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In vivo biocompatibility and *in vitro* characterization of poly-lactide-*co*-glycolide structures containing Levetiracetam, for the treatment of epilepsy

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Abstract: Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and is highly resistant to medication with up to 40% of patients continuing to experience seizures whilst taking oral antiepileptic drugs. Recent research suggests that this may be due to abnormalities in the blood–brain barrier, which prevent the passage of therapeutic substances into the brain. We sought to develop a drug delivery material that could be implanted within the brain at the origin of the seizures to release antiepileptic drugs locally and avoid the blood brain barrier. We produced poly-lactide-*co*-glycolide drop-cast films and wet-spun fibers loaded with the novel antiepileptic drug Levetiracetam, and investigated their morphology, *in vitro* drug release characteristics, and brain biocompatibility in adult rats. The best performing structures released Levetiracetam constantly for at least 5 months *in vitro*, and were found to be highly brain biocompatible following month-long implantations in the motor cortex of adult rats. These results demonstrate the potential of polymer-based drug delivery devices in the treatment of epilepsy and warrant their investigation in animal models of focal epilepsy. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 100A: 424–431, 2012.

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INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent seizures that affects approximately 1% of the population, making it the world's most common serious neurologic condition.[1] It is a particularly challenging condition to manage medically, since over one-third of patients continue to experience seizures despite taking multiple antiepileptic drugs (AEDs).[1] The mechanisms underlying this drug resistance are not well understood; however, one theory with a growing body of supporting evidence is that the blood–brain barrier plays a significant role. There are many features of the normal blood–brain barrier that make it particularly impervious to substances in the plasma, and there is also evidence that repeated seizures further decrease its permeability in patients with epilepsy.[2] Administering higher oral doses of AEDs can overcome this decreased permeability and usually has a beneficial effect on seizures; however, this also produces side effects that prohibit the maintenance of this treatment regimen and is therefore not an appropriate solution to refractory seizures.

Administration of therapeutic substances directly to the pathological region of the brain is a concept that has garnered increasing research attention in recent years. This route of administration has been shown in animal models to permit high drug concentrations in the brain whilst reducing the incidence of side effects.[3–6] These findings initiated interest in implantable drug delivery devices for drug-resistant neurological disorders with focal pathologies, including epilepsy.[7] Such devices permit long-term delivery of AEDs directly to the region of the brain responsible for producing the seizures, whilst drastically reducing the concentration of AED in unrelated areas of the body.

Previous efforts to design drug-eluting implants for neurological disorders have achieved some success using

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biodegradable polymers. These polymers slowly degrade within the body into biocompatible metabolites, and gradually release therapeutic substances into the local microenvironment as the polymer scaffold degrades. Biodegradable polymers also alleviate the need for a second surgery to remove the implant. The most commonly used biodegradable polymer has been poly(D,L-lactide-*co*-glycolide) (PLGA), due to the fact that it is highly biocompatible, its chemistry is easily engineered to alter its biodegradation characteristics, and it has been approved for drug delivery purposes by the United States Food and Drug Administration.

A range of the more conventional AEDs have been incorporated into biodegradable drug delivery devices. One novel AED that is used orally for the treatment of focal-onset seizures in patients with refractory epilepsy is Levetiracetam (LEV). Major side effects of oral LEV treatment include somnolence, headache, and mood changes related to the distribution of the drug throughout the brain following oral administration, and often lead to withdrawal of treatment. It would be of significant benefit to patients to formulate a LEV delivery vehicle that can supply the LEV dosage directly to the seizure focus to eliminate these unwanted side effects. Here, we seek to develop an intracranially implantable drug delivery device capable of releasing LEV directly into the brain parenchyma over an extended time period.

In this investigation, we assessed the response of the brain to a 5-week subdural implantation of thin dropcast PLGA-based films with a maximum loading of LEV to confirm the biocompatibility of the implant materials and design. We then investigated a range of LEV loaded-PLGA dropcast films and wet-spun fibers, to determine the formulation that provided optimal long-term LEV release.

MATERIALS AND METHODS

Materials

Levetiracetam [(S)-2-(2-oxopyrrolidin-1-yl)butanamide] was a gift from UCB Pharma (Brussels, Belgium). PLGA with two different mole ratios of lactide:glycolide were investigated: PLGA 85:15 (films *in vivo*: MW 50,000; Sigma–Aldrich, Australia; and *in vitro* MW 135,000; Purac Asia-Pacific, Singapore) and PLGA 75:25 [(75:25, MW 110,000; Purac Asia-Pacific, Singapore]. Solvents were acetone (Aldrich Sigma) and dichloromethane (DCM) (Chem-Supply Pty). Artificial cerebrospinal fluid (aCSF) was prepared containing sodium chloride (NaCl; 0.866% w/v), potassium chloride (KCl; 0.224% w/v), calcium dichloride ($CaCl_2.2H_2O$; 0.0206% w/v), and magnesium dichloride ($MgCl_2.6H_2O$; 0.0164% w/v) in 1 m*M* phosphate buffer (pH 7.4).

Preparation of LEV PLGA films/fibers

Films for *in vivo* biocompatibility testing were produced by drop casting 1 mL of a solution of PLGA 85:15 (2% w/v) and LEV (4% w/v) in DCM into a Teflon mold (2 × 2 cm) and dried overnight. The theoretical LEV loading was 67% w/w. Blank films were produced by drop casting the same solution without the inclusion of LEV.

Films and fibers were then produced for morphological and *in vitro* LEV release analysis. PLGA films were dropcast in the same manner as described above, by combining PLGA 75:25 or PLGA 85:15 with LEV in DCM at the concentrations given in Table I. LEV-loaded PLGA fibers were made by wet spinning the PLGA-LEV solution through a 1:4 isopropanol:hexane (v/v) coagulation bath using previously described methods.[8,9] Blank films and fibers were produced as described except that LEV was omitted from the polymer solution. Images of these films and fibers were taken using a scanning electron microscope (Jeoul SEM). Samples were prepared for SEM by sputter coating with a thin layer of gold (current 30 mA for 10 s), using a Dynavac Magnetron SC100MS.

TABLE I. Formulations of Polymer Films and Fibers for *In Vitro* Investigation

Polymer	PLGA (w/v)	LEV (w/v)	Theoretical LEV Loading (w/w)
PLGA 85:15	10%	0.4%	4%
		1.16%	10%
		4%	28%
PLGA 75:25	20%	1%	4%
		4%	17%

Evaluation of the *in vivo* biocompatibility of LEV-loaded PLGA films

To investigate biocompatibility, rats were implanted subdurally with blank or LEV-releasing PLGA sheets for 5 weeks, and compared with rats that underwent craniotomies alone. After 5 weeks, the brains were sectioned and examined for evidence of toxic or immune-mediated injury. This involved looking at the morphology of neurons in thionin-stained sections, and examining the activation of macrophages/monocytes and foreign body giant cells (anti-ED1) and the activation and morphology of astrocytes and glial scarring (anti-glial fibrillary acidic protein (GFAP)) using immunohistochemistry.

Animals. Male Sprague–Dawley rats weighing 250–400 g were obtained from Flinders University (Adelaide, Australia). All experiments were carried out under license of the St. Vincent's Hospital (Melbourne) Animal Ethics Committee in accordance with the Australian Prevention of Cruelty to Animals Act (1986), which includes the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Surgical implantation. Rats were anaesthetized with ketamine (70 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and administered carprofen (Rimadyl; 5 mg/kg, s.c.) for pain relief, and received maintenance isoflurane anesthesia throughout the surgery (2% in oxygen). The head of the rat was placed in a stereotaxic frame (Kopf) and a craniotomy created above the right motor cortex (5 × 5 mm, centered over AP: 0 mm, ML: +4.0 mm from Bregma). The central section of bone was removed and a flap cut in the exposed dura. A sheet of polymer (3 × 3 mm) either with ($n = 8$) or without ($n = 6$) LEV was placed on the brain, and the

dural flap and bone piece replaced. The craniotomy site was sealed with dental cement and the scalp sutured closed. Sham surgeries were performed as described, without the placement of a polymer sheet on the brain ($n = 3$).

Tissue preparation. Five weeks after surgery the rats were deeply anaesthetized with sodium pentobarbitone (Virbac, Australia; 200 mg/kg, i.p.) and perfused transcardially with 100 mL of 0.1*M* phosphate-buffered saline (PBS, pH 7.4) followed by 500 mL of 10% neutral-buffered formalin (NBF, Sigma–Aldrich, Australia). The brain was immediately dissected and post-fixed in 10% NBF at 4°C then cryoprotected in 30% sucrose in 0.1*M* PBS (pH 7.4). The brain was then embedded in Tissue-Tek OCT Compound, frozen in liquid nitrogen-cooled isopentane, and stored at −80°C. Brains were sectioned using a Leica CM1850 cryostat. Parallel series of coronal sections were cut at 20 μm throughout the implant site, mounted onto chrome-gel-alum subbed microscope slides, air-dried then stored at −80°C until further processing.

Histology. One series of sections from each brain was stained with thionin. Slides were stained with 0.25% thionin (Sigma–Aldrich, Australia) for 3 min, washed in distilled water (1 min), dehydrated in ascending concentrations of ethanol (50%, 70%, and 95%; 1 min each), 95% ethanol/1% acetic acid (1 min), and 100% ethanol (2 × 1 min), then cleared in xylene (2 × 3 min; Ajax Finechem, Australia), mounted in DPX (Sigma–Aldrich, Australia), and coverslipped.

Immunohistochemistry. To ensure that immunohistochemistry conditions remained identical, sections from all brains were processed simultaneously for each of the antibodies used. Slides were placed into trisodium citrate buffer (10 m*M*, pH 6.0). Antigen retrieval was performed in a microwave (750W) on full power until the solution started to boil, then 2 × 5 min at 10% power. Slides were left to cool in solution (20 min). Sections were washed in PBS (3 × 5 min), incubated in 0.5% H_2O_2 in methanol (15 min), and washed again in PBS (3 × 5 min). Slides were then incubated in 10% normal goat serum [1 h, room temperature (RT) in a humidified chamber]; washed again in PBS (3 × 5 min), before overnight incubations in either anti-GFAP (1:1000; rabbit polyclonal from Chemicon) or anti-ED1 (1:800; mouse monoclonal from Serotec) primary antibodies at 4°C. The following day slides were washed in PBS (3 × 5 min), incubated (30 min, humid chamber, RT) in secondary antibody (Envision$^+$ Dual Link System which contains horse radish peroxidase-labeled polymer conjugated to both goat-anti mouse and goat-anti rabbit immunoglobulins; DakoCytomation, Australia), and washed again in PBS (3 × 5 min). Immunoreactivity was visualized using 0.05% DAB intensified with ammonium nickel sulfate (0.05%) and cobalt chloride (0.05%) in the presence of 0.015% H_2O_2 in PBS (pH 7.2) for 8 min. Subsequently, sections were washed in PBS (3 × 5 min), lightly counterstained in methyl green solution (0.5%) in 0.1*M* sodium acetate buffer (pH 4.2) for 5 min, rinsed in dH_2O, dehydrated quickly through ethanol (95%, 100%, and 100%, 10 dips each), cleared in xylene (2 × 3 min), and coverslipped using DPX. Primary antibody was omitted on the negative control sections. Rat spleen, liver, and infected brain were used as positive control tissue for the anti-ED1 antibody.

Histological and immunohistochemical analysis. Bright-field images were obtained using a SPOT RT-Slider digital camera attached to an Olympus IX70 microscope. Images were captured using SPOT software version 4.0.9 (Diagnostic Instruments).

Thionin-stained sections were examined to assess the macroscopic consequences of polymer implantation. The degree of damage was rated as follows: minor—incomplete disruption of the meninges and disruption of molecular cortical layer; moderate—complete disruption of the meninges and molecular cortical layer; severe—damage extending beyond the meninges and molecular cortical layer. Anti-GFAP and anti-ED1 immunoreactivity was examined in the brain tissue beneath the centre of the implant and compared with immunoreactivity in the contralateral hemisphere, and rated as a mild, moderate, or severe increase in immunoreactivity. A severe increase was defined as a level of immunoreactivity similar to that in the regions of brain that suffered mechanical damage during implantation. To display the results visually, each of the rankings was given a numerical score (minor = 1; moderate = 2; severe = 3) and the mean score and standard deviation was determined for each group (sham, blank polymer, and LEV polymer).

In vitro investigations

To prepare the films for *in vitro* drug release investigations, discs of 2.5-mm diameter were cut using a hole punch, weighed, and placed in separate vials of 300 μL aCSF. To prepare the fibers, bunches of fibers (2-cm length) were cut, weighed then placed in vials of 750 μL aCSF. Vials of films and fibers were incubated in a water bath (37°C). At set time points, three samples were removed and the concentration of LEV in the aCSF was then determined using high-performance liquid chromatography (HPLC). The concentration of LEV was then related to the mass of the film or fibers incubated and the drug release was reported as mass LEV (μg) released per mg of film or fiber.

The HPLC system comprised of a Shimadzu LC-10AT pump, a Shimadzu SIL-10AXL autosampler, a UV Activon Linear 200 detector (set to 220 nm), with Shimadzu LC Real Time Analysis Software. The Atlantis^{TM} T3 (C18) 5-μm column (250 mm × 4.6 mm, Waters) was at RT. The injection volume was 50 μL; the mobile phase was 15% (v/v) methanol and 85% (v/v) Milli-q water (pH 2.8) and the flow rate set at 0.8 mL/min.

RESULTS

In vivo biocompatibility of LEV-loaded films

Examination of thionin-stained sections of sham brains revealed a mild disruption of the meninges, with only 33% showing disruption of deep cortical layers (see Fig. 1). This demonstrated that some damage was caused during opening

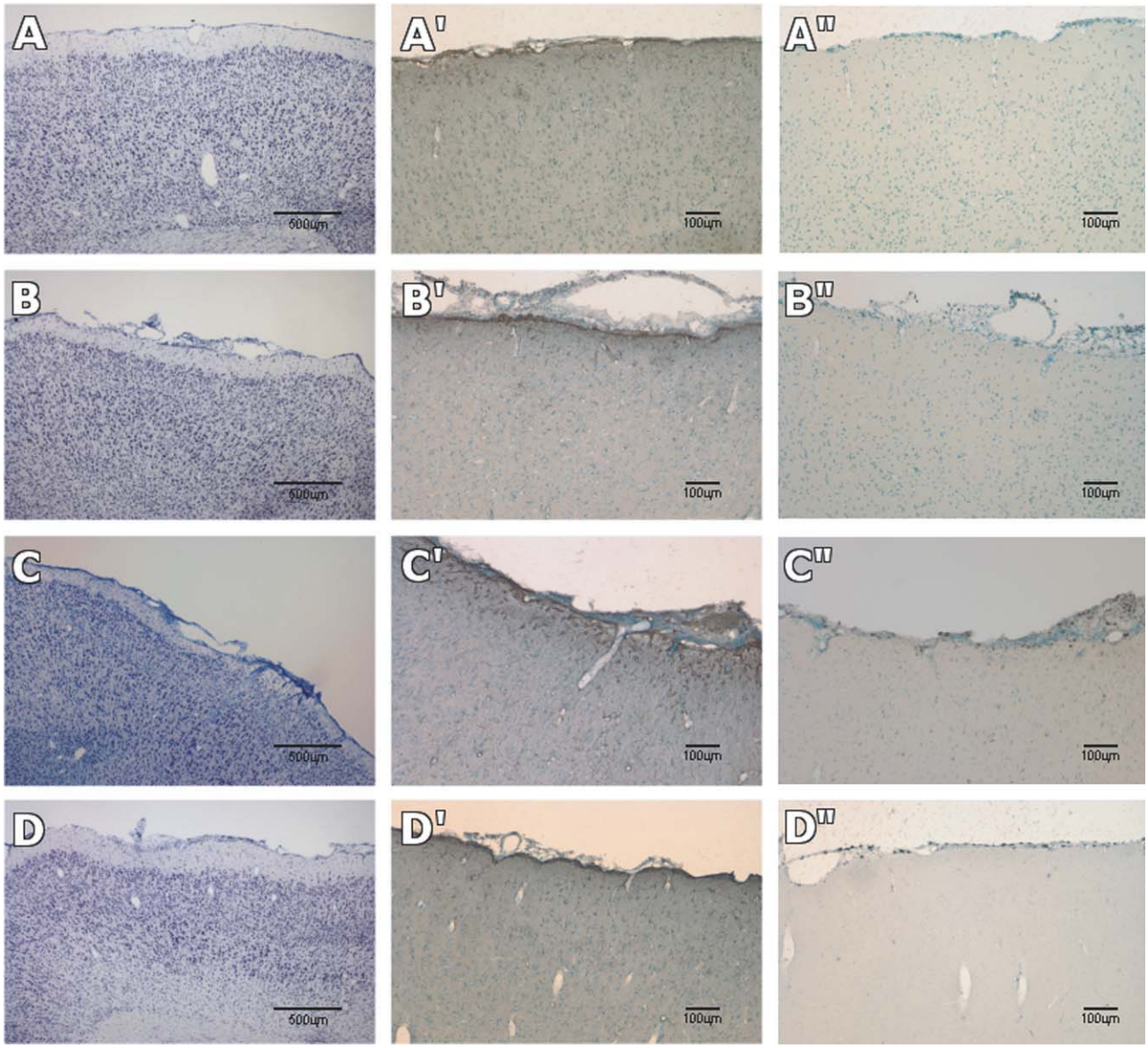

FIGURE 1. Thionin staining and anti-GFAP and anti-ED1 immunohistochemistry in the cortex of rats following 5-week subdural implantations of PLGA. (A–D) Thionin-stained sections. (A′–D′) Anti-GFAP labeled sections; (A″–D″) Anti-ED1 labeled sections; (A–A″) Contralateral left cortex; (B–B″) PGLA implants with a 67% LEV loading; (C–C″) Blank PLGA implants without LEV; (D–D″) Cortex of rats that underwent sham surgeries. Images show no difference in the damage or immunoreactivity in brains that were implanted with polymer films compared with brains that were unimplanted shams or controls. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the dura during surgery. The mean thionin score for the sham group was 2 (Fig. 2). Rats implanted with a polymer sheet showed varying degrees of disruption of the meninges. Fifty percent of rats with blank polymers and 13% of rats with LEV polymers showed moderate damage, and one rat implanted with a LEV polymer showed severe damage (Table II). The mean thionin score for the blank group was 1.5, while that for the LEV-loaded group was 1.4 (Fig. 2).

Examination of anti-GFAP labeled sections from sham rats showed that the surgical procedure alone produced a moderate increase in GFAP-like immunoreactivity at the surgical site (Fig. 1). Reactive astrocytes were observed in the region of the craniotomy; however, no glial scarring was observed at the polymer–brain interface in any of the brains examined. The mean GFAP score for the sham group was 2 (Fig. 2). The increase in GFAP-like immunoreactivity in polymer-implanted rats was not greater than that seen in these sham rats (Table II). The mean GFAP score for the blank group was 1.3, and that for the LEV-loaded group 1.25 (Fig. 2).

Examination of anti-ED1 labeled sections from sham rats showed that the craniotomy and durotomy alone produced a mild expression of ED1-like immunoreactivity, with labeled cells seen in the meninges at the centre of the craniotomy site of all brains (Fig. 1). No multinucleate giant cells were observed in any sections; however, it is worth noting that the polymer material is dissolved from the sections during histological processing and multinucleate giant cells at the tissue–polymer interface may have been removed

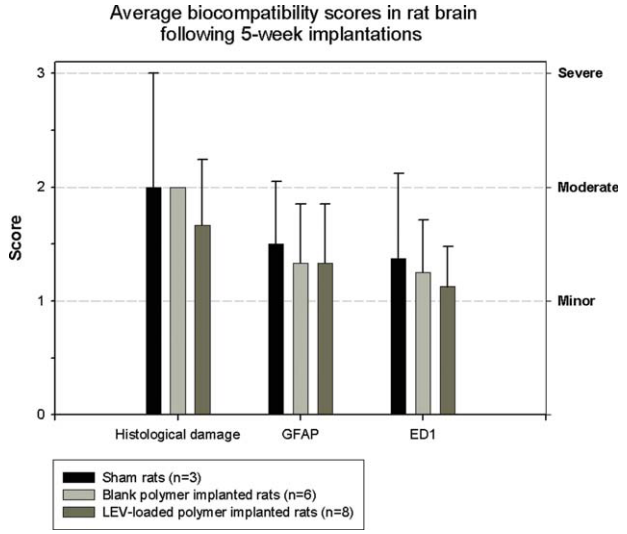

FIGURE 2. Mean biocompatibility scores in rat brains after 5 weeks of polymer implantation. Average scores are shown for sham rats, rats implanted with blank PLGA polymers and rats implanted with PLGA sheets with a 67% w/w LEV loading. Scores are given for histological damage and increases in GFAP and ED1 immunohistochemical labeling. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

along with the polymer, and we therefore cannot rule out the possibility that they may have been present. The average ED1 score for the sham group was 1.7 (Fig. 2). Sections from polymer-implanted rats were similar to those from sham rats. Thirty-three percent of brains implanted with blank polymers and one brain implanted with a LEV-loaded polymer showing moderate immunoreactivity, and none had severe immunoreactivity (Table II). The mean ED1 score for the blank group was 1.3, while that for the LEV-loaded group was 1.1 (Fig. 2).

In vitro evaluation of LEV release from PLGA films and fibers

All films and fibers produced had a smooth, nonporous surface morphology (see Fig. 3). The wet-spun fibers were 25–30 μm in diameter, regardless of PLGA or LEV concentration in solution. Visible markings were evident on the films without LEV [see Fig. 3(A,D)]. Surface adhesion between the film and Teflon mold caused stretching and deformation of the polymer surface as it was removed.

In vitro release of LEV from films with the maximal LEV loading; that is, those used for biocompatibility testing (66.6% LEV w/w in PLGA 85:15), showed that the majority of drug release occurred in the initial hours after incubation. After 1.5 h of incubation, an average of 237.3 ± 44 μg ($n = 5$) LEV was released from each disc, and reached a maximum release after 3 days of incubation at 313.0 ± 48 μg ($n = 3$) per disc.

The LEV release profile from PLGA films with lower LEV loadings are shown in Figure 4(A). PLGA 85:15 films with a 28.6% w/w LEV loading had released 56% of their drug load in the first 24 h of incubation, and 90% by the end of the first week. No further LEV was released after day 20. Lowering the LEV load in the film to 10.4% w/w almost halved the initial burst, with 32% released in the first 24 h, 60% of their drug load was released within the first week, and then gradually the remaining LEV content was released over the next 90 days. Reducing the LEV concentration to 3.8% w/w in the film did not reduce the initial burst further, 30% of their LEV content was released in the first 24 h of incubation, 89% by the end of the third week, and 98% by day 50.

PLGA 75:25 films with a 16.7% w/w LEV loading also released a substantial amount of their drug load in the first 24 h (63%). In contrast, PLGA 75:25 films with a 4.8% w/w LEV loading released only 2% of their LEV content in the first 24 h of incubation. These films then continued to gradually release LEV until day 90, at which point there was a delayed burst wherein the remainder of the LEV content was released, which coincided with observations that the films had undergone bulk degradation.

LEV release profiles from PLGA 85:15 and 75:25 fibers, with similar theoretical LEV loadings as the films, are shown in Figure 4(B). As with the films, both formulations of PLGA 85:15 fibers exhibited a burst of LEV release within 24 h. The burst from fibers with a 28.6% LEV loading was 89%, whereas from fibers with 10.4% w/w LEV loading it was 34%. These fibers with 10.4% LEV gradually and continuously released the remaining LEV until 98 days of incubation.

PLGA 75:25 fibers with a 16.7% LEV loading also exhibited a substantial initial burst, releasing 69% of their LEV content in the first 24 h of incubation. LEV continued to be released rapidly for 1 week with 80% of their initial LEV load detected in solution. PLGA 75:25 fibers with 4.8% w/w LEV released only 28% of their LEV content in the first 24 h of incubation, and then gradually released LEV until a delayed burst occurred at 90 days. This delayed burst correlated with observations that the fiber structure was no longer intact.

Comparing *in vitro* LEV release from films and fibers

To compare the temporal drug release characteristics of the films and fibers, we compared the LEV release profiles of the PLGA 75:25 films and fibers with 4.8% w/w LEV loading. The average rate of release over a subset of timeframes

TABLE II. Histological Scores

Stain Method	Histology			GFAP			ED1		
Score	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Sham ($n = 3$)	1 (33%)	1 (33%)	1 (33%)	0	3 (100%)	0	1 (33%)	2 (67%)	0
Blank ($n = 6$)	3 (50%)	3 (50%)	0	4 (67%)	2 (33%)	0	4 (67%)	2 (33%)	0
LEV ($n = 8$)	6 (75%)	1 (13%)	1 (13%)	6 (75%)	2 (25%)	0	7 (87%)	1 (13%)	0

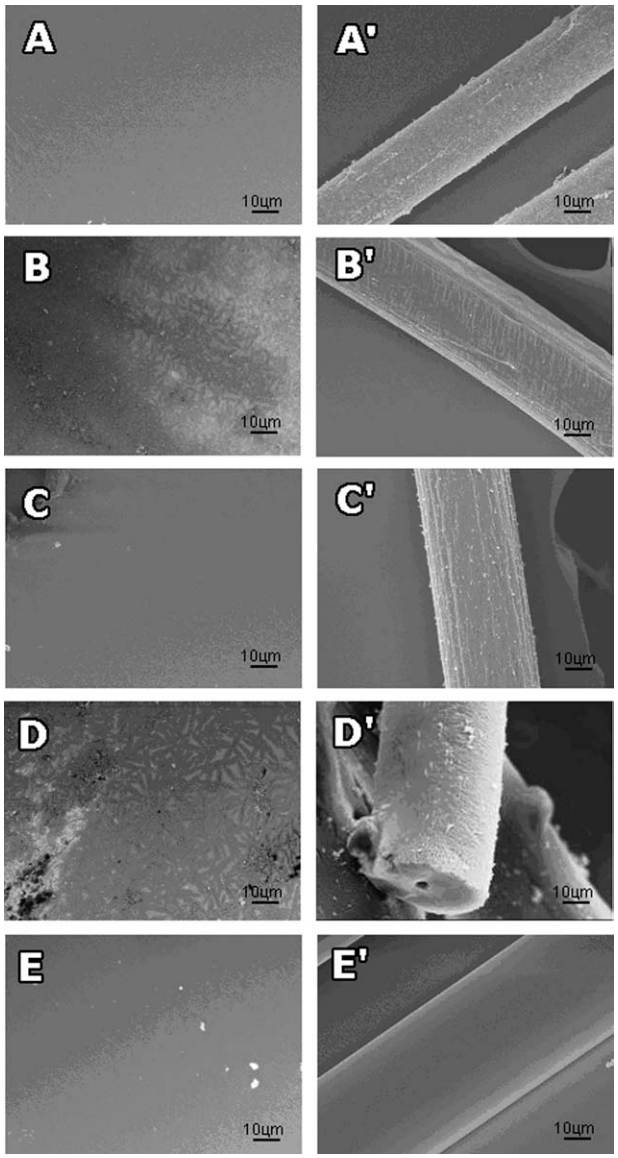

FIGURE 3. SEM images of the surface of PLGA films and fibers, with and without LEV. The column to the left shows films, while the column to the right shows fibers. (A, A′) PLGA 85:15 (10%w/v) without LEV; (B, B′) PLGA 85:15 (10% w/v) with 3.8% (w/w) LEV loading; (C, C′) PLGA 85:15 (10% w/v) with 10.4% (w/w) LEV loading; (D, D′) PLGA 75:25 (20% w/v) without LEV; (E, E′) PLGA 75:25 (20% w/v) with 4.8% w/w LEV loading.

within 90 days was calculated (Table III) and the results demonstrated that the fibers released many times more LEV than the films during the first week of incubation (32.2 μg/mg polymer/day in fibers compared with films 5.1). After 7 days, both films and fibers released similar quantities of LEV (0.2 μg/mg polymer/day) until the last 10 days of incubation when the final burst release from fibers was 1.5 times less than films.

DISCUSSION

We investigated the drug-release characteristics of LEV-loaded PLGA films and fibers *in vivo*, and examined the bio-

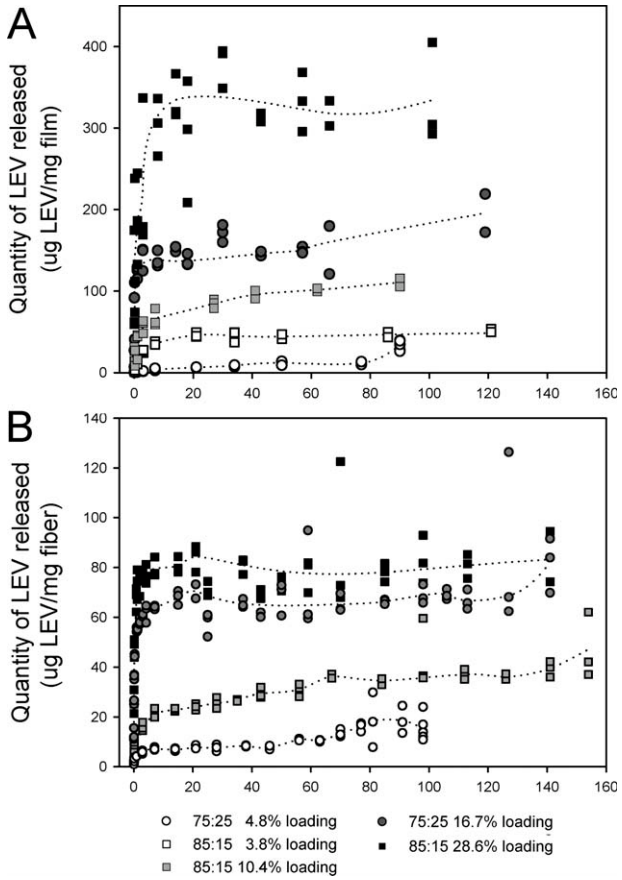

FIGURE 4. Release of Levetiracetam from PLGA films and fibers *in vitro*. (A) Release from PLGA films; (B) Release from PLGA fibers.

compatibility of LEV-loaded PLGA films following 5-week subdural implantations in adult rats.

The results of this study demonstrate that lower initial drug loadings lead to more controlled patterns of drug release, irrespective of the polymer's glycolide content or physical structure. Formulations with theoretical drug loadings of =10% had the smallest initial bursts and thereafter demonstrated gradual, continuous release of LEV, consistent with the published literature.[10–12] We hypothesize that at high drug loadings, there is unlikely to be sufficient polymer present to completely entrap the entire drug load within the polymer matrix, leading to unregulated drug release as soon as the material is immersed in fluid. In contrast, at low drug loadings, the polymer entirely and homogeneously encapsulates the drug such that drug release is primarily dictated by polymer degradation.

TABLE III. Comparison of Mean LEV Release Over Set Time Periods From the Films and Fibers Made From a Solution of PLGA 75:25 (10% w/v) and Levetiracetam (4% w/v)

	Mean Release LEV (μg/mg Polymer/day ± SD)			
Structure	0–4 h	1–7 days	7–80 days	80–90 days
Films	4.46 (±1.4)	0.58 (±0.2)	0.24 (±0.06)	0.37 (±0.07)
Fibers	29.73 (±18.1)	2.52 (±1.6)	0.25 (±0.1)	0.22 (±0.09)

The films and fibers described in this report were capable of delivering LEV in a controlled manner for at least 3 months *in vitro*. A previous investigation described a polymer-based device that released a conventional AED in a controlled manner for over 250 days *in vitro*.[13] The production of implants that release AEDs over a clinically applicable time frame was an important milestone in the field of polymer-based drug delivery; however, the bulky physical structure of the implant necessitated the removal of large portions of the cortex to implant the device. The thin film implants developed in this investigation were implanted onto the surface of the brain without displacing brain tissue and therefore significantly reduced the risk of unwanted neurological deficits. We anticipate that as more and more potent drugs are discovered and made available to researchers, slower rates of degradation can be utilized so that the period of drug release can be significantly extended. In addition, intermediate-term drug delivery systems such as those described in this report may still have several clinical applications, in particular in the post-surgical setting.

This investigation also compared drug release from film and fiber structures. The results demonstrated that the rate of drug release was greater in the fibers during the initial days of incubation, but greater in the films during the final weeks of the study. Fibers may therefore be the preferred structure when drugs with long half lives are chosen, which require a loading dose.

Our study also investigated the biocompatibility of PLGA sheets containing a maximal load of LEV following implantation subdurally in rats. There are two major ways in which an implanted material may be non-biocompatible. The first is that the material or its biodegradation products may be toxic to the cells of the central nervous system (CNS). Toxic injury can be identified by a characteristic set of histological features.[14] In the acute setting, neurons appear pyknotic with disappearance of the nucleolus and loss of Nissl substance in thionin-stained sections. In the chronic setting, neurons undergo necrosis and the necrotic cellular debris activates macrophages/monocytes and astrocytes. Fibroblasts are not present within the CNS and therefore the injury is not healed by a fibrotic scar; rather, a gliotic scar is formed by reactive astrocytes.[14] In this investigation, the polymers were still present subdurally after 5 weeks of implantation and were still undergoing biodegradation; therefore, any acute injury due to toxicity of biodegradation products should have been visible in the brain sections. On examination of the brain slices in our investigation, we found only minimal disruption of the superficial cortical layers of the brain surface, and observed that the degree of astrocyte activation following implantation of LEV-loaded PLGA sheets was no greater than that seen after implantation of blank PLGA sheets or after the sham surgical procedure.

The second way that an implant may be non-biocompatible is if the material is recognized as foreign by the innate immune defenses of the CNS and an inflammatory or immune response is initiated.[15] Microglia and monocytes/macrophages are activated, and initiate a localized inflammatory response in an attempt to destroy the foreign material. Frustrated attempts by the macrophages to engulf and destroy the material result in the fusion of multiple macrophages into more aggressive multinucleate giant cells, a histological hallmark of chronic inflammation.[15] The immune response activates astrocytes which subsequently undergo gliosis and form a glial scar, as for toxic injury. There was no evidence of multinucleate giant cells or glial scarring in any of the brains examined in this investigation. In addition, the degree of microglial activation observed after implantation of LEV-loaded PLGA sheets was no greater than that observed after implantation of blank sheets or after sham surgical procedures.

In summary, there was no evidence of toxic injury or immune-mediated inflammation observed using the methods of investigation employed in this study, indicating that the LEV-releasing PLGA sheets demonstrated excellent brain biocompatibility.

CONCLUSIONS

This study describes the production of biodegradable polymer-based materials with LEV that are (i) very well tolerated by brain tissue after 5 weeks of subdural implantation and (ii) capable of releasing LEV for at least 90 days *in vitro*. The results of this investigation indicate that these materials warrant further investigation into the longevity, long-term biocompatibility, and efficacy of such devices and suggest that biodegradable polymer-based materials are appealing candidates for intracranial drug delivery systems for the treatment of epilepsy.

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