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Dielectrophoretic fabrication of electrically anisotropic hydrogels with bio-functionalised silver nanowires

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We have developed a device based on a microwire network formed from bio-functionalised silver nanowires (AgNWs) through dielectrophoresis (DEP) and hydrogel entrapment. This was achieved by carrying out the DEP assembly of AgNWs in an agarose aqueous solution above its gelling temperature and then cooling to encapsulate the assembled structure within the hydrogel which turns it into an electrically anisotropic material that contains up to 99% water. We have studied in detail the formation of microwires assembled from silver nanowires (AgNWs) in agarose gel, at fixed temperature and AC field voltage, which allowed us to build a "phase diagram" of the microwire assembly as a function of the agarose and AgNW concentration at three different lengths of the AgNWs. In this proof-of-concept study, the AgNWs were functionalised with thiolated biotin, then assembled into microwires by DEP and entrapped into a hydrogel. When exposed to a solution of streptavidin the biosensor device showed a marked increase of the cell conductivity due to compacting of the AgNWs which allows streptavidin detection. This generic type of biosensing device could find applications for detection of other biomolecules, the response to which can be directly converted to an electric signal. Conjugating specific antibodies on the AgNW surface would allow the detection of a matching antigen (*e.g.* protein) in the solution exposed to the anisotropic hydrogel.

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Introduction

Electrically anisotropic materials have been explored for applications in conducting adhesives,[1,2] infusion testing,[3] electronics[2,4] and touch responsive screens.[5] One way to make such materials relies on the controlled assembly of metallic and other conductive nanowires by using optical methods,[6] magnetic fields[7–9] and surface tension forces[10] or hydrodynamic forces.[4] Recent advances in this area also include using dielectrophoretic forces[11] which have been employed to assemble conducting nanowires at electrodes without the need for electrostatic charges or chemical bonding of the assembled particles. The assembly of nanowires by dielectrophoresis (DEP) is an efficient process that can be applied to a number of materials like metals and semiconductors.[10] A range of materials have been assembled in more complex structures by DEP which includes AuNPs,[12–16] AgNWs,[17] SiNWs,[18] CNTs[19–21] and other colloids.[22,23] The DEP assembly of dispersed nanoparticles in aqueous media results from the polarisation of the electrical double layers of the particles in the AC field which align in long chain-like structures that resemble microwires. Often these microwires have a dendrite appearance.[5] Although DEP assembly of colloidal objects in wires is quite a robust process, it suffers a serious drawback that the assembled structures are dispersed after the electric field is switched off[24] due to the Brownian motion of the colloid particles and poor adhesion to the substrate. Various approaches have been suggested to resolve this problem, and make the DEP assembly irreversible, which includes change of solvent,[17] or chemical functionalisation of the metallic nanowires and the substrate.[25]

Recently we reported a novel method to "arrest" the structures formed through DEP assembly, which requires no chemical functionalisation of either the building blocks or the substrate.[26] We achieved this by performing the DEP assembly in agarose solution above its gelling temperature, and then cooling the solution to room temperature in the presence of the AC field. This results in the gel setting around the assembled structures, physically preventing them from breaking apart under gravity or through Brownian motion. We demonstrated this technique in a proof-of-concept study by assembling microwires from AgNWs, with diameters from 50 to 200 nm and lengths of 40–50 μm, followed by entrapping them in an agarose gel. The produced hydrogel was electrically anisotropic due to the formation of an orientated network of silver microwires with resistance ratio across the normal-to-lateral direction of three orders of magnitude.

In the present follow-on paper, we have studied in more detail the role of the agarose concentration in the solution and the concentration of dispersed AgNWs in the successful formation of microwires using the same methodology. The

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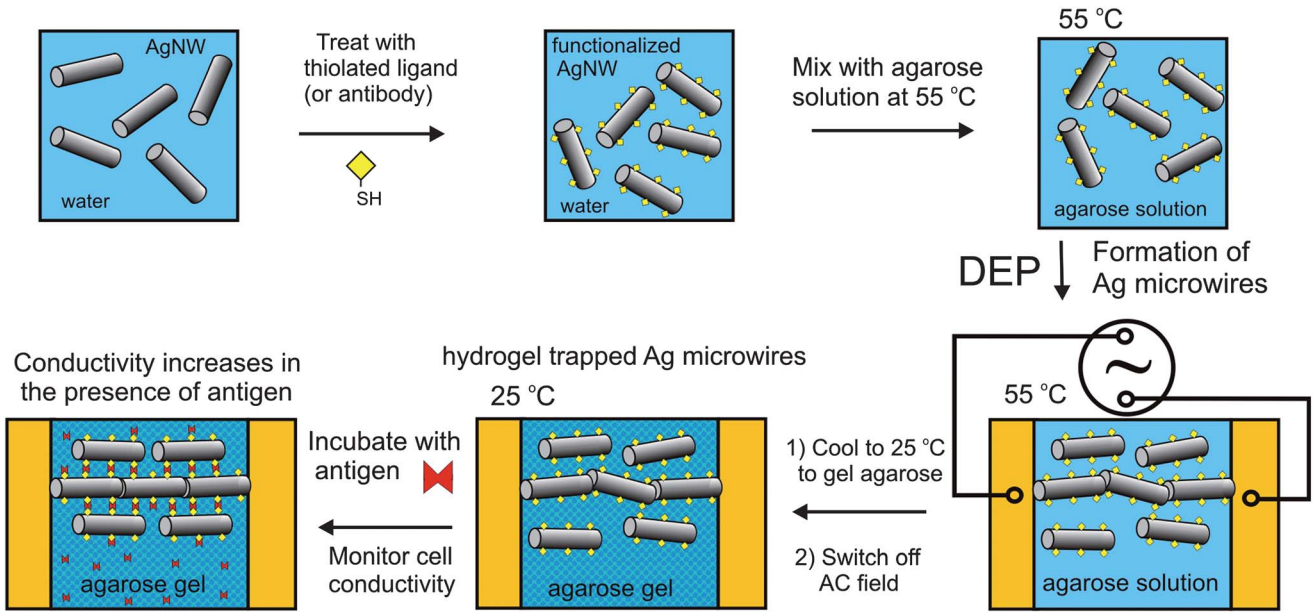

Fig. 1 Schematic of the preparation of anisotropic agarose gels by dielectrophoretic assembly and encapsulation of AgNWs. In our experiments we did a proof-of-concept study by functionalising the AgNWs with thiolated biotin, and after assembling them in microwires by DEP and trapping them in agarose gel we exposed the biosensor to streptavidin solution. The tighter packing of the AgNWs into microwires as a result of the binding of streptavidin to the biotin sites on the NW surface led to an increased conductivity of the assembled microwires due to closer contact of the NW and the suppression of their internal Brownian motion.

results allowed us to produce a "phase diagram" of AgNWs and agarose concentrations for Ag microwire formation. We also took this method further by developing a generic biosensing platform based on hydrogel entrapped microwires of functionalised AgNWs, which can detect the presence of specific bioligands through changes of the microwire conductivity.

The latter is based on induced binding among the AgNWs within the microwire. Fig. 1 illustrates the concept of this biosensor which we explored by functionalising the AgNWs with biotin. We assembled the biosensor microwire-device by using the DEP technique in agarose solution above the gelling point and then gelled the agarose at room temperature to trap the formed structures. When we exposed this biosensor to streptavidin solution, it interlocked the AgNWs further through binding with the covalently attached biotin group which produced a marked increase in the conductivity of the microwires due to additional compacting of their building blocks and further short-circuiting of the AgNWs within the formed microwires. We tested the response of the device against a control aqueous solution of the same salinity in the absence of streptavidin. Similar biosensors can be constructed with a variety of antibodies functionalising the surface of the AgNWs and providing electric conductivity response to the presence of specific antigens (*e.g.* proteins).

Experimental

Materials

Agarose (D5, Hispanagar) solutions were prepared with Milli-Q water by stirring on a hot plate at 90 °C. Silver nanowires (PlasmaChem GmbH, three samples of 50, 100 and 200 nm in diameter and 40–50 μm in length) were dispersed in the hot solution of agarose using high power sonication for 3 min, with a hot water bath at temperature above 50 °C. The hot AgNW dispersion was then degassed and kept in an ultrasonic bath for several seconds before transferring to a hot plate to avoid gelling of the agarose. The cell was built with four copper electrodes (approximately 0.22 mm thick, 20 mm long and 10 mm wide) spaced 4 mm apart and adhered to the glass substrate. Biotinyl tetra(ethylene glycol)undecane thiol (99%) was purchased from Asemblon, Inc. (USA).

Dielectrophoretic assembly of AgNWs in an agarose solution

The DEP assembly cell consisted of a glass microscope slide, to which four copper electrodes were adhered. To study the electrical anisotropy of the formed hydrogels, the electrodes were positioned on the four sides of the cell as described in our previous work.[26] These four electrodes have been labelled A–A and B–B, with the pairs placed opposite and at 90° to each other. As noted in our communication,[26] where the four electrodes (A–A and B–B) were spaced equally from each other, it was found that as well as microwire formation between the oppositely positioned electrodes A–A, occasional assemblies between the electrodes A and B were also observed. This supplementary assembly was considered undesirable, so to prevent it from happening, the electrodes A–A were positioned closer together. The electrodes were thus not equally spaced, with the A–A spacing being shorter than the B–B and A–B spacing. First, we dispersed the AgNWs in a hot agarose solution using ultrasound. After degassing in an ultrasonic bath, the dispersion was quickly transferred to a hot plate where it was kept warm to prevent the sample from gelling. The experimental cell,

specially constructed for DEP, was placed on a heating stage, and warmed to 55 °C. Several drops of the hot dispersion were then placed on the cell between the electrodes, and covered with a warmed glass cover slip. The DEP cell was enclosed in a custom made plastic box to avoid accidental contact with the electrodes during the observations. An AC field was then applied between the two electrodes, resulting in the DEP assembly of the AgNWs into a network of microwires. The AC field was produced using an Agilent 33220A Waveform Generator, which was in turn connected to a Trek amplifier (model 601C).

The DEP assembly of the AgNWs into microwires is a fairly fast process and occurs within a few seconds of application of the AC field. Our observations showed that after the initial few seconds of microwires growth, no further changes were observed, probably due to the depletion of AgNWs from the solution. Therefore we adopted a protocol of looking at the formation of the microwires within one minute of the incremental increase of the AC electric field strength (by 50 V cm^{-1} peak-to-peak increments). Once the microwires were formed (within a minute from the incremental increase of the field strength), the temperature of the DEP cell was lowered from 55 °C to 25 °C (by the microscope heating stage) within 5 minutes to trap the formed microwires by gelling the agarose solution. The temperature was monitored by a non-contact laser thermometer. At this point, the electric field was switched off without the organised structures disassembling due to Brownian motion.

Results and discussion

Phase diagram of the dielectrophoretic assembly of AgNWs in agarose gel

The dielectrophoretic assembly of AgNWs in agarose gel depends on a number of parameters. These are the temperature of the cell, the concentrations of agarose and AgNWs, the diameter of the NWs, AC field frequency and strength, and the length of time that the field is applied for. Here, we have kept the solution temperature at which DEP takes place constant at 55 °C, and used a constant field frequency of 5 kHz. However, the field strengths that were required for DEP assembly were found by varying the voltage, starting at an AC field strength of 50 V cm^{-1} and then rising incrementally in steps of 50 V cm^{-1}. At each voltage change the sample was studied visually, to assess whether any assembly had taken place, for around one minute before raising the voltage again. When the assembly of a microwire between the electrodes was observed, the field was lowered to 50 V cm^{-1} while the sample was cooled. This field strength proved strong enough to maintain the microwire and prevent it from undergoing disassembly while the solution was cooled. For each of the AgNW samples with different diameters (50, 100 and 200 nm), we studied their dielectrophoretic assembly at a range of AgNWs and agarose concentrations. By studying each diameter of the AgNWs, at concentrations ranging from 0.10 to 1.00% w/v, in agarose gel ranging in concentration from 0.50 to 1.25% w/v, we have been able to construct a "phase diagram" for the DEP assembly of AgNWs in agarose solution (Fig. 2). The lowest concentration of agarose that was studied was 0.50% w/v, as lower concentrations did not yield gels with sufficient mechanical strength to prevent the formed microwires from undergoing disassembly when the field was removed. The observations of the assemblies formed by DEP have been split into three types, shown as different coloured circles in the phase diagram. For some systems, a microwire assembled between the electrodes at a given field strength (shown as a full black circle), while other systems showed partial assembly of an incomplete microwire (shown as a broken black circle). However for some systems, no assembly was observed even at very high, up to 1250 V cm^{-1}, field strengths (shown as a full red circle).

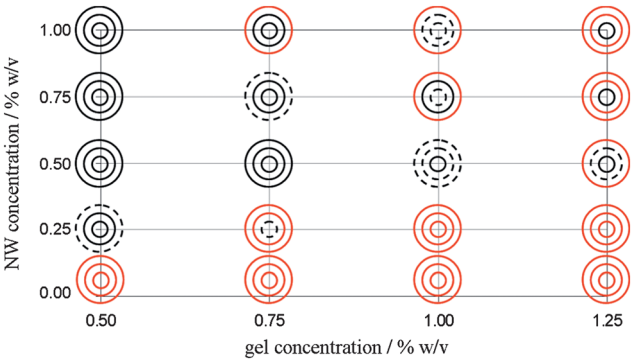

Fig. 2 Phase diagram for the assembly of AgNWs in agarose solution at varying field strengths. Small, medium and large circles represent the 50 nm, 100 nm and 200 nm AgNWs respectively. Full black lines represent assembly between the electrodes, dashed black lines represent partial assembly, and red lines represent no assembly.

Our observations are in line with those of other authors[5] who have grown microwires of gold nanoparticles and other nanomaterials in aqueous solution by DEP. Upon application of the AC field, we observe a growth of individual microwires which starts from one of the electrodes and continues by "adding" new nanoparticles from the solution to the growing tip of the microwire. The process is preceded by increasing the local concentration of the nanoparticles in a close vicinity of the microwire end since the AC field gradient there is the highest. However, one can observe this around the electrodes only at a much lower particle concentration which does not lead to microwire formation.

The phase diagram shows that much of the observed DEP assembly of microwires, both complete and incomplete, generally occurs at intermediate concentrations of the agarose and NWs. These concentrations are approximately 0.50–1.00% w/v agarose and 0.50–1.00% w/v AgNWs, though not all combinations of these concentrations resulted in assembly, as shown by this region being approximately triangular in shape on the phase diagram. At low concentrations of AgNWs (≤0.25% w/v), assembly of a microwire is observed only at low concentrations of the agarose (0.50% w/v). Lowering the AgNW concentration to 0.10% w/v or increasing the agarose concentration above 0.50% w/v results in no assembly being observed. This was attributed to the nanowires not being in sufficient number to reach the percolation threshold under the

dielectrophoretic force and form a microwire, particularly when the concentration and viscosity of the agarose medium is increased.

We can see from the phase diagram that for each concentration of NWs (≥0.50% w/v), as the concentration of the agarose is increased, we observe less assembly. Similarly, for each concentration of the agarose (≥0.75% w/v), as the NW concentration is increased we also start to observe less assembly. The combination of these two observations gives rise to the triangular region of predominantly red circles towards the upper right hand side of the phase diagram, indicating less assembly at high concentrations of the AgNWs and agarose. The observed fall in assembly when the agarose concentration is increased can be attributed to a rise in the viscosity of the medium, preventing the AgNWs from migrating through the solution to the regions of high field strength, even when large fields are applied. When the concentration of AgNWs is increased, the observed fall in assembly may also be attributed to an increase in the viscosity, with several groups reporting the increased effective viscosity of suspensions of rod-like particles.[27,28] The increased concentration of AgNWs might however have another, competing effect on the system – their increased number might favour assembly of a microwire due to closer proximity of the AgNWs. In this case though, it would seem that the effective viscosity increase is the more dominant of the two effects.

These observations have thus far described the general trends in the assembly of AgNWs in agarose solution, regardless of the nanowire diameter. Here we will focus on the effects that AgNW diameter have on the assembly, with regard to the phase diagram in Fig. 2. The results suggest that the assembly of the NWs with the smallest diameter, 50 nm, is more widespread and less affected by AgNWs and agarose concentrations than the larger AgNWs. For example, even at an agarose concentration of 1.25% w/v and a AgNW concentration of 1.00% w/v, we can see that a complete microwire was formed with the 50 nm AgNWs, but not for the larger diameter AgNWs. By fixing the concentration either of the agarose or the AgNWs and studying the assembly when the concentration of the other is increased, we can see that this is a common trend. For example, at a fixed AgNW concentration of 1.00% w/v (see the phase diagram) and agarose concentration of 0.50% w/v, full assembly is observed for each NW diameter. At 0.75% w/v agarose, no assembly was observed for the 200 nm AgNWs, while at 1.00% w/v agarose, only partial assembly was observed for the 50 nm and 100 nm AgNWs. Then at 1.25% w/v agarose, the 50 nm NWs assembled but no assembly was observed for the 100 nm and 200 nm AgNWs.

It seems therefore that when considering the prevention of assembly due to a likely viscosity increase caused by either (or both) an increase in gel or AgNW concentration, the larger NWs are most affected, while the smaller NWs are least affected. This might be explained by considering the properties of the system that might be affected by the diameter of the NWs, such as the number of nanowires at a given concentration and their mass. When considering the three samples with different diameter NWs but at the same concentration, the sample with the NW of 50 nm diameter would contain 4 and 16 times as many NWs as the sample with 100 nm and 200 nm diameter NWs, respectively. So if one of the factors affecting the DEP assembly of the nanowires is their number, and perhaps proximity to each other, then this might partly explain the observed assembly of the smaller NWs when larger NWs will not assemble, at the same NW and agarose concentrations. Larger diameter NWs also have larger volumes, and therefore masses, than those of the smaller diameter NWs. This may increase the sedimentation rate of the larger NWs, resulting in their removal from the area where the dielectrophoretic assembly is taking place.

The phase diagram shows that the DEP assembly of AgNWs in agarose solution seems therefore to be affected by a number of competing/contributing factors. These factors are believed to include the viscosity of the system caused by the agarose and the AgNWs concentration, the number of individual nanowires present in the sample. The results also indicate that the sedimentation of AgNWs from the sample might play a role in the formation of microwires by DEP. How these different factors might affect the assembly of the microwires to varying extents is reflected in the voltages required for microwire assembly. These ranged from 150 to 700 V cm^{-1}, though there appeared to be no apparent trend, for any diameter AgNWs, of the assembly voltage with either increasing AgNWs or agarose concentration in the system.

Optical microscopy of dielectrophoretically assembled Ag microwires

We studied in more detail the microwires formed through DEP, considering the assemblies formed at agarose and NW concentrations of 0.50% w/v. Fig. 3 shows optical microscopy images of the microwires assembled with 50 nm, 100 nm and 200 nm AgNWs.

The 50 nm diameter AgNWs assembled to form a single microwire between the electrodes, though significant branching was observed. This gives the microwire assembly the appearance of a 'web' (Fig. 3A), with no central wire present – just an interconnecting network of microwires bridging the inter-electrode gap. The branches appear to stem from large aggregates of NWs, which can be seen in the microscopy images as dense black areas along the microwire. It was not possible to measure the diameter of the microwire accurately, particularly as some regions of the wire appeared to comprise of aggregates of NWs and the wire was therefore not uniform in size. However, visual observations of some sections of the microwires suggested that they were extremely thin in some places. Fig. 3B shows optical microscopy images of the 100 nm diameter AgNWs, assembled to form a microwire. In Fig. 3B, a single microwire between the two electrodes is shown, with this microwire clearly thicker in diameter than that formed by the 50 nm NWs. This microwire does not appear to be branched, however, consisting of one single microwire despite the apparent presence of some aggregates of NWs at various points along the assembled wire. However, as we have shown before, several incomplete microwires may assemble between the two electrodes, some of which are connected to one of the electrodes and some of which are 'free-standing' in the space between the electrodes. Optical

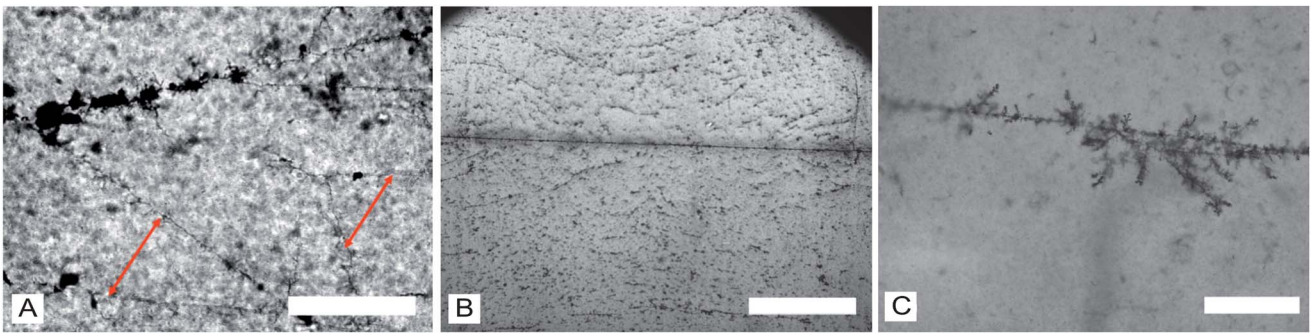

Fig. 3 Optical microscopy images of DEP assembled silver microwires from AgNWs at a concentration of 0.50% w/v in 0.50% w/v agarose gel. (A) DEP assembled microwires from AgNWs of diameter 50 nm. Red arrows highlight a network of very thin microwires. Scale bar 100 μm. (B) Similar microwires DEP assembled from AgNWs of diameter 100 nm. Scale bars represent 1 mm. (C) DEP assembled microwires from AgNWs of diameter 200 nm. Scale bar is 200 μm.

microscopy images of the 200 nm diameter AgNWs are shown in Fig. 3C. The microwire formed by the 200 nm diameter AgNWs appeared to have characteristics somewhere between those formed by the 50 and 100 nm NWs. The wire comprised of a single bridging microwire, similar to that observed for the 100 nm NWs, but a degree of branching was also observed similar to that seen for the 50 nm NWs. The branches, however, did not interconnect to form a 'web' of microwires, instead the branches were quite short, not extending more than 300 μm from the main wire. There is a simple explanation for this lack of trend in this case. It seems that the 200 nm AgNWs are less aggregated in the solution compared to 100 nm AgNWs. We used the same solution and dispersion protocol to disperse the AgNW samples but obviously we have not achieved complete redispersion to individual NWs for the 100 nm AgNWs. This could be due to variation in the surface chemistry of the different batches of AgNWs, data for which are not available from the manufacturer. Therefore more aggregated 100 nm AgNWs behave like they have lower mobility than 200 nm AgNWs.

Electrical properties of DEP assembled microwires and gels

The electrical properties of the gels and assembled microwires were characterised using the electrodes positioned in the cell (labelled A–A and B–B). The importance of having these electrodes in the solution before the assembly took place was illustrated by attempts to remove the gel from the cell, which resulted in rupture of the assembled microwire. This also provided us with a very easy way of monitoring the integrity, or structure, of the microwires, by measuring their electrical properties over time. With the formation of a microwire between the two electrodes, we expected to see a drop in the resistance through the gel – if the microwire then broke or became disrupted in any way, then the resistance would be expected to rise again. This method of monitoring the integrity of the structure of the wire is far more sensitive than simple visual evaluation through optical microscopy.

The resistance of the hydrogel without any added AgNWs was of the order of 10^6 Ω cm^{-1}, and even with the addition of AgNWs, if no dielectrophoretic assembly was performed the resistance remained in this order of magnitude. If dielectrophoretic assembly was performed however, resulting in a microwire being formed between the electrodes A–A, then the resistance of the gel/microwire measured between these electrodes was of the order of 10^2 to 10^3 Ω cm^{-1}. This represents a lowering of the resistance by three to four orders of magnitude, compared to the resistance of the same gel measured through the other couple of electrodes in the sample at 90° (the electrodes B–B). Resistances for the samples with 0.50% w/v agarose gel and 0.50% w/v AgNWs are summarised in Table 1.

As the resistances in Table 1 show, the formation of a microwire between the electrodes A–A has effectively given the hydrogel an anisotropic electrical character, with the resistance of the material lower when measured in this direction than it is when measured at 90° of the DEP electrode pair. Interestingly, we did not find any trend in the resistance of the microwire/gel through the electrodes A–A on the diameter of the NWs. The lowest resistances were measured for microwires obtained with 100 nm AgNWs, which from optical microscopy are believed to be thicker, straighter and less branched than those formed from the 50 nm and 200 nm NWs. We might expect that if the microwire is formed from only single, individually dispersed AgNWs with no aggregates or branching, then the resistance should be dependent on the diameter of the NWs, with the lowest resistances obtained for the largest diameter NWs. In these studies so far however, two major factors have been observed that make such dependence difficult to observe. One factor is that the microwires consist of aggregates of NWs, have variable diameter and contain branches which bridge adjacent microwires. A second factor is that in some cases, supplementary, incomplete microwires have also been formed between the electrodes, which may also contribute to a lowering in the resistance of the hydrogel. In addition, we did not observe an

Table 1 Resistances (per distance between the electrodes) of agarose gels (0.50% w/v) with AgNWs (0.50% w/v) after dielectrophoretic assembly and gelling, measuring through the electrodes A–A (R_{A-A}) and B–B (R_{B-B})

AgNW diameter/nm	R_{A-A}/kΩ cm^{-1}	R_{B-B}/kΩ cm^{-1}
50	4.65 ± 1.15	6470 ± 2490
100	1.15 ± 0.25	4780 ± 575
200	3.15 ± 1.35	6590 ± 1980

established trend in the microwire resistance on the initial concentration of NWs in the hydrogel.

The majority of the resistance measurements were made using a standard multimeter. To further investigate the properties of the assembled microwires, a current–voltage (I–V) plot was obtained for the microwire from 50 nm AgNWs (0.50% w/v) in agarose gel (0.50% w/v). As the current through the assembled silver microwire was found to increase linearly with increasing voltage, from the line slope, we measured this particular microwire to have a typical resistance of 2.3 kΩ cm^{-1}. To assess the stability of the anisotropic hydrogel and the assembled microwire, we monitored the resistance through the electrodes A–A and B–B periodically over several hours. During this time, the hydrogel was maintained at room conditions, with a temperature of approximately 20–25 °C and initial humidity of 25–35%. The mass of the gel was also measured, and is plotted as a percentage of the initial mass (Fig. 4). The data show that, as discussed earlier, the resistance measured through the microwire (electrodes A–A) is approximately 3 orders of magnitude lower than that measured perpendicular to the assembled microwire (electrodes B–B).

This anisotropic nature of the hydrogel remains over a period of just over 4 hours, at which point we observe a sharp rise in the resistance measured through the electrodes A–A. This rise brings the resistance measured through those electrodes close to that measured through the electrodes B–B, showing that the gel has now become isotropic with respect to its electrical character. Visual inspection of the gel after four hours revealed that cracks had formed, leaving large gaps in the gel that had led to the microwire breaking in several places. These cracks were attributed to drying effects caused by evaporation of water from the gel. We can see that the hydrogel loses mass at quite a steady rate over the course of the four hours, with the mass dropping to approximately 65% of its original value at the point at which the electrical anisotropy, and therefore the integrity of the microwire, is lost. To prevent this drying effect from disrupting the microwire and causing the loss in anisotropy from the gel, we conducted another experiment in which we stored a gel with its encapsulated microwire under controlled humidity. These conditions were achieved by placing the DEP cell in a Petri dish lined with tissue paper that had been soaked in Milli-Q water, in an attempt to either significantly slow down or stop the loss of water from the hydrogel. Fig. 4 shows that the electrical anisotropy of the gel was lost after approximately four hours due to drying up of the hydrogel which causes breakdown of the microwires and a decrease in conductivity. By placing the gel in a more humid environment, however, we were able to preserve the electrical anisotropy within the hydrogel to over seven days. At this point the experiment was stopped through user intervention, though the anisotropy would probably have remained for a much longer period of time. This extension of the anisotropic nature of the gel is attributed to the slow down or prevention of loss of water from the gel in the more humid environment. We could not measure the mass of the film accurately, as the mass increased due to water condensing on the cell, but visual inspection by a microscope showed no visible cracks in the gel, even after seven days.

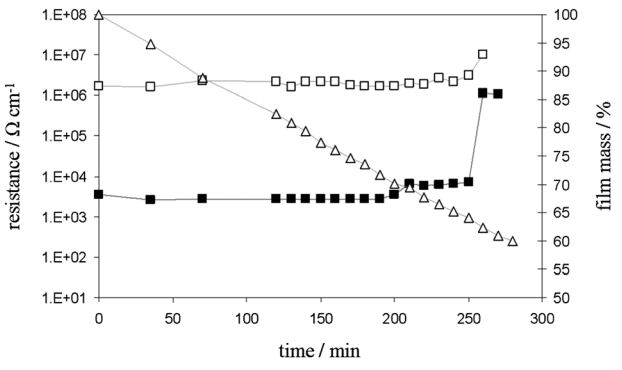

Fig. 4 Plot of the resistance (RHS axis) and the mass (LHS axis) of an agarose gel (0.50% w/v) with encapsulated microwires assembled from 50 nm diameter AgNWs (0.50% w/v) over time. Resistance is measured through the electrodes A–A (filled squares) and B–B (empty squares), while the mass of the film as a percentage of its initial mass is also shown (empty triangles).

Fabrication of a biosensor from AgNWs assembled with DEP

The electrical anisotropy of the gels was then exploited in an attempt to fabricate a novel sensor based on these materials. This was achieved by functionalising the AgNWs with thiolated biotin molecules, with the thiol group acting as an anchor onto the NW surface. After DEP assembly of the functionalised NWs and gelling the agarose, the biosensor was then exposed to a solution containing streptavidin, which has a high binding affinity for up to four biotin molecules. It was envisaged that streptavidin binding to the biotin might result in a change in the electrical properties through the assembled microwire, though not perpendicular to the microwire, hence the use of our electrically anisotropic gels.

AgNWs functionalisation and strategy for biosensor fabrication

The sensor was fabricated as described in the introduction and Fig. 1, and assembled in the four electrode cell used for DEP assembly of AgNWs. The novel part of the method, however, comes from the functionalisation of the AgNWs with biotinyl tetra(ethylene glycol)undecane thiol, prior to their dispersion and assembly within the agarose solution. Sastry *et al.*[29] have previously reported a reduction in the plasmon resonance peak (at ~400 nm) for solutions of silver colloids functionalised with similarly functionalised biotin.[29] They reported that an incubation time as short as one minute is sufficient for functionalisation at a biotin concentration of 100 μM, and 40 minutes at a concentration of 1 μM. In our study, biotin-functionalisation was achieved through incubating the AgNWs (50 nm diameter, 3.0 mg) dispersed in a solution of the thiolated biotin (0.50 mL, 4.2 μM) for 24 hours. The biotin-functionalised AgNWs were washed from the solution of the thiolated biotin three times with Milli-Q water before being suspended in 0.5% w/w agarose solution above its gelling point at a AgNW concentration of 0.50% w/w.

The functionalisation of the AgNWs with biotin was monitored using UV-vis spectroscopy.[29] Fig. 5 shows the UV-vis spectra of AgNW dispersions, both un-functionalised and

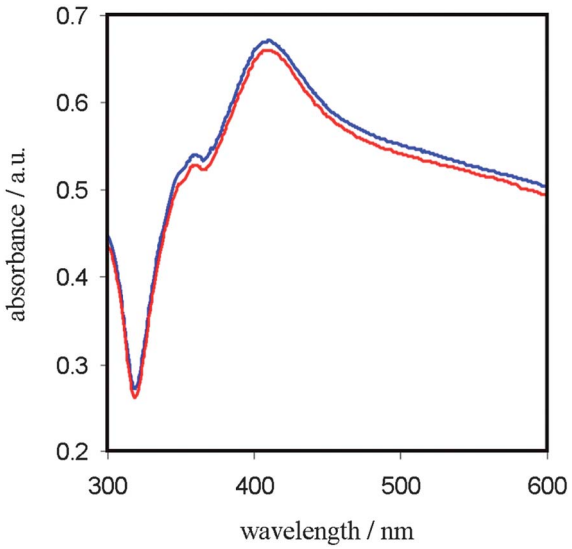

Fig. 5 UV-vis spectra of AgNW (50 nm diameter) dispersions, unfunctionalised (blue line) and functionalised in 1 μM thiolated biotin (red line). The biotin-functionalised AgNWs were washed from the solution of thiolated biotin three times with Milli-Q water. Spectra were recorded shortly after dispersion preparation.

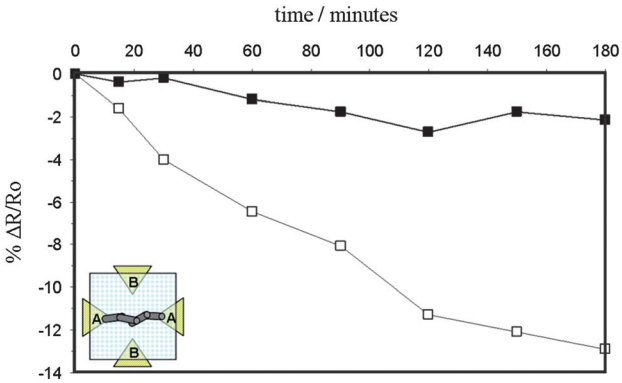

Fig. 6 Plot showing the % change in the resistance of the agarose gel through the electrodes A–A (empty squares) and B–B (filled squares), upon exposure to streptavidin. Inset is a schematic of the electrodes and the anisotropic gel.

functionalised with thiolated biotin, shortly after their preparation. For the biotin-functionalised AgNWs in Fig. 5, there is a small reduction of the peak at ~410 nm, suggesting that a degree of functionalisation has been achieved. The results of Sastry *et al.*[29] suggest that the degree of functionalisation increases over time, so it is envisaged that after 24 hours incubation, additional functionalisation will have taken place. Dielectrophoretic assembly of a continuous microwire was achieved with an AC field of 5 kHz frequency and strength of 250 V cm^{-1}.

Cooling resulted in agarose gelation, entrapping the assembled, functionalised microwire and preventing its disassembly on removal of the AC field.

Sensitivity of the biosensor to streptavidin

The functionalised AgNW gel showed electrical anisotropy, with a resistance of 3.1 kΩ cm^{-1} through the electrodes A–A (through which the field was applied) and 5.67 MΩ cm^{-1} through the electrodes B–B (perpendicular to the field). As observed with the non-functionalised AgNWs, the resistance through the assembled microwire is around three orders of magnitude lower than that observed when it is perpendicular to the assembled microwire. We also washed the hydrogel with the assembled microwires of functionalised AgNWs just in case some physically adsorbed thiolated biotin has leached out of the AgNWs during the DEP assembly process in the agarose solution at 55 °C before setting of the agarose gel. The hydrogel with the functionalised microwires was washed by incubating the cell with the hydrogel in 20 mL of Milli-Q water for 30 minutes. This was repeated three times, changing the aqueous media after each wash to remove the thiolated biotin from the system. The gel was then incubated in 19.5 mL Milli-Q water for 30 minutes, before 0.1 mg streptavidin dissolved in 0.5 mL Milli-Q water was added. Fig. 6 shows the % resistance change over the course of the three hours.

Upon introduction of streptavidin to the solution surrounding the gel, the resistance through the electrodes A–A steadily decreased over the three hour examination period, hence the negative change in the % resistance change shown in Fig. 6. The resistance measured through the electrodes B–B also showed a general decrease, but some rises in the resistance were also observed and the general decrease was not as large as that measured through the assembled microwire (electrodes A–A). By the end of the three hour period, the % change of the resistance through the electrodes A–A was almost 13%, while the % change through the electrodes B–B was just over 2%. Clearly, the microwires have responded to the presence of streptavidin, but have given different responses due to the anisotropy of the gel.

As the microwire is a continuous structure, we might expect that the streptavidin–biotin interaction would result in a greater change in the resistance, compared to measurements made perpendicular to the microwire, where the functionalised NWs remain as discreet or small aggregates. However, washing of the streptavidin from the cell does not decrease conductivity, *i.e.* the sensor is irreversible, probably due to the extremely high binding constant ($K \sim 10^{15}$ mol^{-1} – see ref. 29) of streptavidin to the grafted biotin on the AgNWs. In other words, the compacting of the silver microwires is irreversible and the device is not reusable. Limits of detection are expected to be sensitive to the binding constant of the AgNW functional groups to the complementary bioligands in addition to the device response time. The determination of the latter would require a dedicated study of these factors for several different architectures of the device to optimise response time.

Conclusions

We performed dielectrophoretic assembly of silver nanowires (AgNWs) within an agarose solution above its gelling temperature, followed by gelling of the agarose to encapsulate the formed network of silver microwires which prevents them from disassembling when the AC field was removed. The arresting of

the microwire structures fabricated through dielectrophoretic assembly was achieved without the need to modify the microwire building blocks (AgNWs) or the substrate in any way. We constructed a phase diagram at fixed AC field frequency and temperature of the dielectrophoretic cell, comparing the assembly of the microwires at various combinations of agarose and AgNW concentration, and various AgNW diameters. The assembly and encapsulation of a microwire was dependent on the concentration of both the NWs and the agarose, with assemblies formed at intermediate concentrations of each. With the assembly of a microwire, the resistance through the gel was lowered by three to four orders of magnitude when compared to the resistance measured perpendicular to the formed microwires. The created hydrogels displayed electrical anisotropy, which can be sustained for up to seven days in humid conditions. We were able to fabricate an anisotropic hydrogel responding to streptavidin by functionalising the AgNWs with biotin prior to their assembly. The biotin-functionalised silver microwires assembled using DEP showed a measurable decrease in the resistance when exposed to streptavidin solution. Similar biosensing devices could be developed for a range of other bioligands by functionalising the AgNWs with antibodies and other biomolecules.

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