Gel-based optical waveguides with live cell encapsulation and integrated microfluidics

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In this Letter, we demonstrate a biocompatible microscale optical device fabricated from agarose hydrogel that allows for encapsulation of cells inside an optical waveguide. This allows for better interaction between the light in the waveguide and biology, since it can interact with the direct optical mode rather than the evanescent field. We characterize the optical properties of the waveguide and further incorporate a microfluidic channel over the optical structure, thus developing an integrated optofluidic system fabricated entirely from agarose gel. © 2012 Optical Society of America

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On-chip optical methods for biological applications, varying from biosensing [1–3] to optical trapping and manipulation [4,5], are becoming increasingly prevalent. A majority of such optical devices are fabricated out of rigid materials like silicon or silicon nitride, which do not allow for biomolecular access to the energy rich core field of the optical waveguide. A related area that has attracted increased attention of researchers has been in developing optical devices using hydrogels [6–9]. Hydrogels are attractive because they are biocompatible and can be designed to respond to, or sense, different external stimuli like pH [10,11], temperature [11,12] and light [13]. However, the harsh methods used in fabrication of hydrogel waveguides—for example, femtosecond lasers [6] or UV irradiation [8]—are incompatible with embedding biological material inside the waveguides and are therefore also dependent on the weak evanescent near field for their operation. Here we demonstrate a hydrogel waveguide, fabricated of agarose, that allows for incorporation of biomolecules and cells inside the core of the waveguide.

Agarose, a polysaccharide derived from agar, is a popular material for constructing biocompatible gels for the purpose of DNA separation through gel electrophoresis. Agarose hydrogel is known to be structurally strong enough to be molded into microscale structures [14,15] while providing the additional ability to encapsulate live cells in the gel [14,16]. On measurement of the refractive index of the agarose hydrogel, we found that it varied appreciably with the concentration of agarose in it. A change of concentration by 0.5% (w/v) yields a change of around 0.001 in refractive index—a large enough change for effectively guiding light in an optical waveguide.

In our device, both the substrate and core of the optical waveguide were fabricated from hydrogels made from high strength agarose (USB Agarose High Gel Strength). To fabricate the substrate, a gel solution was prepared by adding powdered agarose slowly to a heated solution of distilled water, until the agarose melted and a clear solution containing 1.5% (w/v) agarose was obtained. The solution was then kept in a water bath at 80 °C for 45 minutes to allow any air bubbles to diffuse out of the solution. The solution was finally poured into small Petri dishes and allowed to cool until a smooth gel surface was obtained. Gel optical waveguides were then created through a soft lithography process using poly (dimethylsiloxane) (PDMS) (Ellsworth Adhesives) stamps [14,15]. An SU-8 master was fabricated on a silicon wafer using standard photolithography techniques and was used to mold the PDMS stamps (a negative replica). The PDMS stamp was exposed to oxygen plasma in a vacuum chamber in order to render it hydrophilic and remove formation of any air pockets or bubbles during fabrication [15]. A 2% (w/v) agarose solution was similarly prepared and a small amount of solution (~500 μl) was carefully pipetted out and then slowly poured on the substrate. The stamp was then slowly pressed upon the hot agarose solution and left to cool in the air for 20 minutes under a small weight (200 g), to allow the solution to gel into the required features. The PDMS stamp was then carefully and easily stripped off from the agarose substrate, yielding the structure shown in Fig. 1(a). The waveguides thus fabricated were designed to be multimoded rib waveguides with cross-sectional dimensions of 130 μm× 130 μm.

The refractive indices of the two different concentration gels—1.5% (substrate) and 2% (core)—were separately measured to be 1.3343 and 1.3357 using a digital refractometer (Sper Scientific). In order to test the gel waveguides, coupling experiments were carried out using a 633 nm (red) laser source coupled into the waveguides using a multimode fiber (Thor Labs AFS105/125Y). Alignment between the fiber and the gel waveguides was facilitated through the use of “fiber guides” designed and fabricated along with the waveguides themselves, removing the need to “cut” the waveguides. The gel waveguides were found to couple light easily, as shown in Fig. 1(b).

An important consideration for optical propagation is the scattering/absorption losses suffered by the light propagating in the waveguide. We quantified these losses in our waveguides through image analysis using ImageJ, an open source image analysis tool. Briefly, we measured the intensity of the scattered light, as seen from an overhead image, along the length of the waveguide. Care was taken to avoid saturation of pixels and to account for any background illumination. Assuming that the scattered intensity is proportional to the intensity of light

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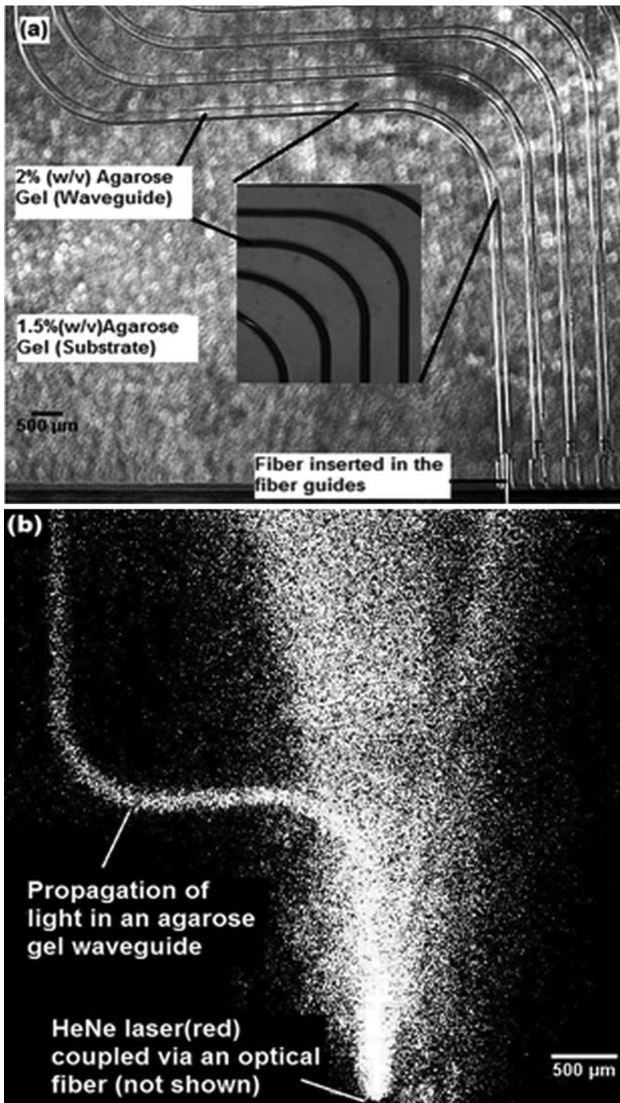

Fig. 1. (a) Agarose gel waveguide with 2% (w/v) gel as core and 1.5%(w/v) gel as substrate (Inset: Magnified view). (b) Gel waveguides with He—Ne laser coupled into the waveguide.

propagating in the waveguides, the intensity map so obtained [and shown in Fig. 2(a)] was plotted on a semi log map in order to determine the loss in dB per unit length. Figure 2(b) shows the measurements done across different waveguides on different chips, and the resultant grouping of loss measurements demonstrates the repeatability of the fabrication process. We obtained an average loss of 13 dB/cm. As comparison, [17] reported losses of 5–10 dB/cm for alginate hydrogels and 2–7 db/cm for synthetic hydrogels for 1.1 mm diameter waveguides. Even though losses could potentially be lowered by using higher concentrations of gel and a better index contrast, it would be accompanied by an increase in stiffness of the gel and a decrease in the pore size of the gel. This would move it away from the stiffness found in actual physiological environments, and the smaller pore size would inhibit the motion of biomolecules, nutrients, etc., through the gel. Additionally, a small refractive index change by increasing the concentration (~0.001) would affect the scattering losses only minimally due to its weak dependence on refractive index [6].

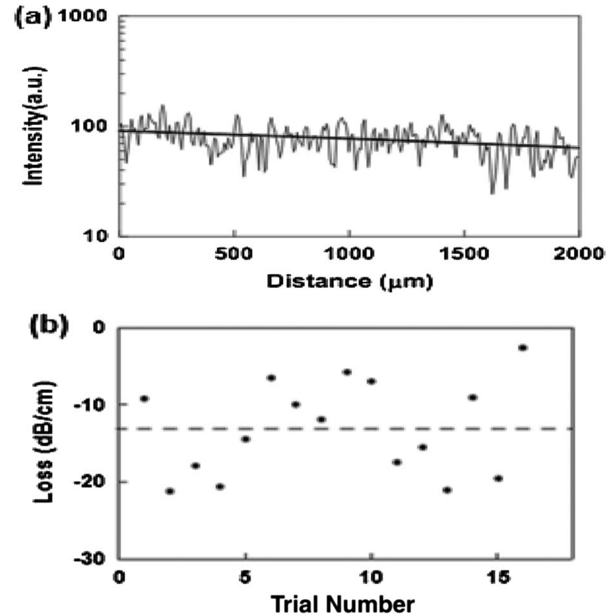

Fig. 2. (a) Intensity variation along a typical gel waveguide for estimation of scattering/absorption losses. (b) Scattering/absorption losses measured across different waveguides. Dotted line indicates average measured loss.

For integrated on-chip bio applications, it would be ideal to incorporate a fluidic channel on the optical chip, thus allowing for flow of analytes or media over the optical elements. Though microfluidic channels using agarose hydrogel have been reported [14,18], those devices consisted of featureless surfaces which were later bonded together. However, adding a microfluidic layer on top of our soft waveguide led to the collapse of the waveguide at the two ends where the walls of the microfluidic channel rested upon the waveguides. In order to overcome this obstacle, we opted for a novel "open-end" microfluidic channel design. As shown in the schematic in Fig. 3(a), both the ends of the microchannel were cut open, after which it was aligned, along its length, with the optical waveguide, so that no part of the microfluidic layer came in direct contact with the optical waveguides. The channels were then sealed, both along the sides and at the open ends, using a low melt agarose solution (USB Agarose–Low Melt) at around 40 °C, which gelled to form a tight sealing. Steel tubing (Small Parts) was used to provide access ports for pumping fluids in through the channel. Figure 3(b) shows the flow experiments performed using blue food color, and demonstrates the efficacy of the fabricated fluidic channel.

In order to demonstrate the biocompatibility of our fabrication process, we embedded live cells in the agarose waveguides. We used the metastatic breast cancer cell line MDA-MB231 for our experiments. The cells subcultured in Dulbecco's modified Eagle's medium (Invitrogen #11965-092) supplemented with 10% horse serum were fed every second day and passaged every three days at a subculture ratio of 2:5. The substrate (1.5% w/v agarose gel) and plasma treated PDMS stamps were fabricated as described earlier. A 4% (w/v) agarose solution was prepared in a similar fashion, and maintained at 70 °C in a water bath for 60 minutes to dissipate

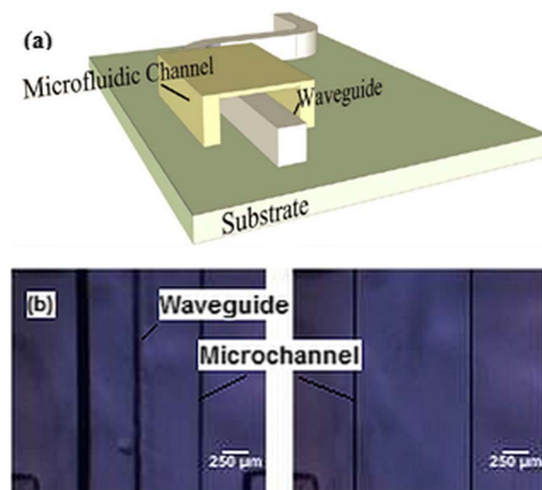

Fig. 3. (Color online) Schematic of an open end microfluidic design (not to scale). Open ends are later sealed with low melt agarose. (b) Flow inside the microchannel: After sealing the channel, a gel waveguide is imaged before (left) and after (right) flowing blue food color through the channel.

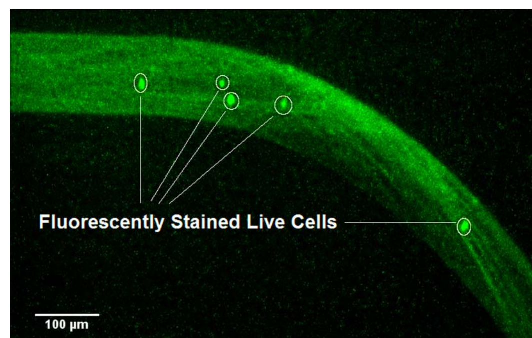

Fig. 4. (Color online) Gel waveguide, with calcein stained cells embedded within the core, coupled to an Argon ion laser (488 nm) and imaged under a fluorescent microscope. Live cells have been encircled for clarity. Supplementary video 1 depicts the movement of one such cell confirming its viability (Media 1).

any air bubbles. A suspension of cells in the subculture media was then mixed with an equal volume of the prepared agarose solution to yield a 2% agarose solution with suspended cells. The fabrication of the waveguides was carried out using the PDMS stamps, as detailed before. The above steps were carried inside a biosafety hood to maintain sterile conditions. The optical waveguides, now embedded with cells, were left under cell media at 37 °C in a 95% air/5% CO_2 incubator for 24 hours. Cell viability was then assessed by calcein AM live cell stain. A 488 nm Argon laser was coupled into the waveguides to excite the calcein stain and was observed under a florescence microscope. As can be seen from Fig. 4, the subsequent image confirms the embedded cells to be alive. We note that the background signal seen from the agarose is due to the scattering of the propagating light. Preliminary measurements indicated that the propagation losses for the cell embedded waveguides were of the same order as before (~12–13 dB/cm). Cell viability was separately confirmed by observing an embedded cell over a period of 12 hours, after incubation within the gel waveguide for 24 hours. Supplementary Video 1 shows the cell in constant movement over the observation period. We believe that the movement is caused by the extension of multiple pseudopodia by the cell in an attempt to attach to the gel matrix. But the absence of adhesive proteins in agarose prevents the cell from actually adhering to the matrix, which could be alleviated by adding adhesive gels, like gelatin [19], to the agarose solution.

In conclusion, we have demonstrated a gel optical platform capable of live cell encapsulation within the core of the optical structures. We have further incorporated microfluidic channel over the waveguides to allow for integrated opto-fluidic operation. We believe that this platform can be extended to perform force experiments on cells, akin to those performed typically by optical tweezers, in a 3D extracellular matrix (ECM)–like environment offered by agarose gel. Additionally, these can also be used for simultaneously sensing any changes to the cell as physical and chemical cues are provided to it. We also found the process to be compatible with DNA encapsulation (results not shown), and we believe it should further allow for a wide variety of biological entities, like proteins, to be incorporated inside optical waveguides.

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