**Electrospun diamond-silk membranes for biosensing applications**

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**Introduction**

Electrospinning generates a 2D or 3D network of fibres,[1] in the form of membranes, with high porosity and high surface area which mimics the extra cellular matrix structure - a network that holds the cells together. Polymer electrospun nanofiber dressings provide a safe method to promote wound healing with the potential to reduce wound infections. However, the human body can, in many cases, quickly reject or react poorly to most polymers used as electrospun membranes.[2] Moreover, these dressings need to be replaced every few days to check the presence of infections, which makes the wound healing process painful and uncomfortable.

We report here the fabrication and characterization of silk fibrous membranes incorporated with nanodiamonds (NDs) for temperature sensing and cell growth during wound healing process. The nitrogen vacancy (NV-) centre in diamond exhibits spin quantum properties that enable the sensitive sensing of small temperature variations.[3] Silk provides a biocompatible and biodegradable[4] network of fibres that will promote healthy cell growth at the wound site. This will result in a dual functional membrane that can track the temperature of the wound site without painful, time consuming, and expensive procedures.

**Aims**

To develop a multifunctional optical material capable of temperature sensing as well as promoting healthy cell and tissue growth at the wound cite.

**Results**

(i) Fabrication of hybrid membranes

The ND-embedded silk fibres are generated through electrospinning. NDs with an average size of 100 nm were mixed with silk aqueous solution. The mixture was filled in a syringe with a 16G stainless steel needle and subjected to high voltage of 13 kV. This produced a jet of fibres that collect as electron membranes on the collector plate. The membranes were either characterized on the substrate (silica) or lifted off for implantation at wound site.

(ii) Optical characterization

The heart of this project is the NV- centre as the quantum sensor in diamond, used to detect and monitor sensitive temperature variations inside an optical silk environment. The schematic of the experimental setup in shown in Figure 1(a). Following excitation with green laser light, the NV- in diamond-silk membranes can decay via red to near infrared fluorescence around 700 nm. A 100 µm2 fluorescence image of the silk-diamond membranes is shown in Figure 1(b). The bright fluorescence (in false color), is generated by the diamonds inside the silk fibres, while the light blue areas in the background represent the silica substrate. When the ground state spin of the centre is excited to the ms = ±1 level (using 2.87 GHz microwaves), the fluorescence output of the defect is reduced slightly, demonstrated by the dip, D, in fluorescence as shown in Figure 1(c). As the temperature changes the position of the dip changes, as indicated by the green and red plots at higher temperatures. For NV- color centres embedded in diamond-silk fibres, the change in the dip is enhanced and was observed to be 95±5 kHz/K.

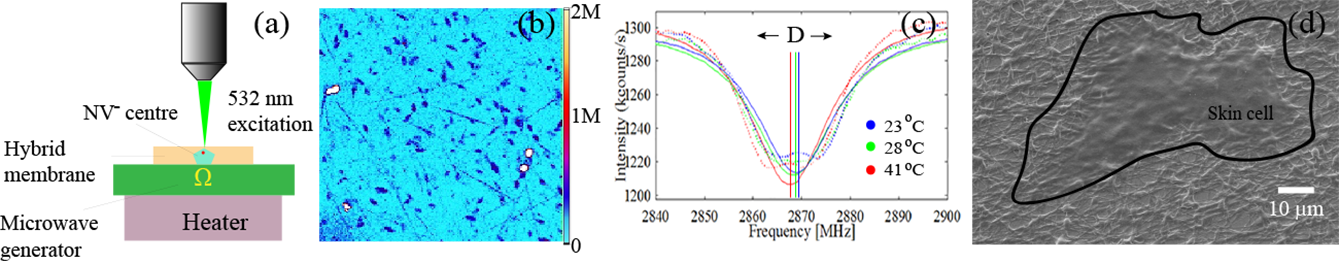


Figure 1: Temperature sensing with diamond-silk membranes (a) Schametic of the experimental equipment to observe ODMR effect for NV- centre embedded in diamond-silk memebranes. (b) A 100 µm2 fluorescence image of the silk-diamond membranes with colour bar representing the emission counts per second. (c) Shift in the ODMR dip for a particular NV- centre at three different temperatures. (d) SEM micrographs of the hybrid silk-ND hybrid fibres on their own and (b) cultured with skin cells.

(iii) Surface characterization and cell regeneration

Morphological studies by scanning electron microscopy (SEM) showed the presence of long fibers with diameters under 800 nm, spun into membranes with high porosity, as shown in Figure 1(d). NDs were encapsulated well inside the silk fibres and did not appear on the surface of the hybrid membranes. The ND-silk fibres were found to be significantly hydrophilic with a contact angle of (65±2)º. The in vitro cell viability test revealed that the silk fibres do not hinder cell attachment, nor the presence of NDs inside the fibres have any adverse effects on cell growth, as is clear from Figure 1 (d). Randomly oriented fibres forming non-woven mat structures are seen providing a porous 2D scaffold for cell growth.

(iv) Antibacterial properties

High levels of Pseudomonas aeruginosa and Escherichia coli bacterial death was observed in ND-silk membranes. The ND-silk hybrid membranes were found to be resistant to the attachment and growth of the bacteria.

Conclusions

The work reports nanodiamond-silk hybrid membranes for biosensing and cell growth in wound healing applications. Enhanced temperature sensitivity is observed for the NV- color centre embedded in the hybrid membranes, which will lead to improved sensing thermal variations associated to biological infections and inflammation. The toxicity of the membranes is tested both in vitro in skin keratinocyte (HaCaT) cells and in vivo in a live mouse wound model. The membranes did not induce any adverse effects to the cell growth and survival. Moreover, the membranes are found to be resistant to the growth and attachment of specific bacteria type. Hence these hybrid diamond-silk membranes have immense potential as multifunctional wound dressings, capable of bacterial killing, healthy cell growth and remote biosensing.

**References**

[1] D. N. Rockwood, R. C. Preda, T. Yücel, X. Wang, M. L. Lovett, D. L. Kaplan, Nature protocols 2011, 6, 10.1038/nprot.2011.379.

[2] S. Houshyar, G. S. Kumar, A. Rifai, N. Tran, R. Nayak, R. A. Shanks, R. Padhye, K. Fox, A. Bhattacharyya, Materials science & engineering. C, Materials for biological applications 2019, 100, 378.

[3] W. W.-W. Hsiao, Y. Y. Hui, P.-C. Tsai, H.-C. Chang, Accounts of Chemical Research 2016, 49, 400; A. Khalid, R. Lodin, P. Domachuk, H. Tao, J. E. Moreau, D. L. Kaplan, F. G. Omenetto, B. C. Gibson, S. Tomljenovic-Hanic, Biomedical optics express 2014, 5, 596.

[4] A. Khalid, A. N. Mitropoulos, B. Marelli, D. A. Simpson, P. A. Tran, F. G. Omenetto, S. Tomljenovic-Hanic, ACS Biomaterials Science & Engineering 2015, 1, 1104; F. G. Omenetto, D. L. Kaplan, Science (New York, N.Y.) 2010, 329, 528.