Ta_2O_5 Waveguides for Mid-infrared Evanescent Sensing of Stem Cell Differentiation

David J. Rowe[1], Nicholas D. Evans[2], Stuart J. Pearce[3], Ganapathy Senthil Murugan[1], James S. Wilkinson[1]

[1] Optoelectronics Research Centre, University of Southampton, Highfield, Southampton SO17 1BJ, UK, d.rowe@soton.ac.uk, jsw@ecs.soton.ac.uk, smg@orc.soton.ac.uk

[2] Institute for Developmental Sciences, School of Medicine, University of Southampton, Tremona Rd, Southampton SO16 6YD, UK, n.d.evans@soton.ac.uk

[3] Nano Research Group, School of Electronics and Computer Science, University of Southampton, Highfield, Southampton SO17 1BJ, UK, sp3@ecs.soton.ac.uk

Abstract: Stem cell differentiation is a fundamental biological process of particular interest for regenerative medicine. Differentiated cells have been characterised using mid-infrared FTIR microscopy but the use of spatially unresolved spectroscopy remains unexplored. Evanescent waveguide sensing may allow non-contact, label-free and real-time monitoring of differentiation. We report preliminary FTIR-ATR measurements which successfully distinguish between lysates of cells grown under different conditions and initial results on the fabrication of mid-infrared Ta_2O_5 waveguides. We also identify potential routes to a wholly self-contained analytical device.

Introduction: The vibrations of molecular bonds give characteristic absorptions in the mid-infrared (MIR) range of the electromagnetic spectrum (wavelengths of 2 to 20 μm), where the absorption frequencies correspond to vibrational resonant frequencies. MIR spectroscopy can therefore be used to identify the composition of a sample or changes thereof.

Evanescent field perturbation of an unclad optical waveguide is a stable and controllable method of performing spectral absorbance measurements. Geometric variation of the waveguide cross-section allows optimisation of field perturbation for maximal sensitivity. Ideally the waveguide should guide a single mode over the range of wavelengths of interest for unambiguous interpretation.

Silicon photonics has been able to take advantage of a wealth of fabrication techniques developed by the microelectronics industry. However, the low transmission of silicon outside the 3 - 5 μm[1] wavelength range severely restricts its use for biomedical sensing in the MIR. The dominant absorptions within this range are those of water and hydrocarbon bonds, which are ubiquitous in organic media and therefore offer limited specificity. In this work we demonstrate tantalum pentoxide to be a viable alternative.

Mid-infrared Ta_2O_5 waveguides: Ta_2O_5 films have previously been demonstrated to offer high refractive index and low loss in the MIR region.[2] Ta_2O_5 offers better biocompatibility, lower chemical reactivity and higher mechanical durability in comparison with alternative materials including Bi_2O_3, HfO_2 and the chalcogenide glasses.

The use of Ta_2O_5 to guide light restricts the choice of substrate material to the halides for suitably high transmission and low index to achieve a strong index contrast over the whole MIR region. Of the most commonly available halides (CaF_2, BaF_2, KBr, NaCl, NaF), CaF_2 was chosen for having the best mechanical rigidity and lowest reactivity, hygroscopicity and cost. Soref's expression[3] was used to choose the design of a single-mode rib waveguide of unclad Ta_2O_5 on CaF_2, resulting in rib height = 4 μm, rib width = 4 μm and slab height = 3 μm.

50 × 50 × 2 mm CaF_2 substrates were obtained from Crystran (Poole, UK) and cleaned for 15 minutes in each of acetone, IPA and DI water with an ultrasonic bath, and then cleaned for 30 minutes in a microwave-frequency oxygen plasma with a plasma asher. Ta_2O_5 thin films were then deposited on the substrates using both a Leybold Optics Helios sputtering tool for reactive sputtering and an OIPT tool for direct sputtering, using tantalum and tantalum oxide targets respectively. The chief advantage of the reactive sputtering process is in using an oxygen sensor to control the oxygen content of the plasma, which ensures the film is not depleted of oxygen and therefore does not need to be deposited

at elevated temperature or annealed. This is particularly important due to the large difference in the thermal expansion coefficients of Ta_2O_5 and CaF_2; direct sputtering cannot be used to deposit adequately oxygen-rich tantala without destroying the film integrity. The slab waveguide loss was measured as 0.88 dB/cm at $\lambda = 1550$ nm using prism coupling (Metricon). Waveguides of 1 to 10 μm width and 100 μm pitch were defined in S1813 photoresist using photolithography and etched to a depth of 1 μm using ion beam milling. The end facets were polished to achieve an optical quality surface finish. Exemplar waveguides are shown in Fig. 1.

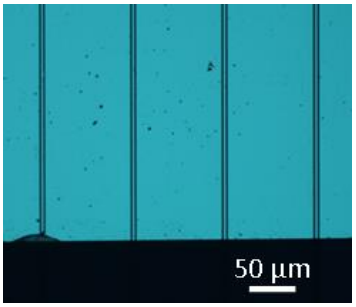

Fig. 1: Polished Ta_2O_5 waveguides on CaF_2.

The waveguides were subsequently aligned with an infrared camera using a HeNe laser and butt coupled to a tunable QCL source. However, optimisation of the polishing procedure included repeatedly mounting and demounting the waveguides on a jig with wax. This gradually introduced irremovable surface defects into the Ta_2O_5 film, which formed scattering centres and prevented waveguiding. MIR characterisation of higher quality Ta_2O_5 waveguides is in progress.

Stem cell differentiation: Cells were used from M57 and M67 human mesenchymal cell lines. Two populations were grown from each line for three weeks: M67 cells were grown in basal and osteogenic media (TLBM and OGM) and M57 cells were grown in basal and adiopogenic media (BM and AM). M67 TLBM and M57 BM remained undifferentiated. M67 OGM differentiated to mineral rich bone-type cells and M57 AM differentiated to fatty cells as expected. Each culture was visually verified with a microscope. The cells were then removed from their respective growth media and resuspended in lysis buffer.

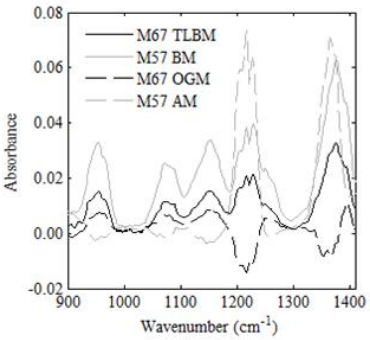

Fig.2: 900–1400 cm^{-1} region of lysate absorbance spectra.

An Agilent FTIR spectrometer was used in conjunction with a ZnSe ATR trough plate for evanescent spectral absorbance measurements. Each cell lysate-lysis buffer mixture was pipetted onto the ATR plate and absorbance spectra recorded in the frequency range 600 – 6000 cm^{-1}. Each measurement was taken relative to pure lysis buffer so the result is effectively that of the lysed cells only. The changes between M67 OGM and M67 TLBM are markedly different from the changes between M57 AM and M57 BM at many points throughout the spectrum. As an example, one region of interest is the peak at 1220 cm^{-1} ($\lambda = 8.2$ μm) shown in Fig. 2. Such differences are probably due to differences in the extracellular matrices from the different growth conditions.

Conclusions: MIR Ta_2O_5 waveguides can be directly integrated with a polymer microfluidic interface, based on previous work[4] and shown in Fig. 3, for continuous flow characterisation. Additional microfluidic functionality for cell growth and lysis[5] should be integrated to allow on-chip stem cell manipulation. The preliminary data on evanescent spectroscopy of stem cell lysates show that the morphology of cells and extracellular matrices does not necessarily need to be known for continuous, non-contact, label-free and real-time monitoring of differentiation. These data also show promise for Ta_2O_5-based integrated MIR optics as a generic biosensing platform and specifically for characterising stem cell differentiation.

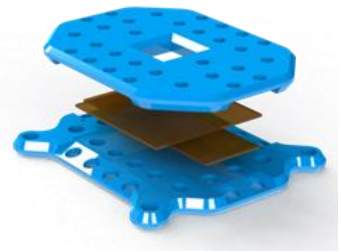

Fig.3: Compression-seal microfluidic manifold.

References

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