PEDOT-containing Implantable Neuroelectrodes in Inflammation Control and Neural Signal Transduction

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Abstract. Implantable neuroelectrodes are used to facilitate the treatments of neurological injuries and diseases such as depression, Parkinson's disease, and epilepsy. However, since the body perceives the neuroelectrodes as exogenous materials, immune responses are triggered, resulting in formation of gliosis around the electrodes. Formation of the scar would impede the transfer of signals and reduce long-term stability. Thus, neuroelectrodes with enhanced biocompatibility, stability, and low impedance are essential for inflammation control and neural signal transduction. This work combines MADA@PEDOT with GelMA to create a composite material. Compared with traditional neuroelectrodes, this combination provides several advantages: 1) the natural origins of DA and gelatin in MADA and GelMA gives them good biocompatibility to support cellular activities, reduce inflammatory responses. 2) GelMA/MADA@PEDOT has strong adhesion to cells and tissues. 3) GelMA hydrogels provide high mechanical flexibility and they can be tuned flexibly to apply to different settings, allowing a smoother and more tailored interface. This work provides a novel implantable conductive hydrogel to reduce inflammatory responses, solve problems with traditional materials, completing the preparation of reliable neuroelectrodes, and providing new insights for the neuroelectrode field.

Keywords: Hydrogel, Implantable neuroelectrode, Inflammation, PEDOT.

1. Introduction

Implantable neuroelectrodes are used to facilitate the treatments of neurological diseases such as depression, Parkinson's disease, and epilepsy that are caused by the abnormality of neurotransmitters. However, the implantation triggers inflammatory responses that would result in the formation of scars around the electrodes. The phagocytosis of inflammatory cells generates a large number of free radical cytotoxic by-products, reactive oxygen species (ROS), which are beyond the body's defense capacity, leading to cellular oxidative stress, intracellular lipid peroxidation, DNA and protein damage, exacerbating neuronal cell damage and affecting the stability of the electrodes. Thus, it is crucial to control the inflammatory responses and ROS. Good electrodes can solve this problem by improving signal transduction and providing a more stable tissue interface. Various biomaterials have been developed to interface electrodes and tissues. From metal electrodes and nanoparticles to conducting polymers and carbon-based materials, more biocompatible and adhesive materials have been developed. However, their stiffness and adhesiveness limit the signal transduction stability and more anti-inflammatory electrodes could still be improved.

Traditional materials present several challenges in the development of implanted neuroelectrodes. Thus, new materials that combine high conductivity, biocompatibility, and stability need to be developed. Polymers such as Poly(3,4-ethylenedioxythiophene) (PEDOT) have good conductivity and are stable. However, they are not biocompatible enough. Hydrogel is a polymer that has been used to overcome the limitations of traditional neuroelectrodes. They are cheap, highly biocompatible, stable, and they have low impedance [1]. Additionally, they are more flexible than traditional materials, allowing the electrodes to match the shape of the tissues. Gelatin methacrylic anhydride (GelMA) is a composite hydrogel which consists of gelatin and methacrylic anhydride [2]. Since GelMA is the product of hydrolyzed collagen, it resembles some essential properties of the extracellular matrices. For example, it has arginine-glycine-aspartic acid sequences that promote cell attachment and matrix metalloproteinase target sequences suitable for cell remodeling [3]. This allows neural cells to proliferate and regrow during the implantation process. Furthermore, GelMA

can crosslink when exposed to light, giving rise to its tunable physical quality, allowing it to adjust cell-material interactions and cellular behaviors [4]. The photocrosslinking property of GelMA allows better temporal control, enhancing the stability and durability of electrodes. Therefore, GelMA is a versatile and ideal biomaterial for neuroelectrode implantations.

Methacrylamide dopamine (MADA) is a polymer synthesized through free radical polymerization of methacrylamide (MA) and dopamine (DA). DA is a neurotransmitter that has a few advantages in the application of neuroelectrodes. As a neurotransmitter, DA can facilitate signal transduction. Electrode surfaces with DA-based materials have high biocompatibility. It allows the signal transduction to be more stable, without causing significant inflammatory responses [5]. The structure of the phenolic hydroxyl group and the phenylamine gives rise to its strong adhesion properties [6]. It can be attached to cells with various surfaces. The small particles of MADA make the polymer network more stable.

PEDOT is a conducting polymer derived from the self-polymerization of 3,4-ethylenedioxythiophene (EDOT) monomers. PEDOT-coated electrodes provide some advantages for neuroelectrode implantations compared with traditional materials. The amino group increases the hydrophilicity of PEDOT, and its low cytotoxicity makes PEDOT a biocompatible material that can minimize adverse immune responses during the implantation process [7]. PEDOT-coated electrodes can increase the charge storage capacity by more than 150 times, compared to the unmodified microelectrode [8]. High charge storage capacity allows more efficient electrical stimulation of neural tissues, thereby providing highly stable electrical stimulation in the long-term. Moreover, PEDOT exhibits lower impedance and high electrical conductivity. Thus, PEDOT is an ideal material for implantable neuroelectrode interfaces.

This work combines MADA@PEDOT with GelMA to create a composite material. Compared with traditional neuroelectrodes, this combination provides several advantages: 1) the natural origins of DA and gelatin in MADA and GelMA gives them good biocompatibility to support cellular activities, reduce inflammatory responses. 2) GelMA/MADA@PEDOT has strong adhesion to cells and tissues. 3) GelMA hydrogels provide high mechanical flexibility, and they can be tuned flexibly to apply to different settings, allowing a smoother and more tailored interface. This work provides a novel implantable conductive hydrogel to reduce inflammatory responses, solve problems with traditional materials, completing the preparation of reliable neuroelectrodes, and providing new insights for the neuroelectrode field.

2. Experiments

2.1. Materials

2.1.1 Chemicals and Reagents

3-Hydroxytyramine hydrochloride (Sigma-Aldrich, H8502, USA), methacrylamide (Sigma-Aldrich, 276685, USA), tetrahydrofuran (Sinopharm, 40058161, China), ethyl acetate (Sigma-Aldrich, 319902, USA), FeCl3·6H2O (Sigma-Aldrich, 236489, USA), gelatin (Sigma, V900863, USA), methacrylic anhydride (Sigma, M543000, USA), photoinitiator LAP (Sigma, 900889, USA), sodium tetraborate (Sinopharm, 10018960, China), sodium bicarbonate (Sinopharm, 10018960, China), Cell Counting Kit-8 (Beyotime Biotechnology, 230063, China), Fetal Bovine Serum (Gibco, A3160801, USA), DMEM (Gibco, 11965-092, USA), Penicillin-Streptomycin-Glutamine (Gibco, 15140-122, USA), Triton X-100 (Sigma, H8502, USA), DCFH-DA (Sigma, D6883, USA), lipopolysaccharide (Thermo, 00-4976-93, USA), interferon gamma (Novoprotein, C746-50, China), vinculin antibody (Proteintech, 66305-1, USA), DAPI (Abcam, Ab285390, USA), phosphate-buffered saline (Meilunbio, MA0015, China), 4% paraformaldehyde (Biosharp, 69080900, China).

2.1.2 Cells

Mouse hippocampal neuronal cell line (HT22) is commonly cultivated through Dulbecco's Modified Eagle Medium (DMEM) with 1% Penicillin-Streptomycin (PS) and 10% Fetal Bovine Serum (FBS).

THP-1 cell line is cultivated through RPMI-1640 with 10% FBS and 1% PS.

2.2. Preparation

2.2.1 Methacrylamide Dopamine (MADA)

10 g $Na_2B_4O_7$ and 4 g $NaHCO_3$ are first dissolved in 100 mL of deionized water, and the oxygen in the system is bubbled with N2 for 30 minutes. Then, 2.5 g DA-HCl is added, and 2.5 mL MA is added to 25 mL THF in a separate container, with a pH higher than 7.5. Finally, the reaction mixture is bubbled at room temperature with stirring for 24 hours. After the reaction, a white slurry solution is formed, then filtered to obtain the solution. The pH of the solution was adjusted below to 2. Ethyl acetate was added to the solution. Then the organic phase was extracted three times. Adding hexyl hydride to the organic solution, MADA was allowed to recrystallize. MADA was washed with water and alcohol three times and freeze-dried overnight.

2.2.2 MADA@PEDOT

MADA@PEDOT was prepared by oxidative polymerization. 0.4g MADA solid was dissolved in 90mL ethanol. $600\mu L$ EDOT and 16 g $FeCl_3 \cdot 6H_2O$ as oxidative agent were added into the solution. The solution was stirred for two days at 500 rpm. After two days, the solution was washed with deionized water for three times, then freeze-dried to obtain the MADA@PEDOT nanoparticles.

2.2.3 Methacrylate Gelatin (GelMA)

4.5g gelatin was added to 150mL deionized water at 42°C to stir at 250 rpm. The pH of the solution was adjusted to 8-9 and added with 4.8g methacrylamide anhydride, then the pH was increased by 0.2-0.4 to react over 24 hours. The completed solution was dialyzed to a 3500 DA dialysis bag. The GelMA solid was obtained after freeze-drying for three days. 0.4 wt% LAP was added into 12 wt% GelMA solution.

2.2.4 Preparation of GelMA/MADA and GelMA/MADA@PEDOT

$50\mu g/mL$ MADA and $100\mu g/mL$ MADA@PEDOT were mixed into 12 wt% GelMA to obtain GelMA/MADA@PEDOT.

2.3. Characterization of MADA and MADA@PEDOT

Dynamic laser scattering was used to measure the zeta potential and hydrated diameter of MADA and MADA@PEDOT through Malvern zetasizer (Zetasizer Nanoseries, Malvern, USA). Before testing, an ultrasonic instrument was used to disperse the nanoparticles.

$500\mu g/mL$ GelMA/MADA and GelMA/MADA@PEDOT were used to measure conductivity through the four-point probe (ST22, Jingge Electronics, China).

A rotational rheometer (Kinexus Pro+, Malvern, USA) was used to measure the mechanical property of GelMA/MADA and GelMA/MADA@PEDOT with an 80 mm parallel plate configuration at 37°C. Dynamic frequency sweeps were performed from 0.1 to 10 Hz to measure G'. The samples went through a process from uncured to cured for 4 mins (UV light was used to cure the samples starting from 1 minute) to compare with neural tissues.

Transmission electron microscope was used to detect the morphology of MADA and MADA@PEDOT nanoparticles at an accelerating voltage of 100kV. The samples were prepared by dropping the nanoparticles dispersion onto the carbon-coated copper grids.

Scanning electron microscope was used to detect the microstructures of MADA, GelMA/MADA, MADA@PEDOT, and GelMA/MADA@PEDOT. The samples were freeze-dried, and a conductive gold layer was sprayed at the voltage of 20 mV for 3 minutes.

2.4. Cells

2.4.1 Cell Viability

HT22 cells were digested by adding 1 mL Trypsin-EDTA to obtain single cells. 2000 cells were planted into the 96 well plate. MADA and MADA@PEDOT were added into the cells (concentration from 0 to 200μg/mL) to co-culture. After 24 hours, the culture media was abandoned and 10% cell counting kit-8 (CCK-8) was added to the 96 well plate to co-culture for 1 hour. The absorbance was tested by the multimode reader (Cytation 3, BioTek, USA) to calculate the cell viability.

$$\text{Cell Viability} = \frac{A-A_b}{A_c-A_b} \times 100\%$$

2.4.2 The Ability of Adhesion by Immunofluorescence staining

5×10^5 HT22 cells were cultured in 3.5cm petri dishes for 24 hours at 37°C in a 5% CO_2environment to allow the cells to attach. MADA and MADA@PEDOT were added to the cells for 24 hours. 0.05% Triton X-100 was used to incubate the cells for five minutes. The cells were washed by PBS twice and 1% anti-vinculin was added into each petri dish. After two hours, the samples were washed with PBS twice, and Hoechst was added to the cells. The samples were taken to the confocal laser microscope (FV300, Olympus, Japan).

2.4.3 Transformation of the Polarization Types of Macrophages by MADA and MADA@PEDOT

THP-1 cells were cultured in RPMI 1640 medium containing 100ng/mL phorbol 12-myristate 13-acetate (PMA) and 10% FBS at 37°C in a 5% CO_2 for 48 hours to induce their differentiation into mature macrophages. After washing the cells with PBS, 10mL of RPMI 1640 medium containing 20 ng/mL $IFN-\gamma$ and 100ng/mL LPS was added, and the cells were further cultured at 37°C in a 5% CO_2 for 48 hours to induce polarization to M1 macrophages. RPMI 1640 culture medium containing 1% FBS and MADA, MADA@PEDOT were prepared separately. MADA and MADA@PEDOT were added to M1 macrophages at $50\mu g/mL$ and $100\mu g/mL$, separately to co-culture for 24 hours. Flow cytometry detection (Cytoflex S, Beckman, China) was used to detect the content of macrophage phenotypes. The cells were washed with PBS twice. 0.5% CD86 (APC) and CD206 (FITC) antibodies were used to dye the cells for 30 minutes at room temperature. The cells were washed by PBS once before testing.

3. Results and Discussions

3.1. Characterization of MADA and MADA@PEDOT

In this study, we used Dynamic Light Scattering (DLS) analysis to characterize the hydrodynamic size, particle size distributions, polymer dispersity index (PDI), and zeta potential of MADA and MADA@PEDOT nanoparticles (NPs). The DLS measurements reveal that MADA NPs exhibit a hydrodynamic diameter of approximately 403.7 ± 55.7nm, while the MADA@PEDOT NPs display a larger diameter of 580.3 ± 48.3nm, showing PEDOT's ability in modifying surface structure to increase charge capacities (Figure 1A). Moreover, the PDI values are 0.130 for MADA and 0.314 for MADA@PEDOT, suggesting that PEDOT coating provides a broader particle distribution (Figure 1B, C). Similarly, in the case of MADA and MADA@PEDOT, the zeta potential is approximately 6.2 for MADA and 17.8 for MADA@PEDOT, suggesting that PEDOT addition increases the stability of the overall structure (Figure 1D).

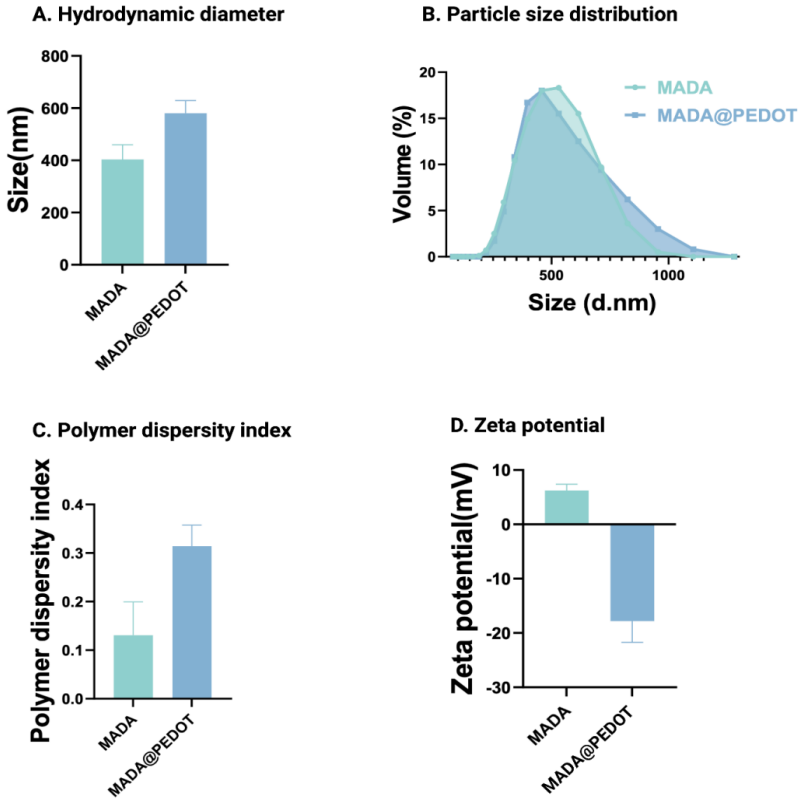

Figure 1. Characterization of MADA and MADA@PEDOT

3.2. Characterization of GelMA/MADA and GelMA/MADA@PEDOT

Figure 2A shows that the conductivity of GelMA is 0.39S/m, while the conductivity of MADA@PEDOT/GelMA is 1.21S/m. This indicates that hydrogels with the addition of MADA@PEDOT NPs have a pronounced, 3.1 times increase compared with GelMA. Figure 2B shows that photocrosslinking starts at 60 seconds. At 240 seconds, G' and G'' of GelMA are 17.34Pa and 2770 Pa, respectively, and the G' and G'' of MADA@PEDOT/GelMA are 8.34Pa and 3780Pa, respectively. The elastic component decreases with the addition of MADA and PEDOT coating, while the plastic component increases.

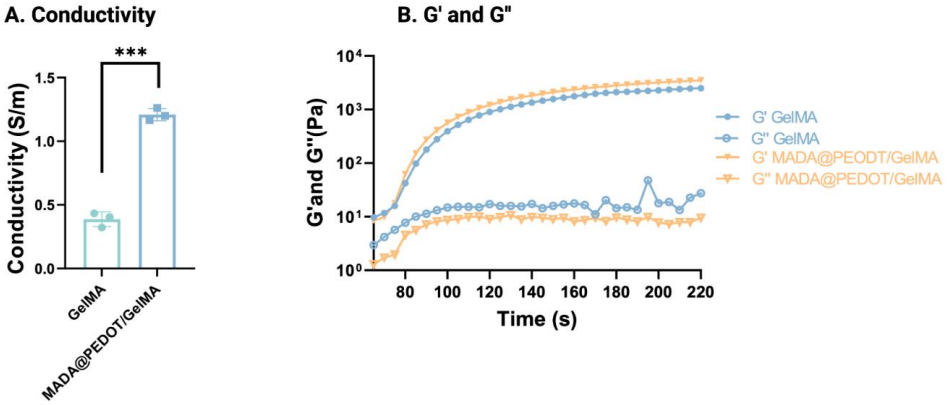

Figure 2. Mechanical properties. (A) *** $p < 0.001$, $n = 2$. (B) G' and G'' of GelMA and MADA@PEDOT/GelMA

3.3. Biocompatibility of MADA and MADA@PEDOT

The culture medium containing different concentrations of MADA and MADA@PEDOT ($0\mu g/mL$ - $200\mu g/mL$) was prepared for HT22 cells. By CCK-8 kit, figure 3A shows over 200 $\mu g/mL$ of MADA has a pronounced cytotoxicity on HT22 cells. For MADA@PEDOT, the concentration of $50\mu g/mL$ promotes cell proliferation on HT22 cells (Figure 3B).

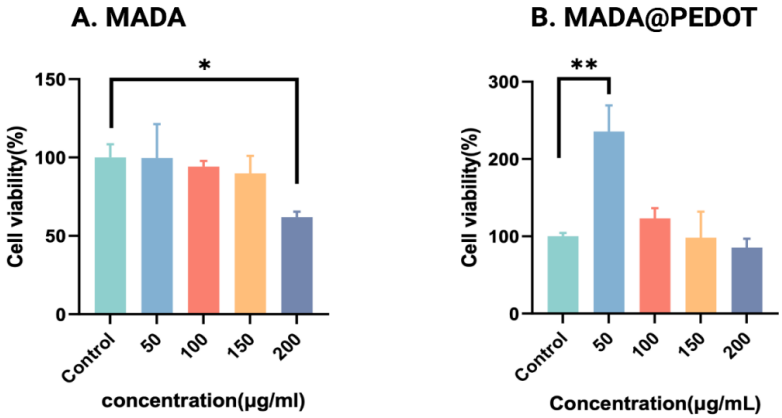

Figure 3. Viability of HT22 cell culture with different concentrations of CCK-8 through multimode reader in (A) *p < 0.05, n = 5. (B) **p < 0.01, n = 5

3.4. Adhesion of GelMA/MADA and GelMA/MADA@PEDOT

TEM (HT7700, HITACHI, Japan) was used to detect the morphology of MADA and MADA@PEDOT. Figure 4A shows that the unmodified MADA NPs contain smooth surfaces and are relatively uniform in structure, while PEDOT coating in Figure 4B indicates that the NPs contain more layers and exhibit a rougher surface. SEM (S-4800, HITACHI, Japan) was further used to reveal the surface topography of MADA and MADA@PEDOT NPs, as well as the hydrogel GelMA and GelMA/MADA@PEDOT. GelMA (Figure 5C) has a porous structure with spaces in between. With the addition of MADA and the coating of PEDOT, GelMA/MADA@PEDOT presents a more interconnected network, benefiting the proliferation and growth of neural tissues (Figure 5D).

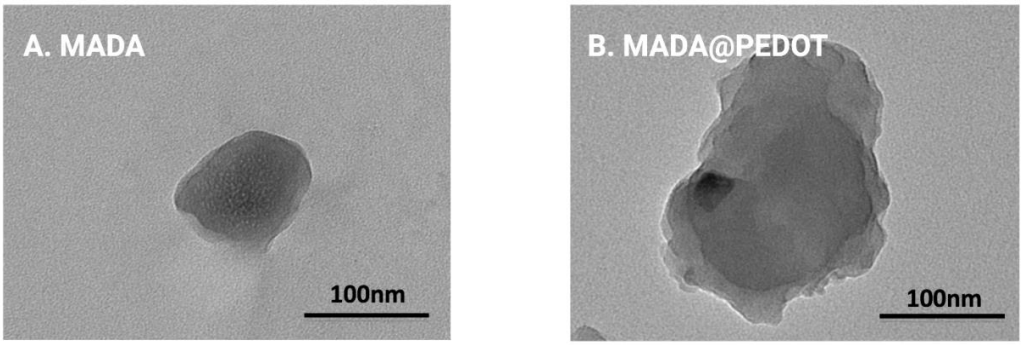

Figure 4. Morphology of MADA and MADA@PEDOT

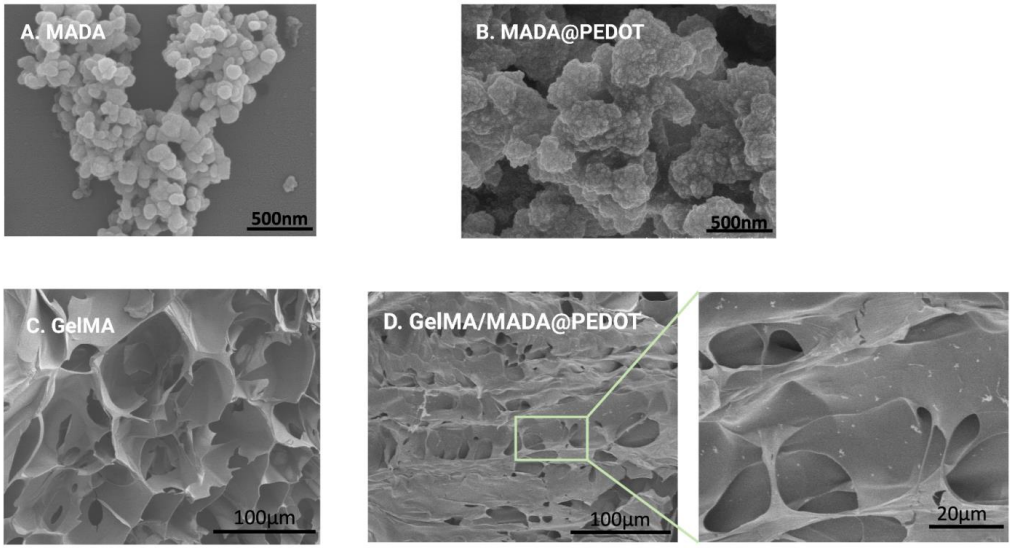

Figure 5. Surface topography of GelMA/MADA and GelMA/MADA@PEDOT

3.5. GelMA/MADA@PEDOT Modulate the Inflammatory Phenotype of Macrophages

Macrophage polarizations were studied through the flow cytometry (FACS) analysis of THP-1 cells. Figure 6 shows a high percentage of cells expressing CD86 (Q1: 82.0%) and low percentage expressing CD206 (Q3: 17.7%), indicating a predominant M1 macrophage population for the control group. There is a decrease in CD86 expression (Q1: 68.4%) and a substantial increase in CD206 expression (Q3: 31.3%), showing that M1 gradually polarizes into M2 phenotypes. Moreover, PEDOT coating induces polarization towards M2 phenotype, as cells expressing CD86 increased (Q1: 38.6%) and CD206 decreased significantly (Q3:60.3%). The Q3/Q1 trends increased from a low ratio of 0.2 in the control group, to an intermediate ratio of 0.5 in MADA, and finally a high ratio of 1.5 in the MADA@PEDOT group. The increasing trend shows MADA@PEDOT promotes macrophage phenotype transformations, which is helpful in controlling inflammation for tissue integration and impairment.

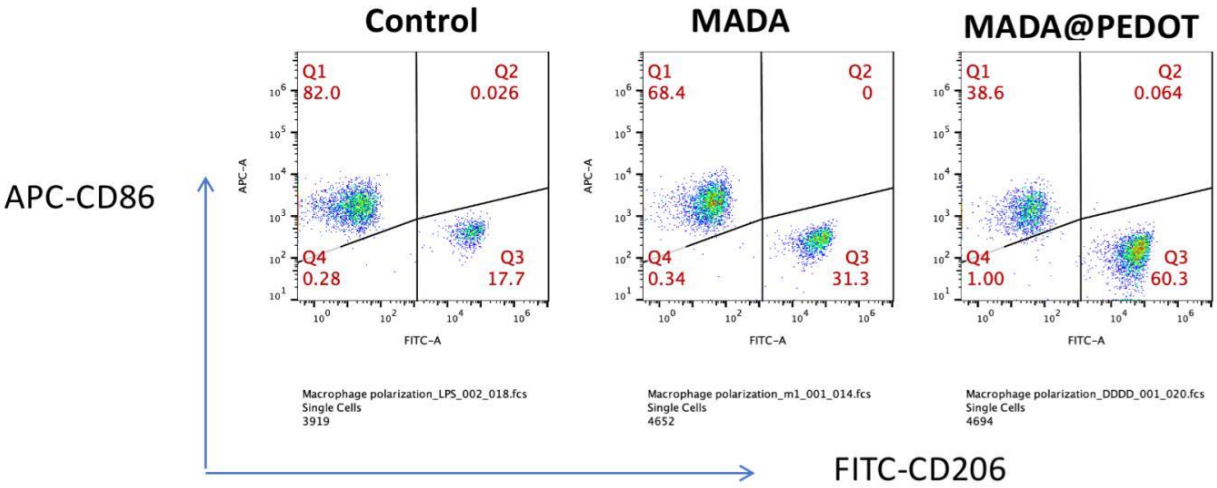

Figure 6. Flow cytometry of THP-1 cells for transformations of macrophage phenotypes

Immunofluorescence staining shows that MADA can increase the secretion of vinculin in HT22 cells and can maintain this high secretion of protein after PEDOT coating (Figure 7).

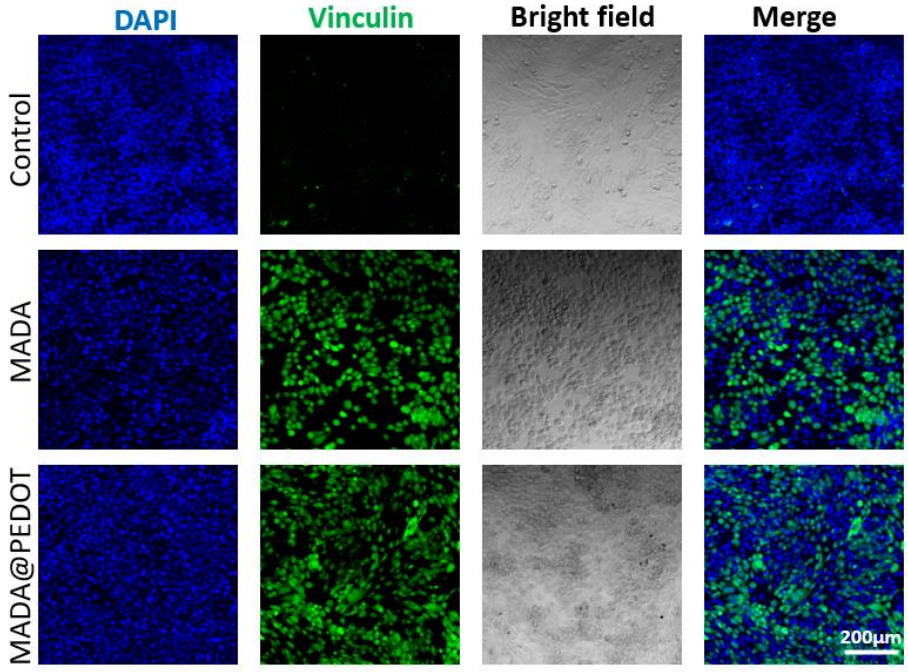

Figure 7. Immunofluorescence images of HT22 cells in GelMA/MADA and GelMA/MADA@PEDOT. Cells were stained with Hoechst (green), and cell nuclei (blue)

4. Conclusion

This work prepares MADA@PEDOT nanoparticles through oxidative polymerization and mixes it with GelMA to create a light-crosslinking hydrogel neuroelectrode (GelMA/MADA@PEDOT). The hydrogel mimics neural tissues in terms of mechanical properties, with improved conductivity that helps with neural signal transduction and its porous structure provides the matrix of neural cells. GelMA/MADA@PEDOT also shows great biocompatibility and anti-inflammatory ability, as well as enhanced cell adhesion. Therefore, GelMA/MADA@PEDOT neuroelectrodes help with the development of neurological diseases repairment.

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