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Poly-HEMA as a drug delivery device for *in vitro* neural networks on micro-electrode arrays

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Abstract

Delivery of pharmacological agents *in vitro* can often be a difficult, time consuming and costly process. In this paper, we describe an economical method for *in vitro* delivery using a hydrogel of poly hydroxyethyl methacrylate (PHEMA) that can absorb up to 50% of its weight of any water-solubilized pharmacological agent. This agent will then passively diffuse into surrounding media upon application *in vitro*. An *in vitro* test of PHEMA as a drug delivery device was conducted using dissociated rat-cortical neurons cultured on micro-electrode arrays. These micro-electrode arrays permit the real-time measurement of neural activity at 60 different sites across a network of neurons. Neural activity was compared during the application of PHEMA saturated with cell culture media and PHEMA saturated with bicuculline, a widely used pharmacological agent with stereotypical effects on neural activity patterns. Application of PHEMA saturated with bicuculline produced a gradual increase in concentration *in vitro*. When the minimum effective concentration of bicuculline was reached, which was found to be 0.59 μM using the diffusion properties of PHEMA, it produced the rapid almost periodic synchronized bursting characteristically associated with this agent. In contrast, the application of PHEMA saturated in culture media alone had no effect on neural activity reinforcing its inherent inert properties. Since PHEMA is nontoxic, can be molded into a variety of shapes, quickly manufactured in any laboratory and is inexpensive to produce, the material represents a promising alternative to drug delivery systems on the market today.

1. Introduction

Micro-electrode arrays (MEA), a grid of electrodes imbedded in a culture dish for multichannel-electrophysiological recordings, have become an important and popular research tool for investigations measuring the activity of populations of electrically active cells. An MEA, as shown in figure 1, allows the investigator to detect both individual action potentials from single neurons or field potentials produced by large ensembles at different locations across a network. Hence, these systems are often used for the study of population-wide dynamics during both spontaneous or elicited activity in a wide variety of cell lines such as cortical, hippocampal and spinal neurons [1–5], cardiac myocytes [6], and visual processing in the retina [7, 8]. Moreover, cells cultured on MEAs can survive for months or even years [9]. Because of this, activity can now be measured for hours, days or even weeks [10]. In fact, these features are now leading to the development of diagnostic tools such as biosensors to examine the pharmacological effects of various drugs or toxins [3, 11–14]. Pharmacological experiments present a problem, however, in that an agent or drug must be delivered to the MEA culture in a sterile and controlled manner.

One common delivery technique uses a perfusion system that circulates a perfusate (e.g., culture media) across the culture that has been mixed with the agent of interest. This permits the simultaneous measurement of the effects of a pharmacological agent on neuronal activity even as the drug concentration is varied [15]. While perfusion systems have distinct advantages, they are often elaborate and expensive

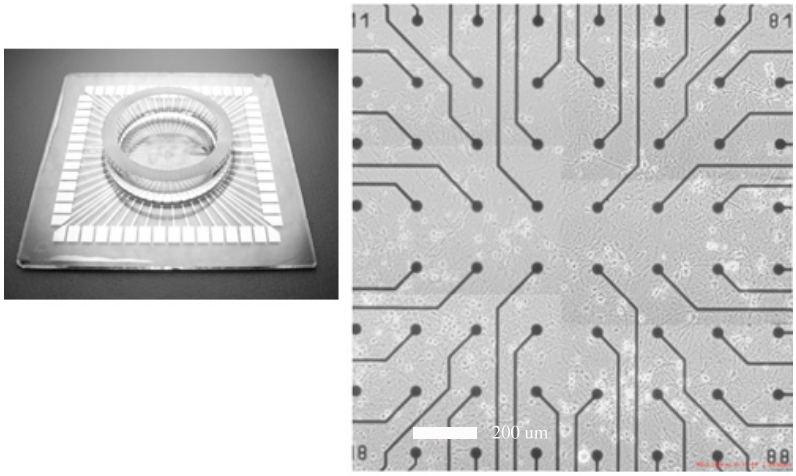

Figure 1. A micro-electrode array from multichannel systems (left) and a magnified view of the array with cultured E18 rat-cortical neurons. Sixty electrodes arranged in an 8 × 8 grid measure changes in electrical potential produced by neurons growing across the surface (SiN) of the array. Each electrode (TiN) is 30 μm and spaced 200 μm apart and can detect the action potentials produced by neurons located within approximately 20–60 μm radius of each electrode.

devices to create, problematic in terms of sterility, flow and temperature control, and are often cumbersome to operate.

Hydrogel polymers represent a promising alternative that can achieve similar results to a perfusion system without the expense or complications inherent with the use of perfusion systems. Hydrogels are cross-linked polymeric structures that become swollen with water. Unlike perfusion, hydrogels absorb any water-solubilized agent, acting as an internal drug reservoir for passive diffusion to the target tissue. One hydrogel, poly hydroxyethyl methacrylate (PHEMA), is particularly promising for drug delivery due to the fact that when fully swelled at 40% water content, it has a similar density and water composition to that of living tissue [16]. PHEMA is also biologically inert, optically clear, nonionic (suitable for blood contact), resists degradation, is permeable to metabolites, non-absorbable, can withstand autoclave sterilization and can be molded to a variety of shapes for applications in the laboratory. This material is also inexpensive, simple to manufacture in any laboratory, sterile and easy to use making it an attractive alternative to the more costly and cumbersome perfusion style systems.

Pharmaceutical applications for hydrogels including PHEMA have become a major topic of investigation dating back to the work of Wichterle and Lim [17–19]. The basic principle is to dissolve a water-soluble drug in solution that is then absorbed by the hydrogel. When placed into an environment that lacks this agent a concentration gradient is formed producing an outward diffusion of the drug into the surrounding environment [16]. Ideally, the material or method used to deliver the pharmacological agent should itself not influence neural activity. Within neural culture this is an important attribute for any system since a wide variety of variables can affect ongoing neural activity. For example, changes in temperature due to differences in the carrier-solution's temperature, differences in ionic concentrations [20] or its gas content can produce robust changes in activity. Furthermore, if the culture must be used again either for repeated trials or over multiple days, each component of the delivery system must be sterile. In contrast, PHEMA is chemically inert, non-bioactive material that can be sterilized.

The ability of poly-HEMA to deliver bioactive agents to neural cultures was evaluated by comparing the effect of poly-HEMA soaked in bicuculline on neural activity measured on MEAs. The effect of bicuculline, which produces a well-known stereotypic response in neural culture, was then compared to poly-HEMA soaked in culture media alone. Bicuculline is a phthalide isoquinoline bioactive agent derived from *Dicentra cucullaria* which is a flowering plant common in North America. Bicuculline is a specific competitive agonist of GABA [21]. Direct injection of bicuculline into the motor cortex of behaving monkeys produces rhythmic dystonia which is characterized by involuntary muscle contractions that cause involuntary movement and twisting of body parts [22]. *Ex vivo* application of bicuculline into culture results in stereotypic rhythmic bursting of neural activity [23] and increased burst duration *in vivo* [13].

Therefore, the application and diffusion of bicuculline using poly-HEMA should result in both synchronous rhythmic bursting and increased burst durations when applied to neural cultures. Moreover, as the diffusion of bicuculline out of the poly-HEMA should take several seconds, the concentration of bicuculline in the media surrounding the neurons should gradually increase resulting in a gradual increase in its effect on neuronal activity. Hence, it may be possible to plot a dose dependency curve before the concentration reaches equilibrium. In contrast, the application of poly-HEMA alone should have no effect on activity. The following details the development, construction and testing of this hydrogel as a drug delivery system using neural cultures grown on MEAs.

2. Methods

2.1. Synthesis of PHEMA

Synthesis of PHEMA from its monomer is a relatively simple procedure, which requires approximately 1 h to produce in the laboratory. Four milliliters of ultra pure water and 7.5 mg of 2,2′-azobisisobutyronitrile (AIBN Sigma cat# 441090) initiator was added to 4 ml HEMA monomer (Sigma cat# 128635) in a 50 ml test tube [24, 25]. The reaction mixture was blended by mechanical agitation and then pipetted as needed into a sterile reusable mold created from Teflon©. The mold is used for baking the reaction mixture during polymerization and creating the desired shape for application in culture. Teflon© was chosen because of its machinability, nonstick surface for HEMA removal from the mold and sterilization via an autoclave. The mold was then placed in an oven at 90–95 °C for 1 h to allow polymerization of the material to occur. At lower temperatures the reaction will slow and at temperatures above 100 °C the PHEMA may develop bubbles in the solid that forms, interfering with the rings homogeneity. Alternatively, a sandwich of two glass plates and an o-ring can be used to produce a sheet of PHEMA to reduce the presence of bubbles in the PHEMA. However, the drug delivery ring will then have to be formed by being punched from the PHEMA sheet using sterile stainless steel punches. The final product after baking should be an odorless transparent solid, as an odor would indicate incomplete polymerization of the monomer.

After removal from the oven the PHEMA ring must be removed immediately from the mold. If allowed to cool within the mold the PHEMA will gradually become brittle and difficult to remove. The PHEMA can be removed using a sterile pick to impale the ring and pull it from the mold. If the ring does become brittle or cracks, placing the ring in the oven immersed in a small quantity of tissue culture water will soften the ring. Once removed, the ring was placed in a sterile 50 ml test tube and sealed for later use. The rings can be kept in this sealed sterile environment for an indefinite amount of time until hydrated.

The release of a dissolved agent over time from a monolithic polymer can be quantified by the early-time and late-time approximations for diffusion [16, 26]. These combined equations predict the rate of release from a slab of thickness l (in cm), with a known diffusion coefficient ($cm^2\ s^{-1}$) and initial concentration. The early-time equation accounts for the first 60% of the release where the late-time equation accounts for the later 40% of release. Derived from these equations are two approximations (1) and (2) that predict per cent of an agent's initial concentration released over time, represented by concentration at time t M_t divided by the initial concentration M_0. This model has a proven accuracy reliable to better than 1% [27]. Using this model for diffusion with a thickness (l) of 0.1 cm and where the diffusion coefficient for bicuculline (D) is 2.61×10^{-9} $m^2\ s^{-1}$ (calculated from the Wilke–Chang equation [28]), we produce the predicted diffusion curve for bicuculline from our drug delivery rings, as shown in figure 2. Varying the initial concentration of bicuculline that the ring is soaked in will then determine the diffusion rate and drug concentration over time from the

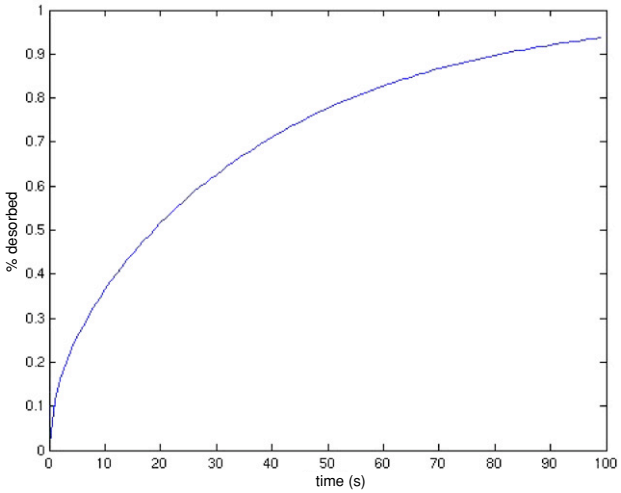

Figure 2. Diffusion curve plotted from derivations of the early-time (equation (1)) and late-time (equation (2)) equations for bicuculline from our PHEMA drug delivery rings. This curve predicts the per-cent release of bicuculline from a known initial concentration in the PHEMA ring over time.

PHEMA rings. This model for the diffusion of bicuculline from PHEMA will be tested and used to find the minimum effective concentration of bicuculline *in vitro*.

$$\frac{M_t}{M_0} = 4\left[\frac{Dt}{\pi l^2}\right]^{\frac{1}{2}} \qquad \text{early-time equation} \tag{1}$$

$$\frac{M_t}{M_0} = 1 - \frac{8}{\pi^2}\exp\left[-\frac{\pi^2 Dt}{l^2}\right] \qquad \text{late-time equation.} \tag{2}$$

One additional feature of PHEMA is that the diffusion rate can also be manipulated by adding cross-linking agents during manufacture. Cross-link density is the effective number of cross-links per original polymer chain where a cross-link is the junction between two polymer chains. Addition of cross-linking agents increases the cross-link density of the PHEMA resulting in a decrease in diffusion rates and a decrease in equilibrium water content of the hydrogel [16]. In the following experiments, only the native cross-linking resulting from the HEMA polymerization process was tested.

2.2. Creation of a ring

An example of a freshly made ring using the glass mold, optically clear, 2 mm thick and with a diameter of 14 mm is shown in figure 3. The volume of a ring when hydrated is 0.25 ml. The ring was designed to be smaller in diameter, when swollen, than the inner diameter of the MEA dish (20.3 mm). The device was manufactured as a ring rather than a disc to prevent the PHEMA from contacting the culture of neurons located at the center of the dish. This prevents abrasion of tissue within the culture and prevents the PHEMA from influencing dissolved gas transport to and from the culture media. Before an experiment, the PHEMA ring must be hydrated in culture medium in an incubator to allow carbon dioxide concentration and temperature of the ring to reach equilibrium, becoming equivalent with the

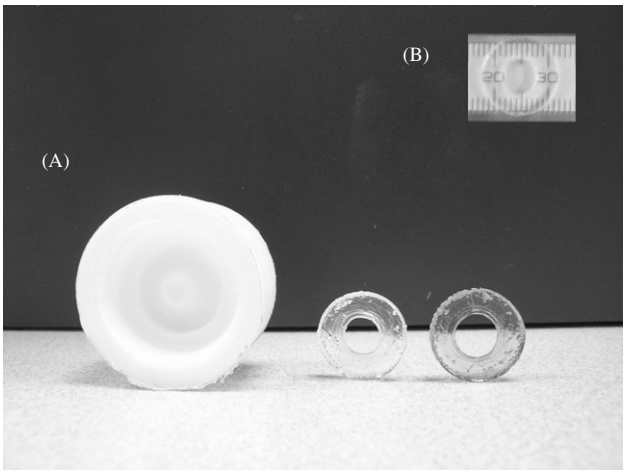

Figure 3. (A) Teflon mold with a new and media-saturated poly-HEMA ring. The Teflon mold was used to create the rings in this study. Note the optical clarity, with a few bubbles, and rigidity of the ring. (B) An example of a ring made using the glass plate mold that better demonstrated the optical clarity of the PHEMA due to the absence of bubble imperfections.

media surrounding the tissue within the MEA. Sufficient time must be also allowed for the equilibrium water content of the PHEMA to be reached. It is recommended to prepare the rings with medium a few hours before experimentation. Pharmaceutical or bioactive agents should be added to the media in the desired concentration before the equilibrium process is initiated allowing the agent to diffuse into the hydrogel. While placing the PHEMA ring into an MEA care must be taken not to touch the neural culture and to use sterilized forceps. The outer surface of the ring must be rinsed with medium before application to remove surplus liquid containing the pharmaceutical agents and to prevent delivery of excess agent from the surface of the material.

2.3. Dissociated neural cultures

The cultures used to test the PHEMA consisted of cortical hemispheres from E17–E18 Wistar rat embryos (obtained from Brain Bits Inc.) that were digested using the Papain Dissociation System (Worthington Biochemical Corporation) and mechanically triturated separating the neuron's soma from the surrounding connective tissue. After dissociation, approximately 50 000 cells were placed in the center of an MEA, as shown in figure 1, that has been coated with polyethyleneimine (PEI) [29] and laminin (Sigma) [30]. The neurons were cultured in medium consisting of 90% Dulbecco's modified Eagle's medium (DMEM) (Gibco cat# 10569-010) and 10% equine serum (HyClone cat# SH30074.03). The medium was allowed to equilibrate to 5% carbon dioxide and 35.5 °C temperature in an incubator before use. The medium in each culture was exchanged twice per week.

Neurons prepared in this way rapidly begin to reestablish a network, forming synapses to neighboring cells and producing spontaneous action potentials within the first few days after placement within the MEA [31]. As the network matures over the next two weeks, the neurons begin to express the full complement of receptors and have matured after one month *in vitro* [32–34]. All cultures were older than 30 days *in vitro* in this experiment.

2.4. Micro-electrode arrays (MEA)

The MEAs (cf figure 1) that were used for this experiment were obtained from Multichannel Systems GmBH. An array of 60 electrodes is arranged in an 8 × 8 grid, spaced 200 μm apart, to measure neural activity over a 1.6 mm^2 area. These arrays consist of a glass wafer over which a gold surface is deposited and etched using photolithography into the pattern shown in figure 1. An insulating layer of silicon nitride (SiN) is then deposited via vapor deposition over the surface providing an electrical barrier between the gold and culture media. 30 μm diameter holes are then plasma etched over the circular pads and titanium nitride (TiN) is plated onto the gold forming a low impedance interface between the recording amplifiers and culture media. Each electrode measures the changes ionic voltage produced by neurons within an approximate radius of 60 μm as they fire action potentials [35]. Conversely, the application of bi-phasic current or voltage pulses (e.g., 100–800 mV, 50–200 mA) will elicit action potentials from neurons near the stimulated channel. Neural activity was sampled and digitized at 25 kHz using Multichannel Systems MEA1060 amplifier and A/D hardware. Action potentials were distinguished from noise using a voltage threshold 5 standard deviations above or below estimated noise levels. Spike events were recorded into a file containing the time, channel, height, width and context information surrounding each event. This spike file was later processed offline to extract information about any bursts that may have occurred. Bursts were detected using a modification of the leaky integrator algorithm described by Gross [23] which is applied to the spike times for each electrode.

The environment both within and surrounding the MEA during recordings was temperature and gas compositionally controlled at 35.5 °C and 5% CO_2 duplicating the environment within the incubator in which these cells were cultured. Media temperature within the array was controlled using Multichannel System's TC02 temperature controller while the surrounding air temperature and gas levels within an enclosure surrounding the recording system (MEA1060 amplifier) was controlled using custom hardware.

2.5. Testing the PHEMA–MEA system with bicuculline

In the first phase of the experiment, neural activity without the presence of poly-HEMA rings was recorded for 10 min across the 60 electrodes to establish a baseline that can be compared with the effects of poly-HEMA with and without bicuculline. The primary measures of neural activity were inter-burst interval and burst duration. The maximum intensity of the burst (determined by maximum average firing rate during the burst) was also used to characterize bursting behavior.

Following baseline recordings, a placebo poly-HEMA ring soaked in medium and equilibrated in a 5% CO_2 35.5 °C incubator, was placed in each of the MEAs and activity was

measured for 10 min. This placebo condition was used to insure that the PHEMA with fresh media does not have an effect on either burst or firing rates.

Data from the placebo were compared to cultures in which the PHEMA ring has been soaked in 100 μM bicuculline. The volume of media in an MEA dish is 1 ml and the volume of a PHEMA ring is 0.25 ml. Thus, the maximum concentration at equilibrium in the dish media using a 100 μM ring, accounting for equilibrium water content in the PHEMA, and the dilution factor into the surrounding media will be 9.1 μM, a 11$\times$ dilution from the soaking solution. Bicuculline removes inhibitory mechanisms from the neurons and should create a highly stereotypic periodic rapid burst. Bicuculline-induced burst activity was compared to baseline and placebo activity. Following each ring application the cultures were then rinsed with fresh culture media and placed in the incubator for later use.

The second phase of the experiment was designed to show how the diffusion properties of the PHEMA rings could be used to create a gradually increasing concentration of bicuculline *in vitro*. Since there were no literature values for the minimum effective concentration of bicuculline, we systematically manipulated the initial concentration to find the range that the first-order diffusion predicted by the early- and late-time equations could be observed. Once the concentration range was found, the model was experimentally tested at various concentrations to predict and calculate the minimum effective concentration *in vitro*.

The following results were analyzed using a one-way analysis of variance (ANOVA). The Type I error for the ANOVA and for any post-hoc tests was set at 0.05.

3. Results

Application of the PHEMA ring saturated in media alone (placebo) did not produce any significant changes in burst activity relative to baseline recordings. In contrast, the application of rings with bicuculline at 100 μM significantly increased both burst duration and inter-burst interval. Figure 4 shows the mean burst duration during baseline, placebo and bicuculline conditions. A one-way analysis of variance was performed on inter-burst interval and burst duration data. There was no significant difference between mean burst durations in the baseline and placebo $F(2,9) = 0.33$. In contrast, the application of rings containing bicuculline significantly increased the mean duration of bursts relative to baseline recordings $F(2,9) = 4.23$.

Figure 5 shows the mean inter-burst interval during the baseline, placebo and bicuculline conditions. While there was no significant difference between baseline and placebo conditions $F(2,9) = 0.33$, there was a significant increase in the inter-burst intervals between baseline and bicuculline treatment with the PHEMA rings $F(2,9) = 4.62$.

Figure 6 shows a raster plot of neural activity for one of the four cultures tested during baseline, placebo and bicuculline conditions. Each plot represents the pattern of neural activity across channels over time. Each point in the plot represents a single action potential occurring near

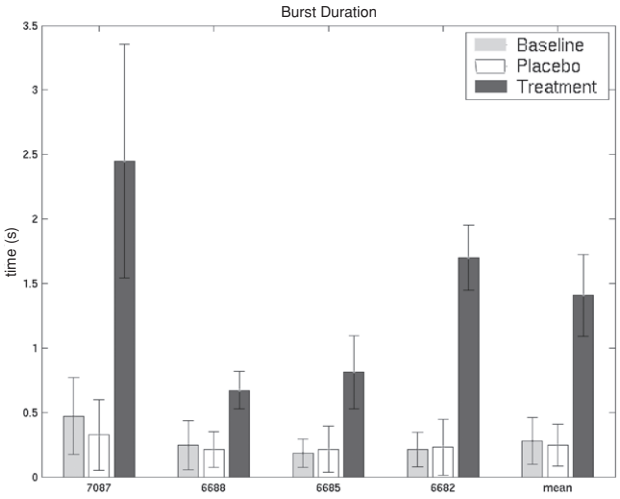

Figure 4. Mean burst duration during the baseline, placebo and bicuculline conditions. (Error bars represent the standard deviation.)

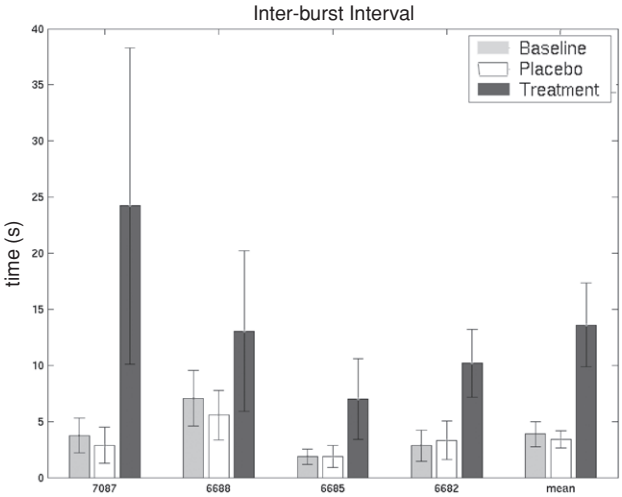

Figure 5. Mean inter-burst interval during baseline, placebo and bicuculline conditions.

the recording electrode (channel on y-axis) over the 10 min recording period. Synchronous bursts of neural activity appear as vertical groupings. During both baseline and placebo poly-HEMA ring recordings both isolated single unit activity as well as periodic bursts of activity are apparent. In contrast, following application of poly-HEMA containing bicuculline, both the duration of bursts and the inter-burst interval have changed which is congruent with the effects reported by others [13].

Figure 7(A) shows the burst durations and inter-burst intervals (IBI) for MEA 6682 during first 100 s of recording. Application of rings soaked in 100 μM bicuculline produced a rapid effect on burst duration. The rate bicuculline diffuses out of the ring is dependent both on the density of cross-linking and the initial concentration of bicuculline contained in the PHEMA ring. Since neural activity should be dependent on the concentration of bicuculline in the surrounding media, a gradual change in the pattern of neural activity/burst

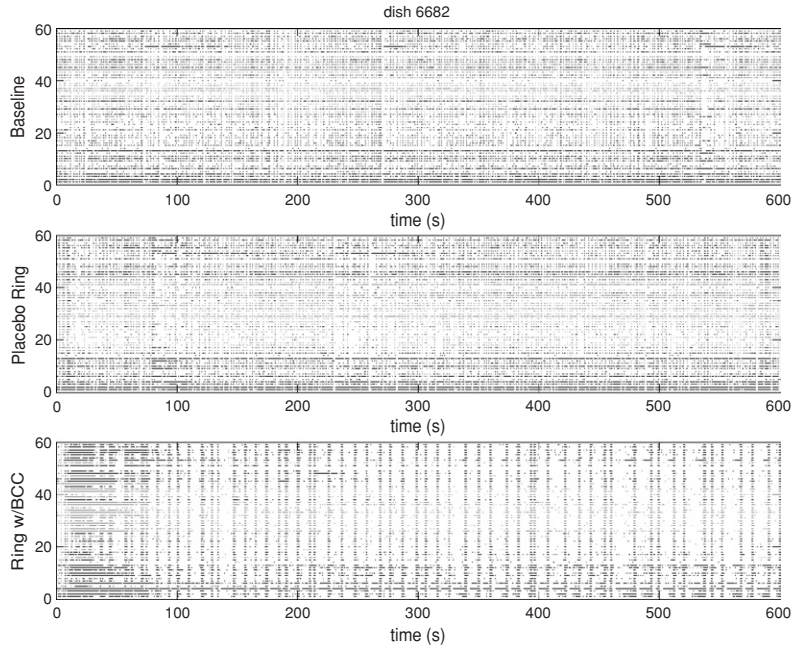

Figure 6. A raster plot of neural activity over time during the baseline, placebo and application of the bicuculline poly-HEMA rings. Each point represents a single action potential. Electrodes were numbered sequentially (left to right, top to bottom) and plotted as channel number on the vertical axis over time on the horizontal axis. Synchronized bursts of activity appear as vertical groupings of points.

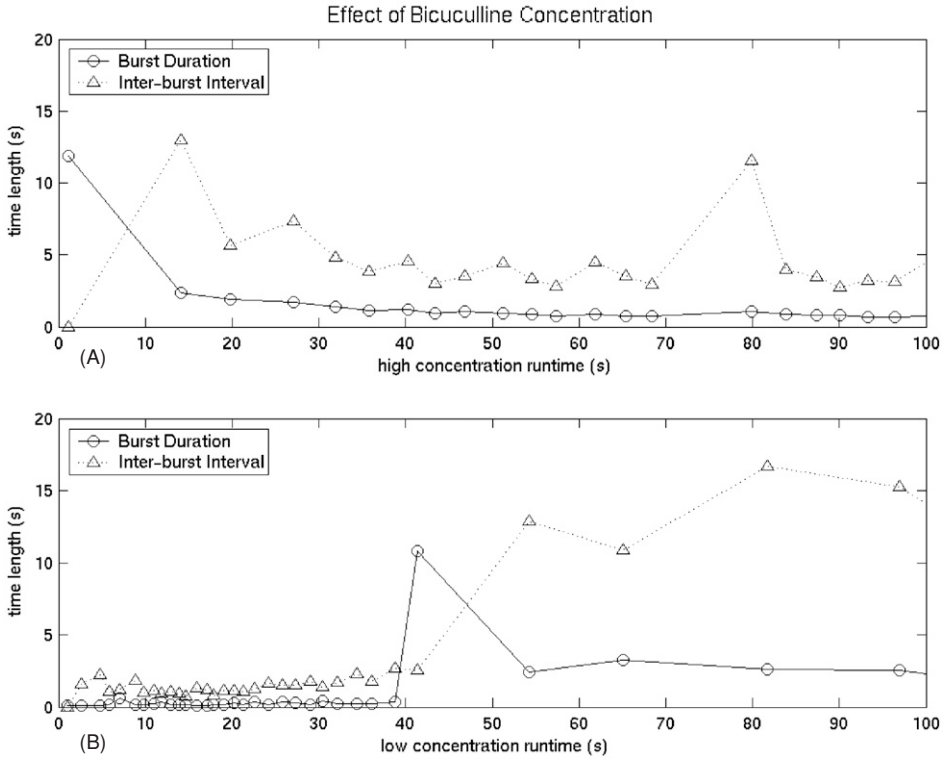

Figure 7. Time-dependent effects of diffusion of bicuculline from the PHEMA ring. Time series over the first 100 s following application of the PHEMA ring showing the mean burst duration and inter-burst interval under high and low bicuculline concentrations ((A) and (B) panels, respectively).

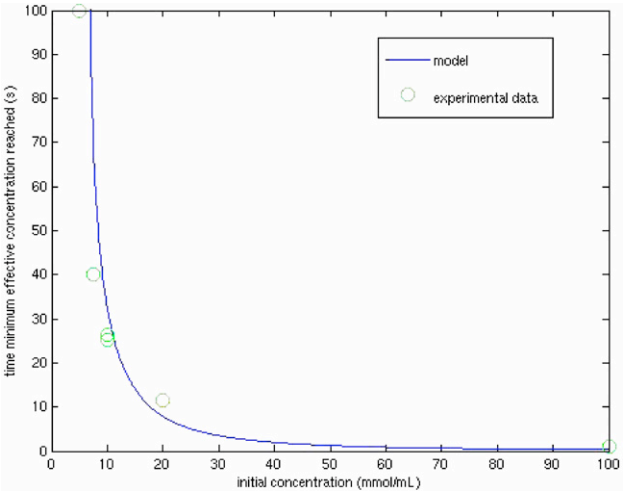

Figure 8. Experimental results for minimum effective concentration and resulting model that predicts how long until the minimum effective concentration is reached by diffusion (equation (3)) *in vitro* for various initial concentrations of bicuculline in PHEMA drug delivery rings. Initial concentration in the PHEMA ring is plotted on the *x*-axis while the resulting delay due to diffusion until the bicuculline takes effect is plotted on the *y*-axis.

characteristics should occur as the drug diffuses out of the ring. The relatively high concentration of bicuculline used of 100 μM produced a rather abrupt change in activity, as the change in concentration to pharmacologically active levels would be rapid.

To determine if this dose dependency could be detected, two MEA cultures were tested with poly-HEMA and bicuculline at a much lower concentration of approximately 5–10 μM. Figure 7(B) shows the effect on neural activity of a poly-HEMA using this lower concentration bicuculline. In contrast to higher 100 μmol l^{-1} concentrations in figure 7(A), a roughly ten-fold decrease in initial concentration resulted in a delayed yet sudden increase in both inter-burst interval and burst duration which is consistent with a slow release of bicuculline into the surrounding media until a minimum effective concentration threshold was achieved.

Once the effective concentration range was established, the first-order release of bicuculline could be observed on a reasonable time scale and with more precise concentrations. Thus, additional trials were conducted at initial concentrations of 5, 10 and 20 μM bicuculline and compared to the previously calculated diffusion model to determine the minimum effective concentration of bicuculline, which was found to be 0.59 $\pm$ 0.11 μM *in vitro*. The time at which the sudden onsets of increased burst durations and inter-burst intervals, as shown in figure 8, were detected was used

$$t = \frac{\pi}{D}\left(\frac{11M_{\mathrm{mec}}l}{4M_0}\right)^2$$

early-time dose–response derivation (3)

$$t = \frac{l^2}{-D\pi^2}\ln\left[\frac{\pi^2}{8}\left(1 - \frac{11M_{\mathrm{mec}}}{M_0}\right)\right]$$

late-time dose–response derivation (4)

to determine and calculate the minimum effective concentration of bicuculline *in vitro*. The minimum effective concentration was also used to derive a dose–response model from the early- and late-time equations (equations (3) and (4)), where M_{mec} is the minimum effective concentration and M_0 is the initial concentration in the PHEMA ring, for bicuculline using our PHEMA rings. This is shown in figure 8 along with the experimental data. Note that the 5 μM initial concentration did not produce any change in the culture activity. The maximum concentration of bicuculline at equilibrium for this concentration of 0.45 μM is less than the minimum effective concentration. This represents one extreme of the diffusion curve in contrast with the experiments at 100 μM that reach minimum effective concentration in less than 1 s.

4. Discussion

This experiment tested the ability of one class of hydrogel, poly hydroxyethyl methacrylate (PHEMA), to act as an efficient *in vitro* drug delivery device. Rings that were molded from PHEMA in the laboratory were soaked in 100 μM bicuculline solution. These rings were then immersed in the culture media on an MEA containing spontaneously active neural culture. Once placed into culture, a concentration gradient forms between the ring and surrounding media resulting in a slowed diffusion of the drug, bicuculline, into the surrounding media. Unlike a bolus application (e.g, via pipette) in which the concentration is initially high (near the droplet) and decreases as the agent rapidly diffuses into the surrounding media, the PHEMA ring produces a controlled diffusion with an initial low concentration, which systematically increases over time. Changes in the concentration of bicuculline resulted in longer burst durations and longer inter-burst intervals, which is consistent with the well-known effects of this drug. In contrast, the application of PHEMA rings soaked in culture media alone had little effect on the pattern of neural activity. Comparison of activity before and after administration indicated no difference in either burst duration or in inter-burst interval supporting the notion that PHEMA is a nontoxic, gas permeable and nonionic biopolymer [16] that is inert in neural culture.

One of the interesting features of PHEMA is the ability to control the diffusion rates of the test agent either through manipulation of initial drug concentration or via addition of cross-linking agents during manufacture. Unfortunately, at the high concentration used in testing the PHEMA ring there was little evidence of the slow changes in the pattern of neural activity indicative of the gradual release of bicuculline into the ambient media surrounding the neurons. At initial concentrations of 100 μM, the effect of the bicuculline was almost immediate resulting in longer burst durations and longer inter-burst intervals. In fact, this might be expected given the high initial concentrations used in this experiment, coupled with a burst measure of neural activity of a relatively infrequent event (i.e., bursts).

The second phase of experiments addressed this issue and the initial concentration of bicuculline was reduced until a slow first-order release could be observed. This was done by trial and error, as the minimum effective concentration

of bicuculline was unknown prior to the experiment. The decreased concentration in the first trial resulted in a 40 s delay in the onset of stereotypic bicuculline activity. As the bicuculline diffused from the PHEMA ring, the ambient concentration surrounding the neurons gradually increased, reaching an effect dosage level resulting in modified burst characteristics. More importantly, this supports the notion that diffusion is the primary mechanism for drug delivery from the PHEMA rings. However, although each PHEMA ring was rinsed of excess drug before application, some of its effect may have been due to residual bicuculline remaining on the surface of the material following saturation. When studied further at various initial concentrations, it was found that the diffusion model for bicuculline could be used to calculate the minimum effective concentration for bicuculline, found to be 0.59 ± 0.11 μM *in vitro*. Once this concentration was found, the time at which the minimum effective concentration would be reached could be readily predicted at various initial concentrations using a dose–response model derived from the early- and late-time equations.

There was also no evidence of infection or contamination following the use of the rings. The proper use of sterile procedure is key to preventing exposure of the rings, cultures and medium to infectious agents. In fact, the predominate cause of failure during the development process of these rings was due to poorly molded rings that physically abraded the neural tissue as the ring was place in culture. One solution was to make the inner diameter of the rings larger which limits the potential contact with the neural tissue. When manufactured correctly in a sterile environment, the PHEMA drug delivery ring proves a viable cost-effective alternative to a perfusion system for drug delivery.

Similar to other demonstrated uses for *in vivo* polymeric drug delivery systems, the PHEMA rings proved to be an inert, non-bioactive material that can effectively deliver pharmaceutical agents using diffusion. This material can easily be used in the laboratory and molded into a wide variety of desired shapes or more advanced designs that take advantage of these properties to produce complex medical devices. For example, by varying the cross-link density of the polymer, the initial concentration of the agent being delivered, or through the use of layering the material, these devices could be used to deliver multiple agents at different diffusion rates over time. The diffusion properties of the PHEMA can be used to inexpensively establish reliable dose dependency curves as well as establish minimum effective concentrations for various agents, a feature that could have many uses in the pharmaceutical, biosensor and neuroscience fields. Additionally, the polymer PHEMA used for these rings can be copolymerized with other polymers to give it various properties not explored in this research. With modification these copolymers could possibly be used to solublize, stabilize (e.g., by creating distinct hydrophobic and hydrophilic regions in the polymer matrix for respective nonpolar and polar agents) and deliver agents that are hard to work with due to their chemical properties. Being fairly inexpensive these devices could be made and placed in pretreated drug and media solutions and packaged similarly to disposable contact lenses as a sterile one-time use drug delivery device for *in vitro* neural culture.

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