Available online at www.sciencedirect.com
SCIENCE DIRECT
Biomaterials 27 (2006) 5368–5376
Biomaterials
www.elsevier.com/locate/biomaterials

In vitro model of glial scarring around neuroelectrodes chronically implanted in the CNS

Vadim S. Polikov[a], Michelle L. Block[b], Jean-Marc Fellous[a], Jau-Shyong Hong[b], W. Monty Reichert[a,*]

[a]*Department of Biomedical Engineering, Duke University, Durham NC 27708-0281, USA*
[b]*Neuropharmacology Section, National Institute of Environmental Health Sciences, 111 Alexander Drive, Research Triangle Park, NC 27709, USA*

Received 25 May 2006; accepted 22 June 2006
Available online 13 July 2006

Abstract

A novel in vitro model of glial scarring was developed by adapting a primary cell-based system previously used for studying neuroinflammatory processes in neurodegenerative disease. Midbrains from embryonic day 14 Fischer 344 rats were mechanically dissociated and grown on poly-D-lysine coated 24 well plates to a confluent layer of neurons, astrocytes, and microglia. The culture was injured with either a mechanical scrape or foreign-body placement (segments of 50 μm diameter stainless steel microwire), fixed at time points from 6 h to 10 days, and assessed by immunocytochemistry. Microglia invaded the scraped wound area at early time points and hypertrophied activated astrocytes repopulated the wound after 7 days. The chronic presence of microwire resulted in a glial scar forming at 10 days, with microglia forming an inner layer of cells coating the microwire, while astrocytes surrounded the microglial core with a network of cellular processes containing upregulated GFAP. Vimentin expressing cells and processes were present in the scrape at early times and within the astrocyte processes forming the glial scar. Neurons within the culture did not repopulate the scrape wound and did not respond to the microwire, although they were determined to be electrically active through patch clamp recording. The time course and relative positions of the glia in response to the different injury paradigms correlated well with stereotypical in vivo responses and warrant further work in the development of a functional in vitro test bed.

Keywords: Neural prosthesis; Foreign-body response; In vitro test; Cell culture; Brain; Biocompatibility

1. Introduction

While the promise of using thought controlled robotics to treat paralysis is very exciting, the electrodes implanted in the brain to record neuronal signals have failed to create a stable, long-term interface for obtaining the necessary control signals [1–3]. It is generally believed that a well defined, although poorly understood tissue reaction against the recording electrodes is the primary cause of the signal degradation experienced over time [4]. The in vivo response to the implantation of a chronic electrode can be divided into two stages: the initial acute response to the mechanical injury of insertion, and the chronic response resulting in a glial scar [4,5]. As the electrode is inserted into the cortex, its path severs capillaries, extracellular matrix, and glial and neuronal cell processes. Activated, proliferating microglia, the immune cells of the CNS, appear around the implant site as early as 1-day postimplantation as they migrate towards the injury site to initiate wound healing and debris clearance [5–7]. Astrocytes are also activated within the first few days and upregulate glial fibrillary acidic protein (GFAP) as far as 500 μm away from the injury site [5]. This initial reaction is transitory however, as electrode tracks are not found in animals after several months when the electrode is inserted and quickly removed [8–11].

If an implant is chronically present, a foreign-body reaction is observed, although it is unclear whether such a reaction occurs due to unresolved acute inflammation, the toxic nature of the implant material, chronic micromotion between the implant and the neural tissue, or through some

*Corresponding author. Tel.: +1 919 660 5151; fax: +1 919 660 5362.
E-mail address: reichert@duke.edu (W.M. Reichert).

0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.06.018

other mechanism [5,9,12–14]. This reaction is characterized by the presence of both reactive astrocytes and activated microglia [5,8,15,16]. Microglia will cluster around the implant in a reactive tissue sheath and will persist for the life of the implant [5,14,16,17]. The astrocytes surround this inner core of microglia in an encapsulation layer referred to as the "glial scar" [5,10,15,17–22]. Imaging studies in the first 2 weeks after insertion have revealed a reactive astrocyte region surrounding the implants extending out 500–600 μm [5]. This region decreased over time, but the layer of cells immediately adjacent to the implant become denser and more organized. The mesh of astrocytic processes becomes stronger and more compact until a complete glial scar is formed at around 6 week post-implantation.

The biological testing of neuroelectrodes has been almost exclusively performed in vivo, where microelectrode designs aimed at overcoming the tissue response are implanted into an animal model (typically rat cortex), followed by sacrifice of the animal at various time periods, and assessment of the extent of the tissue reaction to the implant (see recent review [4] and references therein). Yet, after more than two decades of in vivo testing, the mechanisms behind the signal degradation of chronically implanted electrodes remain unclear. The neural implant field would benefit from an in vitro system capable of dissecting the complicated mechanisms behind implant failure and that allows high throughput testing of new neuroelectrode designs.

This paper presents an in vitro cell culture system that has been adapted from a culture system used to study neuroinflammatory processes for the past 15 years. This culture contains all of the brain cell types known to play a major role in the tissue reaction, and that successfully recreates many of the hallmarks of glial scar formation. The cellular responses of neurons, astrocytes, and microglia to injury were characterized using immunocytochemistry. Mechanical injury, in the form of a scrape to the confluent cellular layers, and chronically placed stainless steel microwire, mimicking the presence of a foreign body, resulted in cellular responses that were similar to those documented in vivo. This system will be employed as a useful tool for the neuroelectrode biocompatibility field in understanding the causes of implant failure.

2. Materials and methods

2.1. Reagents

Cell culture ingredients were obtained from Invitrogen (Carlsbad, CA, USA). Monoclonal antibodies against the CR3 complement receptor (OX-42) and against MAP-2 were obtained from Chemicon (Temecula, CA, USA). Polyclonal antibody against IBA-1 was obtained from Wako Chemicals USA, Inc. (Richmond, VA, USA). Polyclonal antibody against GFAP was bought from DAKO Corporation (Carpinteria, CA, USA). Monoclonal antibody against vimentin was bought from Sigma–Aldrich (St. Louis, MO, USA). The Vectastain ABC kit and biotinylated secondary antibodies were purchased from Vector Laboratories (Burlingame, CA, USA). 50 μM diameter stainless steel microwire was bought from A-M Systems (Carlsborg, WA, USA). Secondary antibodies with fluorescent tags Alexa 594 and Alexa 488 were bought from Molecular Probes (Invitrogen Corporation, Carlsbad, CA, USA).

2.2. Animals

Timed-pregnant Fisher F344 rats were obtained from Charles River Laboratories (Raleigh, NC, USA). Housing and breeding of the animals were performed in strict accordance with the National Institutes of Health guidelines at the National Institutes of Environmental Health Sciences (Research Triangle Park, NC, USA).

2.3. Primary mesencephalic neuron-glia cultures

Neuron-glia cultures were prepared from the ventral mesencephalic tissues of embryonic day 13–14 rats, as described previously [23]. Briefly, dissociated cells were seeded at 5×10^5/well into poly-D-lysine-coated 24-well plates. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air, in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), 10% horse serum (HS), 1 g/L glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 μM nonessential amino acids, 50 U/mL penicillin, and 50 μg/mL streptomycin. Seven-day-old cultures were used for treatment after a media change to MEM containing 2% FBS, 2% HS, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 U/mL penicillin, and 50 μg/mL streptomycin. Data shown are representative of at least 3 different culture preparations.

2.4. Scrape (mechanical injury) model

At treatment time, a rectangular area in the middle of the culture well, approximately 2 mm on each side, was cleared of cells with a 2 mm long scrape of the tip of a cell scraper (#3010, Corning Inc., Corning, NY, USA). The injury was inflicted after the media change so that soluble factors released during the injury were present.

2.5. Wire (foreign body) model

Wire was cut into 3–5 mm pieces and soaked in 70% ethanol for at least 30 min, after which it was allowed to dry in a laminar flow hood. At treatment time, 3–4 pieces of wire were placed into each treatment well at random locations using sterile forceps, so that the pieces would sink and rest atop the cultured cell layer.

2.6. Immunostaining

Microglia were detected with the OX-42, which recognizes the CR3 receptor as described [24], or anti-IBA-1 antibody, which recognized a calcium binding protein specific to microglia, astrocytes were detected with an antibody against GFAP, and neurons were imaged by staining with MAP-2 as described previously [25,26]. Immature glia were detected with an antibody against vimentin. Briefly, formaldehyde (3.7%)—fixed cultures were treated with 1% hydrogen peroxide (10 min) followed by sequential incubation with blocking solution (20 min), primary antibody (overnight, 4 °C), biotinylated secondary antibody (1 h), and ABC reagents (1 h). Color was developed with 3,3′-diaminobenzidine. Images were recorded with an inverted microscope (Nikon, Tokyo, Japan) connected to a charge-coupled device camera (DAGE-MTI, Michigan City, IN, USA) operated with the MetaMorph software (Universal Imaging Corporation, Downingtown, PA, USA). Fluorescently labeled cultures were stained in the same way, except a fluorescently labeled secondary antibody was used in place of ABC reagents. Fluorescent images were recorded with an inverted microscope (Nikon, Tokyo, Japan) connected to a charge-coupled device camera (Sensicam QE, Cooke