the negative charge state (NV^-) forms a spin triplet, $m_s = -1, 0$, and $+1$. Upon excitation with green light, the NV^- center produces a broadband fluorescent emission extending into the near infrared region with a zero phonon line at 637 nm. The NV^- center has attracted attention as a potential fluorescent probe for use in biological imaging due to its high quantum yield, robust luminescence, which does not bleach, and the inherent low cytotoxicity of diamond.^[3] The ability to optically manipulate and monitor the electron spin state in the NV^- center is core to its quantum sensing capabilities. Indeed, optical excitation of the NV^- center causes pumping

of the spin into the $m_s = 0$ ground state, as a result of spin dependent radiative and non-radiative de-excitation pathways.^[4] Due to the axial symmetry of the NV^- center the $m_s = \pm 1$ spin levels are degenerate in the absence of a magnetic field and in the ground state are separated from the $m_s = 0$ spin level by an energy of 2.87 GHz.^[4] The photoluminescent (PL) intensity from the NV^- center is dependent on the electron spin state, which is higher when in the $m_s = 0$ state as compared to the $m_s = \pm 1$ spin levels. Measurement of the PL intensity as a function of microwave (MW) frequency enables acquisition of an optically detected magnetic resonance (ODMR) spectrum. Here, the PL intensity reduces when the MW frequency is resonant with the spin transition from $m_s = 0$ to $m_s = \pm 1$. The structure of the ODMR spectrum is extremely sensitive to external perturbations including magnetic and electric fields, temperature, pressure and lattice strain.^[3] This is at the heart of the quantum sensing capabilities of the NV defect, which manifests itself as splitting of the ODMR resonance in the presence of external magnetic fields and shifts in the electronic energy states as a result of electric fields, temperature or strain in the diamond lattice. Quantum sensing is also possible through measurement of the transition rate between the $m_s = 0$ and $m_s = \pm 1$ sublevels, given by the spin-lattice (or longitudinal) relaxation time, T_1 .

There has been a growing interest in exploiting the spin dependent optical properties of the NV center to probe biological systems, most notably in recent years for opto-magnetic imaging in bacterial cultures,^[5] magnetic resonance imaging of spin labelled cell membrane proteins,^[6] optical detection of action potentials in single neurons^[7] and intracellular thermometry.^{[8],[9]} Despite continuing advances in proof of principle studies using NV quantum sensors in biological systems, there is a paucity of studies addressing the significant challenge of implementing these sensors in appropriate growth environments that recapitulate the *in vivo* cell niche. Currently, many sensing protocols utilize NV centers implanted near the surface of single crystal diamond chips as growth substrates to support monolayer cell

culture. Whilst diamond has a low cytotoxicity profile,^[10] diamond chips lack the intrinsic mechanical, structural and chemical signatures to support functional cell behavior compared with more native cell niches. Studies using nanodiamonds containing NV centers have targeted individual cells, but again predominantly for the study of cells in monolayer culture. Both of these approaches do not enable replication of important bioactive cues that occur in the three dimensional *in vivo* environment. Indeed, it is well established in the life sciences that a key component of the *in vivo* environment affecting cell function is the extracellular matrix (ECM), as demonstrated by studies reporting the frequency of postsynaptic potentials in neurons to be significantly reduced in a matrix deficient environment,^[11] reported changes in cell proliferation and gene expression,^[12] and changes in electrophysiological activity in neuronal^[13] and cardiac cells^[14] as a result of changes in ECM stiffness.

Significant work has been carried out over the past two decades to establish surrogate ECM environments to support *in vitro* cell culture, this is particularly so in the area of stem cell culture to promote cell function and differentiation and in Tissue Engineering to provide structural cues to guide regenerating tissue organization. Electrospun polymer nanofibers (ENF) are synthetic scaffolds that can replicate essential properties of the ECM to support functional cell behavior *in vitro*. The fabrication process enables controlled production of nanoscale dimensions and topography similar to the fibrillar structure of the ECM with tunable fiber thickness, organization and density. Indeed, ENFs have been loaded with detonated nanodiamonds to tailor the mechanical properties and durability of the fibers.^{[15], [16]} The fibers themselves are typically composed of biodegradable polymers that are amenable to functional modifications to enhance cell survival, differentiation and organization.^[17] ENFs can support myocardial regeneration,^[18] functional neuronal development^[19] and repair of neural injury.^[20] Further, due to the low cost and ease of fabrication of ENFs, a large variety of novel biomedical applications have been developed in recent years, such as the controlled

release of drugs from nanoparticle embedded ENFs^{[21],[22]} and electrical stimulation using conductive ENFs for accelerated maturation of *in vitro* cultured neurons.^[23]

This work describes the fabrication and implementation of ENFs embedded with fNDs for optical quantum sensing to address the need for biological systems that incorporate high precision biosensors to monitor cell function, together with environments that mimic *in vivo* cellular niches. Specifically, protocols and methodologies are established to embed fNDs into ENFs composed of poly(lactic-co-glycolic acid) (PLGA) nanofibers. The resulting nanofibers were characterized using fluorescent microscopy and Scanning Electron Microscopy (SEM) and the sensing capabilities of the embedded fNDs assessed via ODMR, longitudinal spin relaxometry and detection of external, non-biological time varying magnetic fields. The growth of neural stem cells (NSCs) on the nanofibers was compared to control cells cultured on planar glass substrates. Herein, a new approach to quantum sensing in therapeutically relevant live cells is demonstrated in a physiological-like cell niche using fNDs embedded in ENFs.

2. Results and Discussion

2.1. Characterisation of fND embedded ENFs

The composition of solutions for electrospinning notably affected fND aggregation (**Figure 1a**). Visual inspection of solutions prior to electrospinning reveals distinct aggregates of fNDs for chloroform, acetyl nitrile:chloroform (1:2) and Ethyl Acetate:Chloroform (1:2) mixtures. In the case of the mixture containing a 2:1 ratio of chloroform to methanol the solution is visually uniform indicative of uniform dispersal of fNDs, it should be noted that due to the size of the fNDs (100 nm) individual particles are not resolved. Further, fluorescence microscopy of the Rhodamine-B labelled ENFs, without fNDs, demonstrates the formulation containing a 2:1 ratio chloroform to methanol to produce fibers with a low rate of blebbing as compared to the other solutions studied (Figure 1b). Based on this finding, all

subsequent experiments were performed using ENFs spun from a solution containing a 2:1 ratio of chloroform to methanol.

Figure 2 contains a macroscale image of the fND embedded ENFs, images obtained using differential interference microscopy (DIC) and fluorescence microscopy to highlight the co-location of the fNDs and fibers, along with SEM images and data from image analysis of ENFs with and without fNDs. Light microscopy images demonstrate the uniform distribution of fNDs within the ENFs (Figure 2a). Qualitative inspection of the SEM images indicates the addition of fNDs had little impact on the organization and size of ENFs (Figures 2b and 2c). This is further evidenced through the comparable average fiber diameters, 365 nm ($SD \pm 182 \text{ nm}$) without fNDs (Figure 2d) and 306 nm ($SD \pm 184 \text{ nm}$) with fNDs (Figure 2e). It should also be noted that the final ENF coverage, density, thickness and fiber diameter can be tuned by adjusting experimental parameters such as the distance between the syringe needle and the collection plate, the applied voltage across the electrodes and the spinning time. Overall, these results demonstrate effective fabrication protocols for uniform embedding of fNDs in ENFs.

Figure 2: Morphological characterization of fND embedded ENFs. a) Macroscopic image of whole fND embedded ENF matt with inset showing left to right fNDs and ENFs visualized with widefield DIC, fluorescence microscopy and a merged image, b & c) Scanning electron microscopy images of control (b) and fND embedded fibers (c), d & e) Histograms displaying the distribution of fiber diameters for control and fND embedded fibers.

The effect on PL and quantum sensing of embedding the fNDs in the ENFs was investigated through a comparison of drop cast fNDs with fND embedded ENFs. **Figures 3a and 3b** display fluorescent microscopy images obtained from fNDs drop cast on a glass coverslip and fND embedded ENFs (FOV $220 \mu\text{m} \times 220 \mu\text{m}$). Of note is the low background signal from

the PLGA fibers, which are known to be autofluorescent. The PL emission spectra were also collected using a UV-Vis spectrophotometer (Flame, Ocean optics) to compare the emission from the drop cast and embedded fNDs. The spectral features are comparable with both cases showing the NV⁻ zero phonon line at 637 nm. The reduction in PL intensity from the fND embedded fibers is attributed to the optical scattering properties of the PLGA fibers. An important result from a quantum sensing point of view is the successful acquisition of an ODMR spectrum from fNDs embedded in ENFs (Figure 3d). This is characterized by a minimum in PL intensity centered around 2.87 GHz, with a split indicative of lattice strain, and a full width at half maximum of 20.3 MHz, consistent with nanodiamonds containing an ensemble of NV centers.^[9]

2.2. Longitudinal spin relaxation measurements

Results from measurement of the longitudinal spin relaxation times (T_1) of between 40 and 50 individual clusters of fNDs of comparable intensity and size are shown in **Figure 4**. Overall, in agreement with previous reports,^{[24],[25]} a broad distribution of T_1 relaxation times is observed in all fNDs studied which is attributed to variations in the size, shape and dispersivity of individual fNDs as indicated by the histograms. The results also demonstrate an almost two fold reduction in T_1 in the case of embedded fNDs ($T_1=57 \mu\text{s} \pm 8 \mu\text{s}$) as compared to drop cast fNDs ($T_1=103 \mu\text{s} \pm 9 \mu\text{s}$). Multiple effects are envisaged to contribute to this reduction, amongst them differences in the local chemical environment and changes in the relaxation rate of the metastable singlet states.^[25]

The successful implementation of T_1 relaxometry using the fND embedded ENFs provides a pathway to multifunctional NV based quantum sensing in an environment which recapitulates the nanoscale architecture and topography of the cell niche. Protocols previously implemented on NV implanted diamond demonstrating quantification of paramagnetic species in solution, analogous to the detection of endogenous free radicals in living cells

could be performed using the fND embedded ENFs.^[26] Furthermore, recently reported protocols using polymer encapsulation of fNDs to achieve gadolinium based sensing of pH and redox potentials using T_1 relaxometry could also be performed.^[26] It is noted that for this application, there is rapid drop off in target signal strength as a function of distance from the ND surface, thus optimization of ENF fabrication process would need to be considered for such applications including the use of hollow fiber, porous constructs. In addition, while the sensing capabilities of the fNDs embedded in the ENFs was compromised, through reduction in the T_1 relaxation time as compared to bare fNDs, this could be mitigated by investigating different polymer compositions and electrosinning parameters affecting fiber density, thickness, and distribution.

2.3. Time resolved detection of external magnetic fields generated by a permanent magnet

The protocol for fast, simple determination of external magnetic field strength is shown schematically in **Figure 5a**. The method relies on the comparison of PL intensity acquired with and without MWs. Experimentally, the protocol involved acquisition of two consecutive images of PL intensity, one with MWs applied at 2.87 GHz and one in the absence of a MW field. The ratio of the two PL images varies with magnetic field strength. A calibration curve was generated by correlating the percentage ODMR contrast to magnetic field strength as measured by a transverse Hall probe located on the microscope stage in the same position as the fND embedded ENFs (Figure 5b). This approach provides several advantages for wide-field magnetic sensing and imaging of nanodiamonds, firstly no external magnetic field or complicated MW pulse sequences are required. Secondly, the protocol is robust, fast and can readily be implemented in cell culture systems. The sensing capabilities of fND embedded ENFs using this protocol was demonstrated by moving a neodymium permanent magnet mounted on a translation stage above the microscope stage. At each position of the magnet

the contrast between the images with and without MWs was calculated using the ratio of the averaged PL intensity in the corresponding images.

Experimentally the external magnetic field was varied between 2 μT and 4mT. Figure 5b shows a dynamic range of up to 1mT, with contrast saturating at higher magnetic field strengths. A minimum detectable magnetic field of $4.8 \mu\text{T} \pm 0.9 \mu\text{T}$ was calculated using the standard error in the mean of points on the calibration curve and the gradient of a line fitted to the steepest point of this curve. Time resolved magnetic field sensing was demonstrated (Figures 5c and 5d) by running the described acquisition protocol over a period of between 10 and 15 seconds and intermittently placing a neodymium permanent magnet in proximity to the fibers. The PL intensity in the presence of resonant MWs changes when the magnet is placed near the fND embedded ENFs is shown in Figure 5c. A corresponding change in contrast is also seen (Figure 5d) demonstrating the capacity of the fND embedded ENFs for time resolved magnetic field sensing with sub-second temporal resolution of 40 ms. This measurement protocol is significant for studies using nanodiamonds as it overcomes the requirement of knowing NV orientation as is the case of magnetometry based on ODMR spectral splitting.^[27]

The measurement sensitivity of embedded fNDs was estimated from analysis of an ODMR spectrum and calculations using the following equation:^[28].

$$\eta = \frac{4h\delta}{3\sqrt{3}g_{nv}\mu_B R\sqrt{N}} \quad (\text{Equation 1})$$

where η represents sensitivity, δ is the full width at half maximum of the ODMR peak, R represents the experimental ODMR contrast, g_{nv} is the g-factor of the NV center, μ_B is the Bohr magneton and N is the number of collected photons per second. Using **Equation 1** and measurements of PL from a single ND within the FOV represented in Figure 2d, the sensitivity of the fND embedded ENFs is estimated to be $3.4\mu\text{T}/\sqrt{\text{Hz}}$. Higher sensitivity

could be achieved through an increase in the number of photons collected via improvement in light collection efficiency, in the number of embedded fNDs and use of a less optically scattering polymer. Sensitivity gains could be achieved via enhanced activation techniques to convert a greater percentage of nitrogen into NV centres in the nanodiamonds.^[29] Isotopic engineering of nanodiamonds would also result in reduced ODMR linewidths,^[30] however ¹²C grown nanodiamonds are yet to be demonstrated. Sensitivity could also be improved by enhancing the contrast, R , which theoretically is significantly higher ($\sim 30\%$) than the one measured here (2 % to 3 %) however, in practice experimental contrast rarely exceeds 10-15% due to broadening from the strain environment of the ND crystal lattice. More efficient delivery of MWs would lead to higher contrast, indeed the literature reports a 5 fold improvement in contrast using a micro strip antenna compared with a copper wire as used in this report.^[31]

Significant improvements in the detection sensitivity of the presented measurement platform could pave the way for the implementation of spatiotemporal electrophysiological measurements on live cells in a physiological-like environment. Indeed, the concept of electrophysiological measurements in cells using NV centers has been explored theoretically and experimentally.^{[32],[33]} Whilst still in its infancy compared to existing methods including patch clamping, whereby deformation of the cell membrane is achieved using a hybrid electrode/pipette tip^[34] and fluorescence methods based on the use of genetically engineered voltage sensitive proteins,^[35] or electrochromic and FRET based dyes,^[36] electrical sensing using NV centers has made significant advances in recent years. Moreover, NV based approaches overcome many of the drawbacks of existing methods such as the inherent invasiveness, low throughput and lack of spatial information associated with patch clamping as well as difficulties associated with fluorescence based sensors, namely the requirement for genetic manipulation, or observed cytotoxicity of many fluorescence based probes.^[37] To

date, a number of studies have considered in detail the different experimental parameters necessary for maximizing measurement sensitivity in NV based sensing of neuronal action potentials.^{[32],[33],[38]} For example, a recent demonstration of optomagnetic detection of single axonal potentials employed a MW lock in detection method to manipulate the NV spin state, citing sensitivity at a distance of 10 μm from the sample and by integrating the signal over a relatively large surface area ($\sim 8\text{mm}^2$) of 15pT/ $\sqrt{\text{Hz}}$. This was more than sufficient to observed the peak fields during axonal firing measured in the region of a few nT.^[33] However, the aforementioned study utilizes an experimental setup consisting of dissected marine worms and squid, in which a central axon running the length of the organism is stimulated using external electrodes and measured in proximity to a planar NV diamond layer. A more recent piece of theoretical work reports the detection sensitivity required to detect electromagnetic fields emanating from signal neurons on a nanostructured diamond substrate^[39]. This study highlighted the detection challenges using NV based sensing. In the work reported here, an uncomplicated measurement protocol is presented that using a sensing system that recapitulates the physiological cell niche to support the growth of a functional network of neuronal cells and has the time resolution to detect coordinated changes in photoluminescence intensity associated the endogenous activity of aggregates of connected neural cells.

2.4. Growth, differentiation and acquisition of ODMR spectra on NSCs cultured on fND embedded ENFs

To demonstrate the potential of fND embedded nanofibers in biological systems NSCs were grown and differentiated into their daughter cells on the fibers. Results demonstrate cells grown for 7 days on the ENFs displayed a morphology typical of the expected differentiated cell phenotypes. The viability of the differentiated NSCs on the fND embedded ENFs, determined from the live dead assay in which the green channel is allocated to live cells and

the red channel indicating pyknotic nuclei in apoptotic cells, was 85% which is in the range previously reported for differentiated NSCs cultured on glass cover slips (**Figures 6a, 6b, 6i and 6j**). The percentage of apoptotic cells, as assessed by the numbers of pyknotic nuclei, was low with percentages of 4 % for cells cultured on the ENFs and 2.5 % for cells cultured on the glass coverslip observed (Figure 6i). Combined these results demonstrate cell viability was not compromised following culture on fND embedded ENFs.

Figure 6c-h show fluorescent images of immunostained cells cultured on glass coverslips and fND embedded ENFs. Marker expression confirms cells underwent successful differentiation into their daughter cell types (astrocytes, neurons and oligodendrocytes) when cultured on glass and ENFs. Figures 6k-m indicates similar proportions of cells were generated in differentiated NSCs on both culture substrates, suggesting no detrimental effect on NSC differentiation by the ENFs. Overall, the fND embedded ENFs provided a suitable substrate for cell growth and differentiation, with no adverse effect on the cell parameters investigated. To demonstrate the utility of the fND embedded ENFs for quantum sensing in live cell environments, cultures of neural stem cells were grown for a period of 21 days, at which point mature networks form. The presence of these active networks was confirmed via live cell staining with fluo5AM, a fluorescence based probe that exhibits higher fluorescence upon binding Ca^{2+} . Ca^{2+} imaging is widely used as a surrogate marker for neuronal electrical activity, as the signaling mechanism itself is mediated by cellular action potentials.^[40]

Figure 7a shows time lapse imaging of calcium flux observed in neuronal cultures after 21 days culture. Dual fluorescence imaging (top left panel) shows a snapshot of calcium stained neurons growing on the fND embedded fibers, further demonstrating the suitability of these substrates for maintaining viable populations of cells for extended *in vitro* culture periods. The colored heat map images in Figure 7a track the progression of calcium flux through the

cellular network over a period of 8 seconds. Inset white arrows highlight regions where the flux of calcium is most prominent for each timestamp. Figure 7b shows a population of single neurons, which can be qualitatively identified via their characteristic cell shape and dendritic outgrowths. The activity was confirmed using time-lapse imaging of intracellular calcium flux (supplementary video 1), after which ODMR spectra were obtained for single fNDs in close proximity to identified neurons in two separate regions (Figure 7bi, inset blue and yellow boxes). Figures 7biii and 7biv show regions containing fNDs in proximity to the neuron cell body and dendrites (inset yellow boxes). fNDs in 7biii are pseudo colored grey to allow ease of viewing of the fNDs that were analyzed. The ODMR spectra for the aforementioned regions are also shown. The acquisition of ODMR spectra from cellular aggregates of fND ensembles is demonstrated in Figure 7c. Here a cell aggregate was identified, the activity assessed (supplementary video 2) and the ODMR spectrum acquired as previously described.

3.0. Conclusion

A quantum sensing platform that mimics the nanoscale architecture and topography of the cell niche has been presented. This work identified and demonstrated formulations and fabrication protocols for the production of polymer ENFs with fNDs embedded along the fibers. The embedded fNDs were used to successfully acquire ODMR spectra, perform longitudinal spin relaxometry and detect external magnetic fields generated by a permanent magnet. The ENFs supported the growth and differentiation of NSCs, with viability after 7 days of culture comparable to cells cultured on control glass coverslips, and coordinated firing of neural signals across the cell network. Overall, this work has provided a new approach to NV based quantum sensing in live cells and in doing so addressed the need for high precision biosensors integrated in environments that mimic *in vivo* cellular niches. Significantly, this technique operates on a millisecond time scale and is accessible to non-

specialists due to the uncomplicated measurement protocol in which changes in the ODMR contrast with and without MWs is monitored on an epi-fluorescent microscope, an approach particularly useful for sensing with randomly oriented nanodiamonds. Furthermore, with improvements in measurement sensitivity this platform could serve a number of applications in the study of *in vitro* biological environments, namely monitoring the synchronous firing of aggregates of neurons. Finally, the establishment of biocompatible scaffolds capable of sensing temperature, pH and free radicals non-invasively during culture and tissue growth would transform many validation and regulatory aspects of cell therapies and provide a route for cell biologists to optimize culture microenvironments non-invasively in real time.

4.0. Experimental Section

Fabrication and characterization of fND embedded electrospun polymer nanofibers

Aqueous suspensions of 100 nm diameter fNDs containing a high dose of NV⁻ centers (>1000/particle) at a concentration of 1 mg/mL were purchased from FND Biotech, Inc. Solutions for electrospinning were prepared by first centrifuging 1 ml of the fND suspension at 21,000 x g for 10 minutes. Next, the supernatant was removed to produce a pellet of fNDs, which was subsequently dried under a nitrogen atmosphere to remove residual water. A range of candidate solutions for electrospinning were prepared by adding a single fND pellet to 0.33 ml solutions of absolute methanol, acetonitrile or ethyl acetate. Each solution was vortexed and added to 0.67 ml solutions of high grade chloroform (1:2 ratio). 80 mg of PLGA (Resomer® RG 505, Poly(D,L-lactide-co-glycolide, Sigma)) was then added to each of the resulting solutions. The solutions were mixed using a magnetic stirrer for 1 hour at 20 °C or overnight at 4 °C, producing a 1 ml viscous solution of milky appearance for electrospinning. Critical requirements for preparation of the final solution are use of PLGA that has not undergone degradation due to long storage times and the use of high grade solvents stored on molecular sieves to avoid the presence of water inhibiting the dissolution of PLGA. Prior to

electrospinning, the solution was sonicated for one hour to disperse the fNDs enabling uniform distribution of the fNDs in the spun fibers. A conventional electrospinning set up was used,^[20] briefly this involved loading the solution to be spun into a glass syringe that was then placed on a syringe pump mounted to a mechanical jack for height adjustment. Two electrodes were constructed, consisting of a metal needle attached to the syringe and a grounded aluminum collection plate. The syringe pump was set to extrude the solution at a rate of 0.08 ml/min. Under a sufficiently large voltage, electrostatic repulsion induced stretching of the droplets leaving the syringe, followed by the formation of a jet of polymer toward the collection plate. As the solvent component of the solution evaporates, continuous fibers of polymer are extruded and collected onto glass coverslips adhered to the aluminum electrode, generating a mat of fND embedded nanofibers. Fluorescent dye, Rhodamine-B dye (Sigma), at a concentration of 1 $\mu\text{g/ml}$ was added to each candidate solution for electrospinning (in the absence of fNDs) to enable assessment, by fluorescent microscopy, of the effect of each solvent mixture on the electrospun fibers. The fND embedded ENFs were also imaged using SEM (Hitachi S45). Here fiber coated coverslips were mounted on aluminum stubs and gold sputtered under vacuum for two minutes prior to imaging. Image analysis was performed using the open source plugin FibrilJ,^[41] to produce histograms of the diameter of fibers and calculate the average fiber diameter in each image. For cell culture work, the coverslips coated with fND embedded ENFs were sealed to 8 well bottomless chambers (Nunc, Sigma) using silicon adhesive and sterilized in a UV chamber (Biorad) for 15 minutes prior to cell seeding.

Optically detected magnetic resonance

Experiments were performed using a wide field epi-fluorescence microscope (Olympus IX83) illuminated with a mercury arc lamp. The emission from the lamp was filtered through a narrow band pass excitation filter (Chroma Technology Corporation) centered at $532 \text{ nm} \pm$

5 nm. The light was then focused onto the backfocal plane of a 1.49 NA Olympus 60x TIRF objective lens, with the fluorescence emission collected through a 633 nm long pass emission filter (Chroma Technology Corporation) and projected onto a sCMOS camera (Photometrics 95 Prime B) producing an image with a 220 μm x 220 μm field of view (FOV). MWs were generated using a Keysight N2581B vector signal generator and amplified using an AR research 20S1G4 MW amplifier. MW transmission to the fNDs was achieved through a 0.125 mm diameter copper wire positioned above the ENF coated coverslip mounted on the microscope stage. Instrumentation and acquisition settings were computer controlled using an Olympus U-RTC control box in combination with Olympus Cellsense imaging software. Continuous wave ODMR was performed by imaging the fluorescent emission from fNDs in the ENFs whilst sweeping the MW frequency between 2.77 GHz and 2.97 GHz with 2 MHz step sizes. Images were recorded for 10 consecutive frequency sweeps and the resulting data analyzed to produce an averaged ODMR spectrum, giving a temporal resolution for a full ODMR spectrum in this instance of 2 seconds. Two point single frequency measurements of the PL intensity were also performed by acquiring two consecutive images, one during the application of a 2.87 GHz MW field and the other without MWs. This measurement scheme was used to dynamically monitor the presence of an externally applied magnetic field. The averaged PL intensity of each image was determined and the difference in the PL intensity related to the magnetic field strength through the use of a calibration curve obtained using a Hall probe. The two point single frequency measurements yield a temporal resolution of 20 ms, an effective increase in temporal resolution of 100x compared with a obtaining a full ODMR spectrum. In effect, the temporal resolution of the system is limited to 2 times the maximum frame rate of the camera. The max frame rate for a full FOV in this setup is set at 100 Hz, however this can be increased to frame rates in excess of kHz if the FOV of acquisition is reduced sufficiently.

Longitudinal spin relaxometry

Longitudinal spin relaxation times (T_1) of NV centers in the fNDs were determined by measuring the PL intensity as a function of time following initialization of the spin states by a pulse of green light. Images were acquired using a custom confocal microscope built around a commercial microscope base (Nikon Ti-U). Pulsed optical excitation was achieved using a 532 nm laser (Verdi, Coherent Scientific) in combination with an acousto-optic modulator (Crystal Technologies), controlled by a PulseBlaster acquisition card (Spincore). The PL emission was filtered by a 560 nm long pass filter (Semrock) in combination with a 650 nm to 750 nm bandpass filter (Semrock) and recorded using a single photon counting avalanche photodiode (PerkinElmer), with the time-correlated photons captured with a multiple-event time digitizer card (Fast ComTech P7889). The microscope and data acquisition were controlled via custom LabVIEW code.

Neuronal cell culture materials

All cell culture reagents and cell culture grade plastics were purchased from Sigma-Aldrich (Dorset, UK) or Fisher Scientific (Loughborough, UK) unless otherwise stated. Basic fibroblast growth factor (FGF-2) was from Peprotech (Rocky Hill, NJ, USA) and epidermal growth factor (EGF) was from R&D systems Ltd (Abingdon, UK). DNase I was from Roche (Welwyn, UK). The LIVE/DEAD Viability/Cytotoxicity Assay Kit was from Fisher Scientific. Primary antibodies were rabbit anti- β -tubulin (Tuj-1) from Biolegend (Cat: 802001, London, UK), rat anti-myelin basic protein (MBP) from Serotec (Cat: MCA409S, Kidlington, UK) and rabbit anti-glial fibrillary acidic protein (GFAP) from DakoCytomation (Cat: Z033429-2, Ely, UK). Secondary antibodies were from Jackson ImmunoResearch Laboratories Ltd (Westgrove, PA, USA). Vectashield mounting medium with 4,6-diamidino-2-phenyl- indole (DAPI) was from Vector Laboratories (Peterborough, UK). The care and

use of all animals used for cell culture were in accordance with the Animals (Scientific Procedures) Act of 1986 (UK) with approval by the local ethics committee.

Neural stem cell derivation, culture and experimental conditions

Neural stem cells (NSCs) were derived from the subventricular zone of P1-3 CD10 mice as previously described.^[42,43] Briefly, after dissection, the subventricular zone was dissociated into single cells through addition of 10% DNase and mechanical dissociation. Single cells were suspended in neurosphere medium (3:1 mix DMEM:F12, 2 % B27, 25 ng ml⁻¹ EGF and FGF-2, 5 ng ml⁻¹ heparin, 50 U ml⁻¹ penicillin and 50 µg ml⁻¹ streptomycin) at 1 x 10⁵ viable cells/mL and 5 mL added to T25 flasks for propagation as neurospheres at 37 °C, 5 % CO₂. Flasks were fed every 2 to 3 days. For passaging, neurospheres were dissociated into single cells using a mixture of Accutase and DNase (9:1) and re-seeded in T25 flasks as before. For experiments, glass rectangular coverslips with or without the ENFs were placed in 60 mm petri dishes and coated prior to cell seeding by application of poly-ornithine (20 %, 1 h, 37 °C), rinsing in PBS, application of laminin (5 µg mL⁻¹, 1 h, 37°C) and then rinsing three times with PBS. Single cells (up to a maximum of passage three) were then suspended at 3 x 10⁵ viable cells/mL in monolayer medium (1:1 mix DMEM:F12, 1% N2, 25 ng ml⁻¹ EGF and FGF-2, 5 ng ml⁻¹ heparin, 50 U ml⁻¹ penicillin and 50 µg ml⁻¹ streptomycin). 3 mL of the suspension was added to each petri dish, which contained one experimental substrate. Once the cells had reached approximately 70 % confluence on the glass substrates (2 to 3 days), the monolayer medium was removed entirely and replaced with differentiation medium (neurosphere medium minus growth factors with addition of 1% foetal bovine serum). Cells were allowed to grow in differentiation medium for seven days with feeding (50% medium change) every 2 to 3 days. Here, cells were either used for the live/dead assay or fixed in 4% paraformaldehyde (20 min at room temperature) for immunocytochemistry.

Live/dead staining and immunocytochemistry

Cells were washed once with PBS. DMEM (without additional factors), containing 4 μ M calcein AM and 6 μ M ethidium homodimer, was then added to the cells. The cells were placed back in the incubator for 15 minutes before immediately being imaged *in situ*. For immunocytochemistry, fixed cells were incubated in blocking solution (5% normal donkey serum [NDS], 0.3% triton-X in PBS) for 30 minutes at room temperature. This was then replaced with appropriate primary antibody solutions which were made up in blocking solution: GFAP for astrocytes, Tuj-1 for neurons and MBP for oligodendrocytes for 16 h at 4°C. Primary antibody solution was washed off using three washes of PBS before addition of blocking solution for 30 minutes at room temperature. The appropriate secondary antibody in blocking solution was then added and incubated for 2 hours at room temperature in the dark. After incubation the samples were washed three times in PBS. Coverslips were then mounted to microscope slides using Vectashield mounting medium.

To demonstrate the utility of the fND embedded ENFs for quantum sensing in live cell environments, cultures of neural stem cells were grown for a period of 21 days, at which point mature networks were assumed to have formed. To investigate the presence of spontaneously active networks live cell staining with fluo5AM, a fluorescence based probe that exhibits higher fluorescence upon binding Ca^{2+} , was performed.

Cell imaging and analysis

Immunocytochemistry imaging was performed using a Leica (Leica Microsystems, Wetzlar, Germany) DM2500 LED fluorescence microscope. Live/dead imaging was performed on a Zeiss AxioObserver.Z (Carl Zeiss MicroImaging mbH, Goettingen, Germany). For quantification of the number of viable cells, three areas from each sample were imaged and green (live) and red (dead) cells were counted with percentage viability calculated as the percentage of green cells of the total number of cells. The proportions of cells displaying cell specific staining and percentage of pyknotic nuclei were also assessed in this way using

fixed, immunostained samples. Nuclei associated with cell specific staining were counted as positive marker expression and the percentage of positive nuclei was calculated compared to the total number of nuclei in the image. Pyknotic nuclei were identified as condensed and/or fragmented nuclei, indicative of apoptosis. Column charts are expressed as mean $\pm$ standard error of the mean. Statistical differences between the two groups were interrogated by an unpaired, two-sided t-test with a threshold of $P < 0.05$ set for any significance.

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Figures

Figure 1. Dependence of fND dispersion and ENF formation on solvent composition. a) Suspensions of fNDs in (i) chloroform, (ii) acetyl nitrile:chloroform (1:2), (iii) Ethyl Acetate:Chloroform (1:2), (iv) Methanol:Chloroform (1:2). Aggregation of polymer is visually apparent in 1a(i), 1a(ii) and 1a(iii). No aggregation is seen in 1a(iv) as indicated by the visually uniform suspension; b) Fluorescence microscopy images of Rhodamine-B labelled ENFs spun with different solvent compositions (i) acetyl nitrile:chloroform (1:2), (ii) Ethyl Acetate:Chloroform (1:2), (iii) Methanol:Chloroform (1:2).

Figure 2. Morphological characterization of fND embedded ENFs. a) Macroscopic image of whole fND embedded ENF matt, spun onto a framed coverslip with inset showing left to right fNDs and ENFs visualized with widefield DIC, fluorescence microscopy and a merged image, bottom scale bar = 2μm. b & c) Scanning electron microscopy images of control (b, scale bar = 20μm and 400nm inset) and fND embedded fibers (c, (b, scale bar = 20μm and 400nm inset), d & e) Histograms displaying the distribution of fiber diameters for control and fND embedded fibers.

Figure 3. Comparison of emission spectra and ODMR signals between drop cast fNDs and fND embedded ENFs. Widefield fluorescence imaging of drop cast fNDs (a) and fND embedded fibers (b), emission spectra for dropcast and fND embedded ENFs (c) and ODMR spectrum acquired from fND embedded ENFs.

Figure 4. Comparison of longitudinal (T_1) relaxation times between drop cast and fND embedded ENFs. (a). Histograms of relaxation times from individual regions within the intensity maps displayed in (a), Time dependent change in PL intensity for dropcast and fiber embedded fNDs.

Figure 5. Detection of external magnetic fields generated by a permanent magnet. (a) Schematic of experimental set up for obtaining magnetic field strength calibration curve and ODMR contrast, (b) Calibration curve relating magnetic field strength to ODMR contrast in fND embedded fibers. (c & d) Time resolved magnetic field detection using fND embedded fibers. Inset red arrow shows the point at which a field is applied by momentarily holding a permanent magnet in close proximity to the fibers.

Figure 7. Real time acquisition of ODMR spectra and visualization of calcium flux using voltage sensitive dyes from differentiated NSCs grown on fND embedded ENFs for 21 days. (a). Dual fluorescence images show fNDs embedded in ENFs (red) and a mixed population of neural cells stained with a fluo-5AM to visualize calcium flux (green). Heat map time lapse imaging shows the flux of calcium over a period of 8 seconds, as reported by fluo-5AM. Inset white arrows highlight areas where flux is most prominent. Single neurons were identified qualitatively (b) via characteristic cell shape and dendrite growth. Snapshots of time lapse data of calcium flux, as reported by fluo-5AM, was used to identify active cells in two separate regions (inset blue and yellow boxes). Higher magnification images are shown (expanded yellow box) showing fNDs located close to the cell body and dendrites, on which ODMR of single fNDs was subsequently performed. Larger cell aggregates (c) and ODMR of fND ensembles was performed in bulk in a region co-localised with areas with a high calcium flux (traced yellow region). Error bars for ODMR spectra in b and c represent standard deviation from the mean of 20ms exposures averaged over a 2s integration time.

A quantum sensing platform that mimics the nanoscale architecture and topography of the cell niche is presented based on the incorporation of fluorescent nanodiamonds containing Nitrogen Vacancies in electrospun Poly Lactic-co-Glycolic Acid nanofibers. The sensing platform supports the establishment of electrically active neuronal networks and measurement protocols presented enable fast detection of optically detected magnetic resonance from live cells.

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