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Development of a New Antibody-Based Diagnostic Approach for the Earlier Diagnosis and Treatment of Alzheimer's Disease

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Abstract

Current diagnostic approaches to neurodegenerative diseases are often flawed as they are often invasive and cannot effectively diagnose early-onset dementia. Antibody-based therapeutics for neurodegenerative diseases are very promising but often lack specificity to certain biomarkers and require invasive methods of administration such as a lumbar puncture. In this study I report a novel quantum-dot (QD) conjugated bispecific-antibody (BsAb) diagnosis system designed for Alzheimer's disease. This structure is easy to synthesize and displays specificity to oligomeric amyloid-beta (A?), which is often present before Alzheimer's symptoms starts to manifest. The bispecific antibody also binds with a weak affinity to transferrin receptors – thus potentially allowing it to cross the blood-brain barrier (BBB) via receptor-mediated transcytosis and reducing the necessity for extremely- invasive means of administration such as a lumbar puncture. The CdTe/ZnS QDs conjugated to the BsAb have multimodal, non-invasive MRI and fNIR imaging capabilities and also displayed allow cytotoxicity to neuronal cells. The synthesized nanoparticles composed of CdTe/ZnS with a Gd-DOTA doped silica shell also displayed therapeutic properties by immobilizing the toxic oligomeric A? and increasing neuronal viability. These novel BsAb-QD structures display promising diagnostic and therapeutic properties and represent an important evolution in neurodegenerative drug design.

I synthesized a novel nanoparticle-bound antibody for the earlier diagnosis and treatment of Alzheimer's disease which proves to be less invasive and more accurate in comparison to existing tests of its kind.

Introduction

The use of antibodies as diagnostics and therapeutics against certain diseases such as cancer has revolutionized modern medicine, both in vitro and in vivo. The two most commonly studied biomarkers of Alzheimer's disease are A? and tau. A? as a diagnostic and therapeutic target has been an area of intense research, with current diagnostic plaques focusing on A? plaques. However, the assemblies of these plaques occur at the end of the A? cycle and do not act as a good biomarker for diagnosing the early-onset of Alzheimer's disease. Many plaque-dissolving drugs have reached clinical trials; however, their inability to produce and therapeutic properties has meant that they have not become FDA approved. Newer research has also suggested that plaques are present in cognitively healthy people and are not as detrimental as previously thought. The BBB is a highly selective barrier that stops 100% of large molecules from entering and 98% of all designed therapeutics are not able to cross the BBB. Antibodies are too large to cross the BBB and so extremely invasive lumbar punctures are used to bypass this barrier whilst administering antibody based drugs. Diagnostic approaches to looking at biomarkers found in the cerebrospinal fluid (CSF) are unreliable as the concentration of the biomarker in the CSF is not significant enough to form a diagnosis.

A? oligomers are the most neurotoxic form of A? and are present in much higher concentrations in the brains of Alzheimer's patients and this increase appears during the earliest stage of the disease, making A? oligomers an attractive biomarker. For the first time, I synthesized a bispecific antibody, composed of an IgM F(ab')2 fragment from an anti-transferrin receptor (TfR) antibody and an IgG1 F(ab')2 fragment from an anti-A? oligomer-specific antibody. I then synthesized CdTe/ZnS silica encapsulated QDs with doped Gd-DOTA shells and carboxyl functionalized the surface. The antibody was bound to the nanoparticle utilizing the covalent bonding between amine and carboxyl groups. Various assays including surface plasmon resonance and confocal microscopy were used to determine the diagnostic and therapeutic potential of the QD-BsAb structure. The QD-BsAb structure demonstrated multimodal MRI and fNIR imaging capabilities as well as a low cytotoxicity to neuronal tissue. It also displayed a low affinity to TfRs, thus potentially allowing it to cross the BBB using receptor-mediated transcytosis – beneficial for a less invasive drug delivery approach. The QD-BsAb structure also displayed therapeutic properties by immobilizing the neurotoxic A? oligomers and inhibiting them from entering neurons. This as a result increased the neuronal viability considerably and shows significant promise for future treatments and drug development.

Results

The F(ab')2 fragment generation of the anti-TfR (IgM) antibody was undertaken utilizing immobilized pepsin and the anti-A?

oligomer-specific antibody F(ab')2 fragments were generated utilizing immobilized ficin. The F(ab')2 fragments were cleaved into Fab' fragments using 2-mercaptoethanol hydrochloride to expose the free sulfhydryl groups. The exposed sulfhydryl groups from the different Fab' fragments were bound using equimolar ratios of Fab' and DTNB (Ellman's reagent).

The success of the BsAb synthesis was determined using HPLC (**Fig 1**). The HPLC was used throughout to monitor the successful synthesis of the BsAb. The single peak present on the 'd' HPLC graph indicated that there were no impurities and free Fab' fragments and that the BsAb had been successfully synthesized.

The synthesis of the CdTe/ZnS silica encapsulated Gd-DOTA QDs has been explained below, however, in short, the QD core was synthesised via the pyrolysis of the CdTe precursors dimethylcadmium and trioctylphosphine telluride. The dots were injected at 350oC and grown at 290oC. Selective size precipitation (SSP) was used to extract QDs of various sizes. The ZnS core was grown from first analyzing the average CdTe radius using TEM (**Fig 2)**. The appropriate ratio of ZnS core to CdTe shell was calculated by taking into account the bulk lattice parameters and the ratio of the shell volume to the core assuming a spherical core. The silica coating was deposited via the Zn/thiol bonds. The silica was doped using chelated gadolinium, where the chelating agent was DOTA. Ethanolamine was used to carboxyl-functionalize the CdTe/ZnS silica encapsulated Gd-DOTA QDs.

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a) Displays the HPLC peak for the different Fab' fragments and present Fc fragments of the anti-TfR antibody. b) This displays the HP

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Fig. 1: Figure 1

a) Displays the HPLC peak for the different Fab' fragments and present Fc fragments of the anti-TfR antibody. b) This displays the HPLC peak for the Fab' and any present Fc fragments of the anti- A? oligomer-specific antibody. c) This HPLC reading was used to monitor the binding success of the two Fab' fragments and any unfiltered Fc fragments were viewed. d) The presence of the single HPLC peak is an indicator that the Fab' fragments have successfully bonded to create the BsAb and any Fc fragments have been filtered.

The near infrared (NIR) region is usually characterized as the region in which the wavelength of light is in-between 700nm and 900nm. The sizes of the QDs are related to the emission wavelength. The unconjugated QDs absorption and emission spectra were read using a microplate reader. The synthesized CdTe/ZnS QDs were compared to the conventional organic dye (OD), Alexa Fluor 790, – which also emits light in the NIR. The spectra analysis shows that the QDs emitted light maximally at 850nm and the OD emitted maximally at 805nm(**Fig 3.)**. The emission peaks are in the NIR, which can penetrate through biological tissue. Furthermore, the use of quantum dots increases the penetration depth to >2nm as described by Hong et al.

These properties make them both suitable for non-invasive fNIR-Spectroscopy. The QDs had a 57x greater extinction coefficient (?) than the AlexaFluor790 (at absorption maxima) and previous research (see **References**) has identified that QDs have fluorescence quantum yields (?) of 40%-90% at room temperature (RT), which is higher than most ODs that emit light in the NIR, ultimately making my QDs significantly brighter. Previous research has well established that QDs are significantly less susceptible to photobleaching due to their inorganic composition.

ODs have narrow absorption spectra whereas the QDs have broad absorption spectra, making QDs more practical as a single excitation source is required. ODs have broader emission peaks with significant emission intensity at wavelengths >850nm whereas the QDs have narrow/asymmetrical emission peaks, enabling easier detection of QDs due to reduced inter channel cross-talk. The QDs have a large 'Stokes Shift' in comparison to ODs, allowing the detection of fluorescence whilst reducing interference. The QDs' large 'Stokes Shift' also reduces fluorescence quenching, resulting in a stronger signal.

The conjugated QDs (QD-BsAb) had slightly different emission properties to the unconjugated QDs. The emission peak was no longer asymmetrical representing fluorescence spikes along other wavelengths, however the emission maxima is in the NIR. The QD-BsAb had 74% emission intensity compared to unconjugated-QDs however due to the high ? and ? of QDs, the QD-BsAb would be significantly brighter than an OD-BsAb. The MRI analysis of the Qd-BsAb was performed when the conjugates were dissolved in PBS at a concentration of 0.4ug/ml. The scans were performed with the T1-weighted mode with TE 8.7 and TR 550. The Gd-DOTA QD conjugate shows up as white on the MRI analysis whereas the undoped DOTA as well as PBS show up as negative. This means that the Gd-DOTA

QD-BsAb can also be used for multimodal diagnosis by means of MRI as well as fNIR Imaging.

Significant fluorescence was emitted from the targeted Aß-oligomers and insignificant fluorescence was detected from other Aß species. The QD-BsAb favoured Aß-oligomeric species and had limited cross-reactivity with other species of Aß, reducing the chances of misdiagnosis. The fluorescence emitted from the QD-BsAb was dependent on the Aß-oligomer concentration and size, suggesting this probe can also determine the stage of the disease by emitting fluorescence based on the aggregation and

quantity of Aß oligomers.

The affinity of the QD-BsAb to certain proteins, Aß oligomers and TfRs, was characterized utilizing surface plasmon resonance (SPR) **(Fig 4.)**. An overlay plot of sensorgrams from the QD-BsAb steady-state affinity analysis was created. The sensor grams were reference subtracted and blank subtracted with the creation of a response versus concentration plot and the subsequent reading of the affinity (Kd). The Kd value for the TfRs was read as 1.54×10-4 moles. This high Kd value suggests that the QD-probe has a low affinity to TfRs but can still engage the TfRs, suggesting that it can cross the BBB utilizing the mechanism of receptor-mediated transcytosis. This low affinity to TfRs can be expected due to the (IgM) anti-TfR antibody Fab' in the BsAb as IgMs tend to have low affinities despite a strong overall avidity.

The QD-BsAb had low Kd values (low-nanomolar (10-9) to high-picomolar (10-10) range) to Aß oligomers of varying molecular-weight, suggesting that the QD-probe has a high affinity to Aß oligomers. This high affinity to TfRs can be expected due to the (IgG1) anti-Aß oligomer-specific antibody Fab' in the BsAb as IgG1s tend to have high affinities. In general, the QD-BsAb had a Kd value to TfRs that suggests it can cross the BBB using the mechanism of receptor-mediated transcytosis and a Kd value that suggests it has a high affinity to Aß oligomers – demonstrating the QD-probe's potential as a diagnostic tool for Alzheimer's disease.

The QD-BsAb displayed very little cytotoxicity to neuronal cells (NIH/3T3), which may be credited to the silica encapsulation and the ZnS shell around the cadmium based core , with cell viability after a 48 hour incubation period being >90% in comparison to the control sample. The use of a CdTe core as opposed to a CdSe core may have also decreased cytotoxicity as CdTe is far less toxic that cadmium and telluride on their own. Oligomers of varying size displayed a significant cytotoxicity to neuronal cells (NIH/3T3), with cell viability being at 25% and 42% respectively in comparison to the control sample. However, the bound oligomers of varying size displayed a decreasing cytotoxicity, with cell viability of bound oligomers being 71% and 83%. As such, the QD-BsAb displays significant therapeutic potential by decreasing the cytotoxicity of amyloid-beta oligomers, the most neurotoxic form of amyloid-beta. This in turn increased cell life by immobilising the toxic species of this protein.

Above shows the results after amyloid-beta oligomers and the QD-BsAb were incubated together for 30+ minutes. The media containing the bound oligomers was then introduced to the neuroectodermal cells and cortical neurons. There was extremely little intracellular amyloid beta as can be seen by the confocal images. With an incubation period of 30 minutes, some amyloid-beta oligomers were still able to enter the cells from the media, however after an incubation period of 2+ hours, very few oligomers were inside the cells. This shows that the quantum dot probe has therapeutic potential by binding to the neurotoxic amyloid-beta oligomers and inhibiting them from entering cells. By inhibiting cellular entry, the neurotoxic Aß was not able to display its usually cytotoxic properties – and this could explain the previous results of the bound Aß oligomers having a decreased cytotoxicity and my QD-BsAb displaying therapeutic effect.

Conclusions

Alzheimer's affects over 47 million people worldwide with 8 million new cases very year. The WHO identifies early diagnosis as essential to treat Alzheimer's however patients are usually diagnosed during the middle or later stages of the disease – after irreversible damage has taken place. Some patients suffering from Alzheimer's do not receive a diagnosis and this disease costed US$604 billion to society in 2010. The molecular 'Trojan Horse' I have developed could potentially be used for the earlier, minimally-invasive diagnosis of Alzheimer's Disease.

By conjugating a bispecific antibody (BsAb) to selective wavelength quantum dots (QDs), I have created a diagnostic probe with promising affinities which can be non-invasively detected. The probe had a low affinity to transferrin receptors (TfRs), thus potentially allowing it to cross the blood brain barrier (BBB) via receptor mediated transytosis. It also had a promisingly high affinity to amyloid-beta (Aß) oligomers – which is beneficial for diagnosis. The CdTe/ZnS QDs used could emit light maximally at 850nm (NIR), thus allowing these probes to be detected non-invasively using fNIR-Spectroscopy.. The QDs were significantly brighter and had various advantages over conventional organic fluorophores. The additional silica encapsulated Gd-DOTA allows the QD-BsAb to be identified via MRI contrast, and thus provides a multimodal platform for diagnostic imaging. The probes successfully targeted Aß oligomers also had very little cross-reactivity with other species of Aß. These results supported my hypothesis as the probes had non-invasive detection properties and had low affinities to TfRs – potentially allowing them to cross the BBB via receptor mediated transcytosis. The QD-BsAb also displayed significant therapeutic properties by decreasing the toxicity of the neurotoxic Aß oligomers and thus increasing cell viability by almost triple.

This biomarker is in significantly higher concentrations in the brains of Alzheimer's patients and the probe had little cross-reactivity with other species of Aß, reducing chances of misdiagnosis. Research has shown that Aß oligomers are the most neurotoxic form of Aß and are a major culprit of Alzheimer's Disease, and by targeting these oligomers, this probe could be used to diagnose and treat the disease before all the damage has occurred – well before typical Alzheimer's symptoms surface. My results also suggest that this probe could be used to determine the stage of the disease by emitting levels of fluorescence dependent on Aß oligomer size and concentration. Due to the potential non-invasive and multimodal detection of the QDs via fNIR-Spectroscopy and also MIR, combined the probes' ability to cross the BBB, there would be no requirement for a lumbar puncture, making diagnosis and treatment with this QD-BsAb minimally invasive.

The QD-BsAb designed and constructed in this report can be further modified to diagnose and treat other neurodegenerative diseases, i.e. current research involves replacing the anti- Aß oligomer-specific Fab' in the BsAb with a Fab' specific to alpha-synuclein (?s) oligomers as these are present in early stage Parkinson's disease. These findings have the potential to benefit millions of people worldwide and so I will aim to collaborate with various research institutes and organisations to help develop this QD-BsAb for further diagnostic and therapeutic use not only for Alzheimer's disease but potentially other neurodegenerative diseases as well.

Materials and Methods

F(ab')2 fragments were obtained using commercially available kits from Life Technologies:

Monoclonal antibody (mAB) Anti-Aß (oligomer and fibril specific) antibody (IgG1) F(ab')2 generation:0.5mL of the antibody (8mg/mL) was added to a previously equilibriated immobilised ficin column and incubated (37ºC) for 25 hours. Generated F(ab')2 fragments were purified with NAb Protein A Column and centrifuged (1000xg) for 1 minute. Flow-through concentration was determined spectrophotometrically by measuring the absorbance at 280nm.

Monoclonal antibody (mAB) Anti-TfR (transferrin receptor) antibody (IgM) F(ab')2 generation:

A previously equilibriated immobilised pepsin column was washed with 8mL IgM F(ab')2 digestion buffer (200ml, 100mM sodium acetate, 150mM NaCl, 0.05%NaN3; pH4.5). The column and 1mL of antibody (1mg/mL) were incubated (37ºC) separately for 3 minutes. Antibody was added to column and incubated (37ºC) for 1.5 hours. Generated F(ab')2 fragments were centrifuged in C30 Concentrator and concentration was determined spectrophotometrically by measuring absorbance at 595nm.

Bispecific antibody synthesis (including Fab' generation)

Antibody synthesis was performed as described by Greg T. Hermanson in Bioconjugate Techniques Second Edition, ISBN: 978-0-12-370501-3

Fab' Generation: 1mL of anti-Aß oligomer-specific antibody F(ab')2 (10mg/mL) was dissolved in 20mM buffer (sodium phosphate, 0.15M NaCl, 5mM EDTA,pH7.4). 6mg of 2-MEA•HCl was added and incubated (37ºC) for 1.5hours. Excess 2-MEA•HCl was removed by gel-filtration. Protocol was repeated for anti-TfR antibody Fab' generation.

Bispecific antibody synthesis: Anti-Aß oligomer-specific antibody Fab' (Fab'A) was added to DTNB (40mg DTNB, 10ml 1MTris?HCl, pH7.5) and incubated at room temperature. Equimolar ratios of Fab'A-DTNB and anti-TfR antibody (Fab'B) were mixed and incubated (37ºC) for 1.5 hours. Reaction was incubated (4ºC) overnight. Bispecific BsAb fraction was purified with Superdex 200 column equilibriated in PBS.

Synthesis of CdTe/ZnS nanoparticles:

Nanoparticles were synthesised with silica encapsulation based on publications from Yang Xu et al. & B. O. Dabbousi et al. However, the protocol described herein further incorporates gadolinium in the outer shell and allows for carboxyl functionalization to allow antibody fragment conjugation.

CdTe/ZnS synthesis:

The preparation of the selenide organometallic precursor (i.e. trioctylphosphine telluride) was achieved by dissolving 0.1 mol of a telluride shot in 100 ml of trioctylphosphine, thereby resulting in a 1M solution of trioctylphosphine telluride. Dimethylcadmium was used as the other organometallic precursor. The CdTe precursor material (also known as quantum dots) was synthesized via the pyrolysis of dimethylcadmium and trioctylphosphine telluride in the co-ordinating trioctylphosphine oxide solvent. Precursors were injected at 350o?C and particles/dots were grown at 290o?C. Selective size precipitation was performed with methanol to collect the particles as powders, and then they were redispersed in hexane. 5g of trioctylphosphine oxide was heated until it reached 190oC under a vacuum and then it was cooled to 60o?C. 0.3 umol of CdTe was dispersed in hexane and transferred into the reaction vessel with the solvent being pumped off.

Hexamethyldisilathiane and diethylzinc were used as the precursors for zinc and sulphide. The average radius of the CdTe core precursors was determined from TEM, then calculating the appropriate CdTe to ZnS ratio. This was done by considering the ratio of the shell volume to that of the core and assuming a spherical core and shell and taking into account the bulk lattice

parameters. Precursors were dissolved in 3mL trioctylphosphine inside an inert atmospheric glovebox. The precursors were loaded transferred into an addition funnel, attached to a reaction flask with the CdTe cores that were dispersed in trioctylphosphine oxide. The trioctylphosphine was heated under an atmosphere of nitrogen; the precursors were then added dropwise to the reaction mixture for 10 minutes at a temperature of 180o?C. The mixture was then cooled to 90o?C, whilst being left stirring for 3 hours; then 5mL of butanol was added to inhibit the solidification of the trioctylphosphine oxide upon the cooling period. The nanoparticles were stored in the solution so that their surfaces remained passivated with trioctylphosphine oxide. When recovered, the powder-formed particles were precipitated with methanol and then redispersed in solvents (e.g. hexane, THF etc.).

Chelated gadolinium (Gd-DOTA) silica encapsulation:

Sodium silicate and mercaptopropyl trimethoxysilane was diluted in deionized water to a final percentage of 0.15% and 0.7%. 0.1mL of dilute mercaptopropyl trimethoxysilane was added to a 10mL solution of CdTe/ZnS nanoparticles and then was shaken for 20 minutes. This allows for the linking of the zinc sulphide shell with mercaptopropyl trimethoxysilane through the Zn/thiol bonds to allow for the deposition of the silica coating. 0.2mL of the previously diluted sodium silicate solution (pH 10) was added, the solution as mixed well and was kept in a dark room at room temperature to allow for the polymerisation of the silica. After 4 hours, the solution was transferred to another vial containing 8mL ethanol (100%) to allow the growth of a thicker silica coating due to the precipitation of the excessive silicate. The silica encapsulated nanoparticles were then precipitated out. The resulting silica encapsulated nanoparticles were added to 10 umol 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) mono-N-hydroxysuccinimide ester for 24 hours at room temperature. The gadolinium chelation to DOTA was achieved by adding two molar equivalents of the gadolinium precursor (Gd3+; GdCl3) for 24 hours at room temperature. The Gd-DOTA doped silica encapsulated nanoparticles were collected by centrifugation and washing.

Carboxyl-functionalization of Gd-DOTA silica encapsulated nanoparticles:

40g of Gd-DOTA silica encapsulated nanoparticles were reacted with 0.05mmol APTES in a 1:2 deionized water-ethanol mixture (12mL, 4mL: 12mL) for 24 hours in room temperature. After being aminated, to convert the terminal amine groups to carboxyl groups, the Gd-DOTA silica encapsulated nanoparticles were twice washed in ethanol and then redispersed in 20mL of anhydrous dimethylformamide with the addition of succinic anhydride (0.06mmol) at room temperature overnight followed by another washing with ethanol twice.

Conjugation with EDC

EDC (1-ethyl-3-(3-dimethylamino) propyl carbodiimide hydrochloride) is a water-soluble carbodiimide crosslinker that activates carboxyl groups for spontaneous reaction with primary amines, enabling peptide immobilisation and hapten-carrier protein conjugation. Conjugation involved covalent bonding of bispecific antibody's amine group to carboxyl groups (as described in Wen-Yen Huang et al.)

Bispecific antibody conjugation to carboxyl-functionalized, Gd-DOTA silica encapsulated nanoparticles:

25mM carboxyl-functionalized Gd-DOTA silica encapsulated nanoparticles were coupled with the bispecific antibody (1mg/mL). An EDC (20mM)/sulfo-NHS (50Mm) was prepared immediately before use. 250µL EDC/sulfo-NHS was added to the solution of carboxyl-functionalized Gd-DOTA silica encapsulated nanoparticles. The reaction was incubated at room temperature for 10minutes and 7µL of 2-MEA was added to quench any excess EDC. 25µL of the bispecific antibody solution was added to the activated carboxyl-functionalized Gd-DOTA silica encapsulated nanoparticles. The reaction was incubated room temperature for 60 minutes. Excess reactants and sulfo-NHS were removed by dialysis against Tris (pH 7.4, 50Mm).

Aggregation

The aggregation protocol of amyloid-beta 1-42 was followed as suggested by the manufacturer (abcam http://www.abcam.com/amyloid-beta-peptide-1-42-human-ab120301.html).

Before use, and prior to opening the vial, it is recommended that the product equilibrates to room temperature for at least 1 hour. Amyloid ? (1-42) human peptide should be initially dissolved at a concentration of 1mg/ml in 100% HFIP (1,1,1,3,3,3-hexafluoro-2-propanol). This solution should be incubated at room temperature for 1 hour, with occasional vortexing at a moderate speed. Next, the solution should be sonicated for 10 minutes in a water bath sonicator. The HFIP/peptide solution should then be dried under a gentle stream of nitrogen gas. 100% DMSO should be used to re-suspend the peptide. This solution should be incubated at room temperature for 12 minutes, with occasional vortexing. The final solution should then be aliquoted into smaller volumes and stored at -80 °C. For a working solution, add 500-1000 ?l of D-PBS (depending on the final concentration to be used) to the peptide stock solution and incubate for 2h at room temperature to allow for peptide aggregation. The molecular weight of the amyloid-beta species was determined by gel electrophoresis.

Surface plasmon resonance

Surface Plasmon resonance was performed using GE Healthcare BiacoreTM, the experimental setup was followed as described by the BiacoreTM Assay Handbook and BiacoreTM Sensor Surface Handbook:

Nanoparticle-probe affinity

Surface plasmon resonance (Biacore) was used to determine the affinity of the bispecific antibody to various targets. A 0.4M EDC/1M NHS solution was added to dextran matrix at flow rate of 10µl/min for 7min to activate surface. TfR solution (ligand, 50µg/mL, PBS diluent) was added at a flow rate of 10µl/min for 7min. 1M ethanolamine-HCl (pH8.5) was added at flow rate of 10µl/min for 7min to deactivate excess reactive groups. Various concentrations of nanoparticle-probe solutions (analyte, PBS diluent) including duplicate-concentrations were used. Unmodified surface was used for reference analysis. Protocol was repeated using Aß oligomers of various sizes as ligand.

Direct-fluorescence assay

Aß monomers, oligomers, fibrils and plaques (100pg/ml-800pg/ml) were blocked in PBS (w/ 5% BSA) in 384 well plates. The probes were added and incubated at room temperature for 1 hour, then washed with PBS-T. Fluorescence was read with a plate reader at 800nm (488nm excitation).

T1 and T2 weighted MRI analysis

The BsAb-QD solutions (Gd-DOTA QdBsAb-4 and Qd-BsAb) were prepared in PBS as a buffer by dissolving the Qd-BsAbs in DMSO (minimum) and then further diluting the solution to produce a 20g/ml stock solution in PBS w/ 1% DMSO. pH was subsequently adjusted to ph7.2 with 0.1M HCl. The different Qd-BsAb solutions (i.e. undoped CdTe/ZnS-BsAb core 4 and Gd-DOTA CdTe/ZnS-BsAb) were both suspended in PBS buffer. PBS was used as the positive control. The Gd-DOTA CdTe/ZnS-BsAb stock solutions (10nm 20g/ml) were used as the negative control to test the efficiency of gadolinium doped QDs for MRI contrast. The undoped CdTe/Zns-BsAb was used as the control.

The comparison between the nanoparticles conjugates Gd-DOTA CdTe/ZnS conjugate and the nanoparticle CdTe conjugate was first carried out. To accomplish this, Whatman filter papers (circular, 15 mm diameter, cat: 1441 150, USA) were soaked in the nanoparticle-peptide stock solutions. The analytes were added into a 96 well plate (500 ?? p/well) for culturing. The MRI scanner used for imaging and analysis was the Siemens AVANTO 1.5T MRI scanner.

Assay kit

A standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay kit (MTT Cell Proliferation Assay (ATCC® 30-1010K™) was used to determine the cytotoxicity of the quantum-dot probe against NIH/3T3 cells.

Immunofluorescence

Two different approaches on (ATCC) HCN-2/CRL-10742 (cortical neurons) and PFSK-1/CRL-2060 (neuroectodermal) were undertaken using double immunofluorescence. This was performed as described by abcam (http://www.abcam.com/ps/pdf/protocols/double%20immunofluorescence%20simultaneous%20protocol.pdf).

Immunofluorescence Protocol:

The coverslips were coated with polyethylineimine at room temperature for 1 hour. The coverslips were rinsed well three times with sterile water for 5 minutes each. The coverslips were allowed to dry completely and were then completely sterilized under UV light for 6 hrs. The C57Bl/Sv129 cells were grown on the glass coverslips and then rinsed briefly in phosphate-buffered saline. The cells were incubated for 30 minutes in PBST (w/ 1% BSA) to reduce unspecific binding. The conjugated primary antibodies (against amyloid beta 1-42 (oligomers and fibrils) and vimentin), which were stored in

the dark to avoid photobleaching, were incubated with PBST overnight at 4oC. The solution was decanted and washed thrice for 5 minutes each in PBS. Cells were also incubated with 0.5 µg/ml of DAPI for 1 minute and then rinsed in PBS. Mounting medium was dropped onto the coverslip and the coverslip was sealed by applying nail polish to avoid drying. The sample was stored in the dark at -20o?C. Confocal microscopy was used to characterise the results of the immunofluorescence.

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