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The fate of ultrafast degrading polymeric implants in the brain

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Abstract

We have recently reported on an ultra fast degrading tyrosine-derived terpolymer that degrades and resorbs within hours, and is a suitable for use in cortical neural prosthetic applications. Here we further characterize this polymer, and describe a new tyrosine-derived fast degrading terpolymer in which the poly(ethylene glycol) (PEG) is replaced by poly(trimethylene carbonate) (PTMC). This PTMC containing terpolymer showed similar degradation characteristics but its resorption was negligible in the same period. Thus, changes in the polymer chemistry allowed for the development of two ultrafast degrading polymers with distinct difference in resorption properties. The *in vivo* tissue response to both polymers used as intraparenchymal cortical devices was compared to poly(lactic-co-glycolic acid) (PLGA). Slow resorbing, indwelling implant resulted in continuous glial activation and loss of neural tissue. In contrast, the fast degrading tyrosine-derived terpolymer that is also fast resorbing, significantly reduced both the glial response in the implantation site and the neuronal exclusion zone. Such polymers allow for brain tissue recovery, thus render them suitable for neural interfacing applications.

Keywords

brain tissue response; biodegradation; bioerosion; ultrafast-degrading polymers; tyrosine-derived terpolymer

1. Introduction

Biodegradable, erodible, and non-degradable polymeric implants have been explored for diverse central nervous system (CNS) bioengineering applications[1–10]. Examples of CNS efforts vary from carriers for electronic and micro-fluidic devices[5–11], scaffolds for tissue regeneration[1, 2, 4, 12–14], and localized drug delivery vehicles via nanoparticles, solid

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constructs, or their combination[1, 3, 15–18]. Both natural and synthetic polymers have been suggested for use in such applications, with varying chemistries, degradation rates, and resorption rates (if any). Recently reported naturally occurring polymers for use in the CNS are silk protein, gelatin and agarose. These were studied specifically for brain computer interface applications, as carriers for delivery of neural prosthetics[6, 7], or substrates for the formation of the electrode itself[19]. Synthetic biodegradable polymers allow the control and adjustment of material properties such as degradation rates, mechanical properties, and lack of immunogenicity. The most commonly studied polymers are the synthetic biodegradable poly(α -hydroxy acids) such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and copolymers thereof (PLGA)[8, 9, 20, 21]. The latter are used to allow for better control of biodegradation properties, where variants such as copolymerization ratio and initial molecular weight can result in faster degradation rates than the homopolymers[22, 23]. Poly(ethylene glycol) (PEG) is an additional synthetic polymer intensively investigated in CNS applications, either in its homopolymer form (as device insertion support construct) [10], or in copolymerized form (for localized drug delivery or hydrogel regeneration scaffolds)[1, 2, 4, 13, 24].

However, in brain tissue applications, the effect of the aforementioned materials and their possible degradation products on adjacent neuronal population in the parenchyma is rarely reported[6–9, 21, 25]. These data are essential for evaluation of the temporal performance of intracortical devices. In particular, it is widely accepted that the persistent immune response to indwelling brain implant (in the form of gliosis) will result in localized continuous neurodegeneration[5, 26, 27].

Here we describe the brain tissue response to degrading polymers of new chemistries. Their synthesis, fabrication into implantable monofilaments, and the evaluation to their effect on surrounding tissue *in vivo* are portrayed. We chose to use the latest sub-group of tyrosine-derived biodegradable polymers as they have been shown to be biologically benign and possess the ability to alter their degradation rates through terpolymer structure which allows for the investigation of the effect of resorption and degradation kinetics on their surrounding tissue *in vivo*[11, 28, 29]. We synthesized and evaluated new types of terpolymers, where desaminotyrosyl-tyrosine ethyl esters (DTE), desaminotyrosyl-tyrosine (DT) and PEG were used as the degradation rate governing elements[11, 28, 29], while PTMC was used as a more hydrophobic alternative component to PEG that allowed for a significant modification of the resorption properties of the final polymer. Furthermore, the potential of these polymers for use in neural tissue applications was studied by way of evaluation of the fate of implanted polymeric monofilaments in rat brains. Assessment of the brain tissue response to the implants were carried out through the observation and quantification of the surrounding cellular response. The performance of the new polymers was compared to the implants made of a 50:50 PLGA polymer, a commercially available and commonly used fast degrading polymer[22, 23], previously studied in brain tissue applications[8, 15]. A significant difference in glial and neuronal response to the changes in polymer chemistries was measured. These changes reported here were materialized through continuous neurodegeneration as a result of indwelling implants, which also is inclusive of the brains ability to recover from the insertion and presence of fast resorbing polymers.

2. Materials and Methods

2.1 Polymer synthesis

All chemicals used were reagent grade. Trimethylene carbonate was obtained from Boehringer Ingelheim USA (Ridgefield, CT). Tin (II) 2-ethylhexanoate and 1,3-propanediol were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Dichloromethane (DCM) and methanol were obtained from Fisher Scientific Co. (Pittsburg, PA). Molecular weights

and polydispersity (PD) were determined using GPC relative to polystyrene standards. The GPC system consisted of a 515 HPLC pump, 717plus autosampler, a 2414 RI detector and Empower 2 software (Waters Corporation, Milford, MA). Two PL gel columns (Polymer Laboratories, Amherst, MA) 1000 and 100,000 Å were used in series. Tetrahydrofuran (THF, flow rate 1 mL/min) or dimethylformamide (DMF, flow rate of 0.8 mL/min) both from Omnisolv, EMD Chemicals, (Gibbstown, NJ) were used as the mobile phase (DMF also contained 0.1% TFA). ¹H NMR measurements were made using a Varian 300 MHz Instrument (Lexington, MA). Preparation of poly(trimethylene carbonate) of Mn 1250 (PTMC1250): Modified literature procedure was used to synthesize PTMC1250 [30]. Into a 100 mL round-bottomed flask were sequentially added 3.32 g (44 mmol) of 1,3-propanediol, 0.259 g (0.64 mmol) of Tin(II) 2-ethylhexanoate, and 51.2 g (500 mmol) of trimethylene carbonate along with a 1" egg-shaped magnetic stir bar. The flask was evacuated and maintained under static vacuum. The flask was immersed into an oil bath maintained at 130 °C using a stirrer/hot plate. The reaction was continued for 5 h under constant stirring. After cooling to room temperature the reaction mixture was dissolved in 200 mL of DCM and the product was precipitated with 1 L of methanol. The supernatant was decanted off and the oily precipitate was stirred with 200 mL of hexane. After removal of hexane layer, the oily precipitate was dried under vacuum at 40 °C for 24 h. GPC of the product (THF) gave a polystyrene equivalent Mw of 2841 Da with polydispersity of 1.36. ¹H NMR (DMSO-d₆, ppm) 4.51 (1, OH), 4.12 (t, (O)CO-CH₂CH₂CH₂-OC(O)), 3.42 (m, HO-CH₂CH₂CH₂-OC(O)), 1.93 (m, (O)CO-CH₂CH₂CH₂-OC(O)), 1.72 (m, HO-CH₂CH₂CH₂-OC(O)). Number average molecular weight (Mn) of 1380 Da was obtained by the comparison of the integration area of the peaks at 4.12 ppm and 3.42 ppm, which is in agreement with the expected theoretical value based on the ratio of the reagents used (1236 Da).

Preparation of poly(45%DTE-co-50%DT-co-5% PEG2000 carbonate): This terpolymer was prepared using procedures of described in Magno et al[28]. Preparation of poly(40%DTE-co-50%DT-co-10% PTMC1250 carbonate): Similar synthetic procedure was used to the one employed for the preparation poly(45%DTE-co-50%DT-co-5% PEG2000 carbonate)[28] with the following modifications: DTE (10.0 g, 28.0 mmol), DTtBu (11.7 g, 30.4 mmol) and PTMC1250 (3.04 g, 2.4 mmol) were dissolved in 150 mL of DCM and 19 mL of pyridine. A solution of triphosgene in 20 mL of DCM (6.5 g, 22 mmol equivalent to 66 mmol of phosgene) was added to the reaction mixture and stirred for a two-hour period at room temperature. After the desired molecular weight was reached, TFA (90 mL) was added to the reaction mixture and stirred overnight. The polymer was precipitated using isopropyl alcohol (IPA, 400 mL) in 4 L industrial blender and then subsequently washed with IPA, IPA: H₂O (1:1) and H₂O. The polymer was dried under the N₂ gas overnight and followed by vacuum drying at 40 °C for 48 hours. The polymer purity and composition was determined by ¹H-NMR (in DMSO-d₆ or CDCl₃). The ratio integration of DTE, DT and PTMC ¹H-NMR peaks was used to confirm polymer composition.

To simplify the naming of the tyrosine-derived terpolymers, the notation E5005(2k) is used to name poly(45%DTE-co-50%DT-co-5% PEG2000 carbonate). Similarly, when poly(trimethylene carbonate) (PTMC) was used, the notation E5010(PTMC) was employed to name poly(40%DTE-co-50%DT-co-10% PTMC1250 carbonate). The PLGA notation was used for the poly(DL-lactide-co-glycolide), with 50:50 ratio of monomers purchased from Sigma (P2191) and was used as received.

2.2 Monofilament fabrication

To allow for a consistent implantable sample preparation, a mold system was created. First, an aluminum positive mold was machined into a 25×100 mm basin with full width ridges measuring 25 mm wide × 0.18 mm tall × 0.18 mm thick, and 10mm spacing between them. Polydimethyl-siloxane (Fisher Scientific, Pittsburg, PA) was molded and set over the

aluminum mold to create a flexible negative mold. Polymer powder was dissolved at 20% w/v in THF with the addition of 0.5% (w/w) of hydrophobic rhodamine B (Sigma, St. Louis, MO) to allow for visualization of the translucent polymers [31]. Using a 27G needle (Fisher Scientific), the solvent was filled into the grooves in the PDS mold and left to evaporate overnight under nitrogen in a fume hood, followed by 7 days of vacuum drying at room temperature. Formed filaments were then trimmed under a dissecting microscope (Fisher Scientific) using a surgical scalpel (Fisher Scientific).

2.3 Polymer degradation and erosion

For *in vitro* evaluations of polymer degradation (loss of molecular weight), monofilament samples were placed in separate scintillation vials containing pre-warmed (37 °C) phosphate buffer saline (PBS, pH 7.4, Sigma St. Louis, MO). Final polymer concentration was of 0.5 mg filament/1.0 mL buffer. Vials were incubated at 37 °C for the duration of the study. At predetermined time-intervals, three samples of each polymer were removed from the incubator and visually inspected for structural integrity. Vials were frozen at −20 °C to stop degradation process and lyophilized to remove all water. Dried samples were dissolved in 1.5 mL of DMF containing 0.1% TFA and filtered (0.45µm filters, Whatman, Piscataway, NJ) in preparation for molecular weight measurements, which were determined as described above. Molecular weights of degraded samples were compared to that of pristine polymer filaments stored at −20 °C in airtight bags. For the evaluation of polymer erosion, Monofilaments were manually inserted into agarose gel capsules placed inside wells within a 6 well plate. Agarose gel was prepared at 0.6% w/w agarose (Sigma, St. Louis, MO) in PBS and cut into 10 mL cubic blocks used as phantom brain models[11] as previously described. This allowed for time dependant observation of the filament integrity within the phantom model. Images of filaments in capsules were taken using an inverted microscope (Axio Observer-D1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) with a 10x objective in phase contrast mode. To avoid dehydration between time points of image acquisition PBS was added to each well, prior to being covered and placed in an incubator at 37°C.

2.4 Sterilization, implantation, and in vivo characterization (monofilaments)

To allow accurate placement and smooth insertion of the monofilaments, guiding insertion catheters were used (see supplementary data). Prior to use, catheters with monofilaments were placed in self-sealing sterilizable pouches and sterilized with ethylene oxide gas (Anderson Products, Chapel Hill, NC) followed by 10 days aeration.

The Institutional Animal Care and Use Committee (IACUC) at Wadsworth Center approved all surgical procedures involving animals. Surgeries were performed according previously described procedures with slight modifications[32]. Briefly, 160 g male Sprague–Dawley rats were anesthetized using isoflurane maintained at 2% (in oxygen) for the duration of the procedure (approximately 60 min) and placed in a stereotaxic holder. Four craniotomy holes were drilled using electric drill (Dremel, Racine, WI), (two on each side of midline, one anterior to bregma and one posterior to lambda). The dura was transected from the area of interest. Using a stereotaxic holder, catheters were accurately placed above the insertion area allowing for smooth insertion of the monofilaments. In each brain, three types of fibers were implanted (E5005(2k), E5010(PTMC), and PLGA) while one craniotomy site was left empty as control. With each animal, the fiber implantation locations were altered to eliminate placement dependant response variability. Cellulose dialysis film (Fisher Scientific) was cut to 5 × 5 mm squares and applied over the exposed tissue, adhered to the skull (Instant crazy glue, Elmer's products Columbus OH) and the skin was closed using surgical staples.

2.5 Tissue processing and immunohistochemistry (IHC)

Animals were sacrificed by first being anesthetized with a ketamine/xylazine mixture, followed by transcardial perfusion and storage of tissue in 4% paraformaldehyde for 24 h[33]. Brain tissue was obtained using previously established procedures[32–34]. Horizontal 100 μ m thick tissue slices were cut using a vibratory microtome (Vibratom®, model 1000, Bannockburn, IL) and stored individually in HEPES-buffered Hanks saline solution (HBSS) containing sodium azide until further use. Sections used for analysis were collected 900–1100 μ m below the dorsal surface of the brain. To minimize variability between samples, IHC was performed on all the tissue slices, using specific labeling for three cell types. Primary antibodies: (1) Astrocytes, rat anti-GFAP (13-0300, dilution 1:200, Invitrogen, Carlsbad, CA) (2) Microglia, rabbit anti-Iba1 (019-19741, dilution 1:800, Wako, Richmond, VA) (3) Neurons, mouse anti-neuron-specific protein (NeuN clone A60 MAB377, dilution 1:250, Millipore, Billerica, MA). Secondary antibodies: (1) Goat anti-rabbit (Alexa Fluor 488 A11008, dilution 1:200, Invitrogen), (2) Goat anti-rat (Alexa Fluor 546 A110081, dilution 1:200, Invitrogen), (3) Goat anti-mouse (Alexa Fluor 594 A21125, dilution 1:250, Invitrogen). Sections were mounted on glass slides with anti-fade reagent (P36930, Invitrogen) for confocal imaging.

2.6 Data collection

Images of histological samples were collected in the form of 3D data sets using a TCS SP2 confocal laser-scanning inverted microscope (Leica, Bannockburn, IL). To minimize variability, imaging of all samples was performed consecutively. The exposure time of each marker was consistent throughout the imaging process using the same laser power and detector and gain setting for each specific antibody stained tissue. Images were stacked into X, Y maximal projections of the entire Z dimension of the sample to allow for evaluation of cellular populations surrounding insertion sites. Images of the insertion site and at least two adjacent lateral fields were collected for each sample. Composite images of extended regions of tissue were formed using the image-processing program ImageJ (National Institute of Health version 1.43) by applying a 3D stitching application. For the analysis of astrocyte and microglia activation, the pixel intensity was calculated as a function of distance from center of the implant site using ImageJ. Individual channels were converted from 32 bit to 8-bit, consisting of 256 shades of grey from black to white followed by a background correction performed by normalizing the average pixel intensity of the whole image to that of the most distant uninjured section. To limit aliasing, automated contrast enhancement was performed to each image. The grayscale intensity of a linear array of line profiles originated from the center of the implant site and expanded laterally with 2.5 μ m spacing generated a spatial intensity profile. A simplified illustration demonstrating this technique can be found in the supplementary data file. The profiles were averaged for each time point and experimental condition (polymer) and the standard deviations were calculated. This allowed for generation of an average intensity profile plot for all collected samples (in arbitrary Fluorescence Intensity units (FI)), and represented the spatial distribution and extent of tissue reaction and infiltration into the implant site. The peak intensities formed at the implant-tissue interface were used as the initial point for plotting GFAP and Iba1 reactivity. Statistical differentiation analysis was performed in accordance to the peak's spatial location and its intensity. We defined a “neuronal exclusion zone - NEZ”[35] using the quantitative radial profile intensity analysis of the NeuN marker surrounding the implant with 3 μ m increments of from the center. A simplified illustration demonstrating this technique can be found in the supplementary data Fig. S2. The spatial locations of the initial readings of NeuN signal were used for determination of the NEZ expansion. To ease the comparison between the various groups, a fluorescent intensity threshold of 40 FI units was assigned as a marker of initial return to normal levels for neural cell population.

2.7 Statistical analysis

Statistical analyses were performed using Addinsoft statistical software. For comparisons involving multiple conditions, one-way standard analysis of variance (ANOVA) was used. When a significant difference was found between groups Tukey's Honesty Significant Difference, (HSD) post-hoc test was utilized to identify pairwise differences. A *p*-value of less than 0.01 was considered significant.

3. Results and discussion

3.1 Polymers synthesis

The chemical structure of the tyrosine-derived polymers and PLGA used in this work are shown in Fig. 1. The weight average molecular weights of each polymer measured via GPC is as follows: E5010 (PTMC) =138 kDa, E5005(2k) =148 kDa, PLGA =111 kDa.

3.2 Polymer degradation and erosion in vitro

Polymer degradation (in form of molecular weight retention) for the E5005(2k), E5010(PTMC) and PLGA is depicted in Fig. 2. The PTMC based tyrosine-derived polymer demonstrated a similar rapid reduction in molecular weight to the PEG-based polymer (E5005(2k) with loss of 80% of polymer chain length within 75 hours. During the same period, the PLGA showed less than 20% loss of its molecular weight. In contrast to the degradation data, the PTMC-based terpolymer did not rapidly erode compared to PEG-based terpolymers (images shown in supplementary data file Fig. S3), and behaved comparably to the slower degrading and slow eroding PLGA. Even though PLGA has been reported to be a fast degrading polyester[23] it is possible that either due to the high initial molecular weight of the polymer used in this study or relatively short period, its degradation and resorption properties were less profound[23]. We used a high molecular weight PLGA for two reasons: (a) so that all three polymer would be similar in their initial molecular weight (over 100kD), and (b) to allow improved mechanical strength of filaments.

PTMC is a hydrophobic molecule compared to the hydrophilic PEG[36] and hence reduced water uptake was expected with the PTMC based terpolymer compared to the PEG based one. The fast rate of E5010(PTMC) degradation can be possibly attributed of the presence of high levels of the DT constituent in its backbone (50 mole percent), the monomeric element we have previously shown to accelerate and govern chain scission[11, 28, 29]. However, the hydrophobic nature of the PTMC possibly led to the E5010(PTMC) lack of erosion compared to the PEG based E5005(2k), since PEG has been shown to accelerate water uptake, dissolution and degradation[11, 28, 29].

3.3 Qualitative brain tissue response to implantable polymer monofilaments

Images of two explanted brains, one and four weeks post insertion, are shown in Fig. 3. The resulting images conform to the *in vitro* data: complete resorption of E5005(2K) and little to no resorption of E5010(PTMC) and PLGA. After one week, the E5005(2K) insertion site showed some swelling at the implant site, while at the 4-week time point it was indistinguishable from control craniotomy (with dura removal) site.

Fig. 4 illustrates representative immunofluorescent images obtained from harvested and processed brain sections at one and four weeks after the craniotomy and implantation of PLGA, E5010(PTMC), and E5005(2K) monofilaments. These sections were processed using immunohistochemistry techniques staining of microglia (blue), astrocytes (orange), neurons (green). Fluorescence intensities were measured for each channel and used for qualitative assessment of the effect of the monofilament on the brain tissue. Based on the visualization at one week post implantation, PLGA and E5010(PTMC) monofilaments were

still present in the tissue. However, tissue processing caused dislodging of the sliced monofilament, completely in the case of the PLGA and partially in the case of the E5010(PTMC) (seen as a light blue colored reflectance). This is evident by an empty space that was previously occupied by the monofilaments. In contrast, the E5005(2K) monofilament was completely resorbed from the area of insertion by one week. Moreover, the space that was previously occupied by the monofilament was significantly smaller compared to the other polymers, and showed signs of cellular infiltration and tissue contraction. The control craniotomy sites showed no tissue loss but still demonstrated a mild degree of gliosis and neuronal exclusion[37, 38]. The trauma caused by the craniotomy and insertion processes is enough to cause tissue damage visible within the one-week time point. Such phenomenon is reported when neural prosthetics are used[35], but less so when tissue scaffolds are inserted to brain parenchyma[12, 14]. An immediate (acute) reactive glial response should be expected regardless of the levels of trauma caused to the brain. Once craniotomy is performed, activated microglia and astrocytes appear near the injury site[37, 38]. Penetration trauma further accentuates this phenomenon, specifically with indwelling implants resulting in a chronic glial response[35, 39]. Brain implant's ability to allow recovery from the trauma (to the extent possible) and minimize the transition from the acute stage to the chronic stage is an advantageous feature.

Visual inspection of tissue four weeks post implantation, showed little, if any, signs of PLGA resorption. However, unlike the one-week sample, the four-week PLGA polymer filament remained intact within the histological slice after processing as indicated by a light blue reflectance of the polymer seen in the implantation hole (Fig. 4, PLGA 4wk). We observed during the tissue slicing and processing that the polymer became softer and more pliable. Thus, the observed denser layer of astrocytes formed at the filament interface (Fig. 5b) could explain why the PLGA monofilament became more stable within the tissue. Kou et al.[15], reported the nearly complete *in vitro* and *in vivo* resorption (via bulk erosion) of PLGA (with molecular weights of 4.5 and 22 KD) within four weeks in brain tissue. The discrepancy in our findings may be attributed to the significant difference in the molecular weight of the polymer used in our study (> 100 KD), which led to the reduction in the rate of erosion[15, 40].

E5010(PTMC) monofilament showed further signs of surface fractioning at four-weeks post implantation. The core of the monofilament was fractured and removed during processing, however the monofilament surface disintegrated into fragments that remained within the tissue. It is possible that the rapid loss of molecular weight (Fig. 2) caused the disintegration of the monofilament into smaller fragments without actually being fully resorbed[41]. It is also possible that the formation of a dense glial response surrounding both the E5010(PTMC) and the PLGA contributed to its full or partial presence within the tissue. This observation is further clarified through the examination of each individual cellular channel, shown in the supplementary data file (Fig. S4).

In contrast, four week insertion sites of the fast resorbing E5005(2k) displayed a significantly smaller damaged area. Cellular infiltration consisting of mostly microglia and astrocytes (blue and yellow stains) can be seen in the area previously occupied by the resorbed monofilament. The size of the astrocytic sheath surrounding the insertion site appears to be smaller after four weeks when compared to the one-week post implantation and other tested monofilaments.

3.4 Quantitative brain tissue response to implantable polymer monofilaments

Quantification of the above-described observations was performed through the measurement of the cellular response to the implanted monofilaments as grey scale intensity versus. distance, from the peak intensity to the area of background intensity. This allowed for

evaluation of the size of each implant, contribution of each cell type to the reactive response, and evaluation of cellular infiltration into the implant site (in the E5005(2K) case).

Within one week, the microglia intensity shown in Fig. 5a indicated a slightly higher (though statistically insignificant) cellular response to the slow resorbing PLGA and E5010(PTMC) monofilaments (80 FI vs. 70 FI). The microglia response gradually reduced to the normal tissue levels further away from the implanted monofilament, spanning approximately 250 μm from the peak. This phenomenon is a typical response to a foreign material insertion into the brain parenchyma[5, 26, 32, 39, 42]. A temporal reduction in the state of activation of microglia is expected after implantation with silicon and microwire devices[39, 43]. However, in the case of PLGA and E5010(PTMC) monofilaments, the temporal extent of microglia response did not change with time and was similar in both one and four-week tissue samples. This retention of microglia response can possibly be attributed to the fact that the slow resorbing polymers are constantly secreting their degradation products to the surrounding tissue, resulting in constant activation of microglia for the removal of the degraded material[26].

The E5005(2K) instigated a different kind of microglia response (Fig. 5a). One-week post implantation, large number of activated microglia were present at the center of the implant site, at the space previously occupied by the fully resorbed polymer. After four weeks, the microglia signal was subdued not only in its intensity (from 80 FI to 55 FI), but in its spatial extent as well, indicated by FI intensity return to normal background levels 100 μm away from the center (compared to 250 μm after one week). This reduction in the extent of microglia response possibly indicates tissue recovery from both the injury and presence of the degraded monofilament. In addition, this microglia response was comparable to the control craniotomy sites indicative to the benignity of the E5005(2k) and its degradation products.

Fig. 5b illustrates both spatial and temporal difference of the extent of astrocyte reaction to the fast and slow resorbing monofilaments. In all cases, the intensity of astrocyte signal (GFAP) as a function of distance from the implant was somewhat reduced from one to four week. In addition, the data suggest that the slow resorbing PLGA and E5010(PTMC) exhibited the formation of a more compact sheath of astrocytes surrounding the monofilaments. This was evident by the increased peak in GFAP intensity at the monofilament interface (from approximately 30 FI units to 45 FI units) and a sharper reduction further away (return to normal levels at approximately 280 μm compared to 350 μm at the one week time point). This phenomenon is expected for indwelling brain implants[44]. The control sites exhibited similar levels of astrocyte intensity when compared to the E5005(2k) after the first week of implantation. However, the control had a smaller spatial distribution (return to normal levels at 80 μm compared to 120 μm). After four weeks, the control's peak intensity and spatial extent were reduced, though not entirely. The E5005(2k) did not show a reduction in peak intensity after four weeks, but did demonstrate a reduction in spatial expansion of astrocyte response (return to normal levels at 150 μm compared to 220 μm).

Unlike glial cells, mature neurons do not proliferate, and are possibly the most important cellular indicators to the extent of damage caused by the insertion of the foreign body into the brain. Moreover, the neural response to implanted devices is considered as an imperative parameter in design and evaluation of neuroprosthetics[5, 27]. Calculated NEZ for all tested conditions are presented in Fig. 5c. To ease the qualitative comparison between conditions, we depicted a 40 FI units threshold, as an indicator of the neural tissue return to approximate normal levels. One week post implantation, possibly due to the sheer physical presence of the E5010(PTMC) and PLGA filaments, a reduction in the measured neuron intensity was

seen. The region of neural exclusion roughly conforms to the area of maximal activation of astrocytes and microglia, measured as complete neuronal loss at 80 μm and 120 μm away from implant center for the E5010(PTMC) and the PLGA, respectively. The threshold crossing for these materials was observed at 150 μm and 200 μm . Four-week post implantation, unlike the glial response that remained the same or even subdued, the NEZ increased compared to one week. The crossing of threshold levels was at approximately 280 μm away from the implant site for both slow resorbing polymers. This data indicates a continuous neural regression and degeneration, possibly triggered by the constant or prolonged presence of activated microglia[27].

In the case of fast resorbing E5005(2K), its effect on NEZ was more comparable to that of the control craniotomy sites rather than that of the other monofilaments. After one week, complete neuron absence was observed 30 μm away from implant center with threshold crossing 200 μm away. At four weeks, an upward shift of neural levels occurred, indicated by presence of neurons at the center of implantation site (x-axis origin) and threshold crossing 100 μm away. This result can be explained by the rapid elimination of the E5005(2k) monofilament from the implantation site, along with tissue shrinkage and infiltration. The craniotomy sites demonstrated a similar trend to the E5005(2k), though a lesser extent of neuronal loss was seen, which is in agreement with measured findings both for neuronal loss and glial response instigated by both craniotomy and dura removal[35, 37, 38]. The E5005(2k) permitted tissue infiltration and presence of neurons at the implant site, in a form that resembles the control sites rather than the slow eroding polymers. Even though the polymer degrades rapidly it is possible that the removal of the degradation products is supported both by their metabolite and non-toxic nature[45, 46], and by the fluid influx in brain tissue[47]. It is possible that the rate of polymer degradation and erosion, surpasses the rate of transition of the acute glial response to the chronic one[5, 26], thus allow tissue recovery. As we have previously suggested[11], such a material property may be a useful feature in the field of neuroprosthetics. The E5005(2k) could allow for neuronal proximity when used as a degradable coating or carrier for neural electrodes, a highly desired feature for neural prosthetics (i.e. where action potentials cannot be recorded from neurons that are distanced over 150 μm from the electrode[48]). Moreover, such polymer, while causing minimal collateral damage, could potentially be used as implantable device for localized drug delivery devices within various tissues.

4. Conclusions

We have demonstrated the versatility in the synthesis and resulting degradation and erosion properties of tyrosine-derived polycarbonates through a new terpolymer compositions containing poly(trimethylene carbonate). The effect of polymer implant resorption on the fate of surrounding neural tissue was found to depend on the rate of implant resorption. The favorable fast resorption and degradation of E5005(2k) resulted in the most rapid and efficient tissue recovery at the implant site. The ultrafast degrading but slow resorbing nature of the E5010(PTMC) did not result in measured differences in tissue response compared to the slow degrading slow eroding PLGA. In addition, a continuous insult to glial cells was observed deeming both slow resorbing polymers, regardless of degradation rate, unsuitable for applications where immediate tissue recovery is necessary. These findings lead us to conclude that the E5005(2k) is a strong candidate for localized therapies in the central nervous system. Specifically, E5005(2k) –based devices offer the potential for neural prosthetic applications, where the neuronal survival at the implant site, along with minimization of distance from implant to neuronal soma are crucial requirements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Chemical structure of (a) poly(DTE-co-XX%DT-co-YY%PEG(Wk) carbonate), (b) poly(DTE-co-XX%DT-co-YY%PTMC(Wk) carbonate), and (c) poly(M%lactic-co-N%glycolic acid)

Fig. 2.
Molecular weight retention of E5005(2k), E5010(PTMC), and PLGA.

Fig. 3.

Explanted rat brains one and four weeks after monofilament implant. Sites were implanted with monofilaments of E5010(PMTC), PLGA, or E5005(2k). Control sites were surgically prepared for microfilament implant, but no implant made. Polymers were labeled with rhodamine (Sigma) to allow for visualization of the translucent polymers. Rhodamine signal (pink) is present at the implant sites for monofilaments of the E5010(PMTC) and the PLGA at both one and four weeks post implant. Similar to control locations, no rhodamine signal was observed at the E5005(2k) implantation sites at both one and four weeks, indicative of the ultrafast degradation and absorption characteristics of this polymer.

Fig. 4.

Representative confocal images of brain sections at depths of 1000–1100 μm from the cortical surface immuno-labeled for neurons (NeuN-green), astrocytes (GFAP-orange), and microglia (Iba1-blue) showing brain reaction surrounding implantation sites. (Top) Images of tissue samples prepared one week post implantation from brain regions implanted with PLGA, E5010(PTMC), E5005(2k), and controls surgically prepared for microfilament insertion, but not inserted. Dislodging of PLGA and partial dislodging of E5010(pTMC) are observed. Signs of tissue infiltration into E5005(2k) are evident. All sites demonstrate elevated levels of microglia and astrocyte (Bottom) Images of tissue samples prepared four weeks (4 wk) after implantation. Remaining PLGA filaments (blue “shadow” in center) and partial fractioning of E5010(PTMC) are observed. Further closure of E5005(2K) site is evident. All implant sites demonstrate high levels of activated microglia and astrocytes. Scale bars=100 μm

Fig. 5.

Cellular distribution around implanted monofilaments. Quantitative line-profile intensity analysis expressed by the fluorescence intensity as a function of distance. Data are presented as mean + variance of measurement (SE) expressed as vertical lines. (a) Distribution of microglia (Iba1). (b) Distribution of astrocyte (GFAP). (c) Distribution of neurons (NeuN) resulting in neuronal exclusion zone. Dashed line represents threshold for comparison of initial return to normal neural expression levels. The presence of neurons at the center of the implant site after four weeks for the E5005(2k) is indicated by fluorescence reading at the origin of the x-axis, similarly to the control site. The two other polymers showed neural presence only at distances of 100 μm and further